Abstract

Background: Dysphagia and cognitive problems, both common after stoke, may affect dietary intake increasing the risk of malnutrition. Malnutrition has adverse effects on body composition especially in conditions that escalate the stress response in the body and may be associated with immobility such as stroke.

Study objective: The objective of my study was to understand the prognosis of malnutrition on post cardiovascular disease (CV) outcomes, understand body composition changes after stroke assessed using multi-frequency bioelectrical impedance analysis (MF-BIA) methods, examine the utility of MF-BIA in diagnosing dehydration in stroke patients, and validate MF-BIA selected body composition estimates against the reference method Dual X-ray absorptiometry (DEXA).

Methodology: To understand the prognosis of malnutrition on post CVD outcomes I carried out a systematic review and meta-analysis examining the association between selected markers of malnutrition on outcomes. The systematic review is presented in Chapter 2 of this thesis. Chapter 3 presents an observational longitudinal study that describes body composition changes after ischaemic stroke and their prognosis on outcomes. Ischaemic stroke patients admitted to an acute unit were prospectively recruited between January-July 2011. Body composition variables (BioScan 920-2, Maltron International Ltd, Essex, United Kingdom) were measured on admission and discharge. Results were descriptively presented stratified by type of feeding regimen, type of stroke and stroke severity. Validated follow up questionnaire were sent to participants by post to understand body composition changes association with their health and quality of life.

In chapter 4 the diagnostic accuracy of MF-BIA BioScan 920-2 in diagnosing dehydration after stroke was examined for several diagnostic cut offs of current and impending dehydration. In chapter 5 external validation of MF-BIA BioScan 920-2 fat free mass and fat mass estimates against reference method DEXA was examined using ten participants data. Bland and Altman analysis for understanding the agreement between two methods of clinical measurement was carried out.

Results: Undernutrition (assessed using nutrition assessment tools) were associated with mortality post cardiovascular event. Other findings are presented in Chapter 2. Fat free mass loss, and fat mass gain, protein mass loss, muscle mass loss, and body cell mass loss were observed in patients on modified diet (soft/mashed diet, pureed diet, nil-by-mouth feeding regimen). Sample size was small to generalize a conclusion on the association between body composition changes in acute stay and outcomes. MF-BIA BioScan 920-2 did not show diagnostic accuracy in diagnosing dehydration in stroke patients. MF-BIA BioScan 920-2 fat free mass and fat mass estimates were in agreement with their corresponding estimate from the reference methods DEXA.

Conclusion: My study was novel as it provided new information with regard to body composition changes in acute stroke while utilizing new validated equipment in estimating body composition component of fat free mass and fat mass. My study also aimed to investigate new non-invasive methods to diagnose dehydration in stroke patients. It contributed new knowledge that can be useful in future research, sample size calculation, and can help researchers in the field to determine minimally clinically significant differences for similar research and targeted intervention clinical trials.

Measuring Nutritional Status, Hydration and Body Composition Changes in Acute Stroke

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“Dedicated to my mother Mayya and Father Wasfi for giving me a normal childhood in abnormal surroundings in Palestine….”

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PRESENTATIONS:

M W Kafri, A H Wilson, D Doherty, J F Potter, P K Myint, L Hooper. **Diagnostic accuracy of bioelectrical impedance analysis (BIA) for water-loss dehydration after stroke.** Oral presentation at the British Geriatric Society Spring Meeting 2012, Llandudno, Wales.

MW Kafri, L Hooper, D Doherty, JF Potter, PK Myint. **Type of feeding regimen and body composition. Poster presentation at the British Geriatric Society Spring Meeting 2012, Llandudno, Wales.**

PUBLICATIONS:

Mohannad W Kafri, Phyo Kyaw Myint, Danielle Doherty, Alexander Hugh Wilson, John F Potter, Lee Hooper. **The diagnostic accuracy of multi-frequency bioelectrical impedance analysis in diagnosing dehydration after stroke. Med Sci Monit** 2013; 19:548-570.

Mohannad W Kafri, John F Potter, Phyo Kyaw Myint. **Multi-frequency Bioelectrical Impedance Analysis for assessing fat mass and fat free mass in stroke or transient ischaemic attack patients.** European Journal of Clinical Nutrition. In Press.

Chapter 1: Understanding impact of stroke, the role of nutritional status, and the utility of body composition assessment in stroke care

1.1 Introduction

1.1.1 Stroke epidemiology

Globally cardiovascular diseases (CVD) are the leading cause of death (1), with stroke being one of the major CVD. According to 2008 figures, stroke contributes to ~36% of total CVD mortality (1). In the United Kingdom there were over 190,000 deaths from cardiovascular diseases with stroke contributing 43,142 deaths of which 33,896 were over the age of 75 (2). Despite such statistics, better preventative strategies resulted in a reduction in stroke incidence in the first decade of the twenty first century. Stroke incidence in England dropped in 2005-2007 from 193 to 178 (per 100,000) in men and from 152 to 139 (per 100,000) in women. Scotland followed the same trend with a drop in stroke incidence between 2000 and 2009 from 277 to 202 in men and from 208 to 160 in women per 100, 000 population (2). A recent cohort (n= 32,151) of patients with a first stroke confirmed these findings and suggested that stroke incidence decreased from 1.48/1000 per person-year in 1999 to 1.04/1000 per person-year in 2008 ($p<0.001$); a 30% reduction (3). The same study reported 12.5% increase in stroke prevalence between 1999 (6.40/1000) and 2008 (7.20/1000); $p<0.001$ (3). The decrease in stroke incidents (2, 3) accompanied by reduced stroke mortality (4, 5) suggest that more people survive stroke and are left to bear its burden.

1.1.2 Stroke Pathophysiology

There are two main types of stroke namely ischaemic stroke and haemorrhagic stroke. In both types of stroke the blood supply to the brain is compromised, but in two different manners. In haemorrhagic stroke the blood supply to the brain becomes inadequate due to bleeding into the brain and in ischaemic stroke the blood supply to the brain becomes interrupted due to a blockage as a result of thrombosis or embolism of an artery. Reduced blood supply to the brain damages parts of the brain tissues resulting in neurological impairment (6). In both types of stroke, the loss of cerebral function occurs and the symptoms usually last for more than 24 hours (7).

Ischaemic stroke (infarct) can be further classified depending on the site and the vascular territory of the brain affected, and based on modalities of functional deficit. One of the most well known classification is the Oxfordshire Community Stroke Project (OCSP) classification by Bamford and colleagues which classified cerebral infarction as Lacunar Infarct (LACI), Partial Anterior Circulation Infarct (PACI), Posterior Circulation Infarct (POCI), and the Total Anterior Circulation Infarct (TACI) (8). This classification does not provide the underlying pathology albeit LACI are usually due to small vessel disease. The underlying pathological process leading to an ischaemic stroke varies. Causes can range from plaques in large arteries known as atherosclerosis which embolises to brain (arterio-arterial embolism), or an embolus from the heart known as cardio-embolism that could occur as the result of conditions such as arterial fibrillation, or a small vessel disease related to old age such as hyaline arteriosclerosis of blood vessels supplying blood to the brain, or due to unknown causes (9, 10).

1.1.3 Risk factors of stroke

There are many risk factors for stroke. Examples of stroke risk factors include but are not limited to age, sex, ethnicity, family history, previous or current co-morbid conditions, lifestyle, or certain treatments and therapies.

The probability of stroke is directly correlated with age and sex. The 10 year average probability of stroke incidence in men and women, with no previous stroke, is directly correlated with increasing age and differ between men and women. For example the probability of stroke for those aged 55-59 years was 5.9% and 3.0% for men and women and it increased to 7.8% and 4.7% for men and women aged 60-64 years respectively; showing continuous increase with age with respect to sex differences (11). Although family history is suggested to increase the risk of stroke, a systematic review and meta-analysis suggested that it was difficult to interpret the results due to large heterogeneity between studies, potential bias, and insufficient details (12). Nevertheless large scale studies of long term follow up suggest that the risk of stroke maybe increased with parental history of stroke (13, 14).

Co-morbid conditions such as hypertension, diabetes, atrial fibrillation, and small artery disease can increase the risk of stroke. Extensive review of observational studies suggests that hypertension greatly increases the risk of stroke. An increase in blood pressure can be associated with an at least 30% increases stroke risk (15) with risk increasing by 90% and 65% in men and women respectively (11). Studies examining the effect of blood pressure reduction suggested that a reduction in blood pressure may reduce the risk of stroke by at least 20-30% (16), (17). Clinical Trials on anti-hypertensive therapies also provide an idea on the impact that hypertension can have on the risk of stroke. Lawes and colleagues systematic review and meta-analysis of trials examining the risk reduction of stroke in anti-hypertensive drug users compared to placebo and no treatment suggested a 30% reduction in stroke risk in anti-hypertensive drug users (18).

Condition such as diabetes can increase the risk of stroke. The incident of stroke was 62.3 and 32.7 per 1000 for diabetic and non-diabetic men respectively, with a relative risk of 2.0 (95% CI: 1.4 to 3.0) in participants with diabetes compared to those with no diabetes (19). These finding were further confirmed by a systematic review and meta-analysis of 102 prospective observational studies which suggested at least a 50% increase in the risk of stroke in participants with diabetes compared to those with no diabetes (20).

Risk of stroke can also increase due to other conditions such as atrial fibrillation (AF) and small vessel disease. The calculated probability of stroke from Framingham study suggested that the risk of stroke in patients with atrial fibrillation increased by 83% in men and by more than three fold in women (11). Wolf and colleagues reported an almost six fold increase in the risk of stroke in men and women with Atrial fibrillation compared with those who did not have AF (21).

Earlier review of observational studies suggested that the risk of hormone replacement therapies (HRT) on stroke was inconsistent (22) however more recent meta-analysis

suggested hormone replacement therapy may increase the risk of stroke by more than 20% (23).

Lifestyle in terms of habitual physical activity and dietary preferences can also impact the risk of stroke. Long term longitudinal cohort studies suggested that the risk of stroke can increase substantially reaching up to 50% in smoker compared to non-smokers (24). Review of previous studies examining the risk of smoking on stroke also suggested that smoking can increase the risk of stroke up to 50% when compared to non-smokers (25). Similar to smoking, stroke risk increases with excessive alcohol consumption. Systematic review and meta-analysis of observation studies (cohort and case-control) suggested that heavy and excessive alcohol consumption (more than 60 g/day) increased the risk of stroke by 64% compared to non-drinker whilst moderate alcohol consumption of <12 g/day was found to reduce the risk of stroke by 17% compared to abstainers (26).

A diet high in sodium and saturated fatty acids, and low in potassium can increase the risk of stroke (27). A meta-analysis examining the risk of stroke in high salt consumers (diet high in sodium) compared to low salt consumers, suggested that high salt intake increases the risk of stroke by 23% (pooled relative risk 1.23, 95% CI 1.06 to 1.43; P=0.007)(28). In contrary, meta analysis of systematic evidence (1966-2011) suggest the opposite with a risk reduction of stroke by 11% for every 1 g increase in dietary potassium consumption per day (29).

Another nutrient that was under investigation was saturated fatty acids. A meta analysis of prospective cohort studies suggested that the risk of stroke did not increase with higher consumption of saturated compared to those in the lower quintiles of saturated fat consumption (30). However these finding do not necessarily mean that the potential risk of saturated fat such as trans-fatty acids should be ignored. Saturated and trans fatty acids increase the ratio of total: high density lipoprotein (HDL) Cholesterol (31), a risk factor for stroke (16).

Physically active lifestyle as opposed to sedentary lifestyle can reduce the risk of stroke. Physical activity improves blood flow to the brain and contributes endothelium relaxation (the inner membrane of blood vessels) resulting in protection from stroke (32). A long term follow up study suggested that a physical activity as simple as walking can reduce the risk of stroke (33). These finding were further confirmed in a systematic review and meta-analysis that suggested that in physically active individuals stroke risk decreased by at least 20% compared to people with a sedentary lifestyle (34).

Risk factors of stroke are many and efforts were made to understand them resulting in reduced incidence of stroke (see introduction). Equally important is to improve stroke outcomes once it occurs. In the next section I will present the prevalence of malnutrition in stroke patient and its prognosis on outcomes.

1.1.4 Stroke outcomes and burden

The majority of those experiencing stroke are older than 65 years (35). In a 13 years follow up study, it was reported that life expectancy and average quality of life (QoL) loss after ischaemic stroke in people older than 65 years old regardless of gender was 8.7 and 8.3 years respectively (36). Fate of younger people who experience stroke is not different. Up to 12% of strokes do occur in 15-45 years old population (37). Keppelle et al 1994 documented that in their long term follow up study (mean follow up 6 years, median 5.6 years, range 2 months to 16 years) of 15-45 years old with stroke only 49% were still alive at the end of the follow up period, 42% returned to work, and quality of life as evaluated by Short Form-36 (SF-36) survey was reduced (38).

Compared to those with no previous stroke, medcial admission risk increased by more than two fold in those with pervious stroke (HR: 2.6; 95% CI 2.2-3.0) (39). Further, rehospitalisation after stroke is not uncommon. One study reported that 25% (n=129) of stroke patients were readmitted with stroke during a 12 month follow up period post hospital discharge with a mean length of hospital stay of 23±31 days at rehospitalisation (40). A reported 33% rehospitalisation rate within the first year after stroke was

observed due to complications such as infections, recurrent stroke, or other cardiovascular events in a study of 2,657 stroke patients (41). Recurrent events after stroke are one of the major contributors of rehospitalisation with a reported incidence rate of 105.4/1000 and 52/1000 during the first year and after the first year post ischaemic stroke (42). Recurrent stroke not only contribute substantially to rehospitalisation with a suggested rate >20%, but also to disability with 48% of rehospitalised patients who were not disabled by a prior stroke becoming disabled as suggested by a decrease in the average Barthel index score ($p<0.001$) and National Institute of Health Stroke Scale ($p<0.001$) (43). The risk of death or disability also increases with recurrent strokes compared to a first ever stroke; (OR=9.4, 95% CI 3.0-30)(44).

A huge economic burden is inflicted by stroke in the UK given that 300,000 stroke survivors live with disability and require care. Therefore, the burden of stroke on UK economy is considerable. Annual direct costs of stroke are 2.8 billion in the UK which included diagnostic costs, inpatient and outpatient care costs, and community care (45). Informal care costs of stroke are 2.4 billion which are defined as costs of caring for stroke survivors whether by patient's families or care homes. The costs of lost productivity and disability due to stroke outcome, indirect costs, are estimated be at 1.8 billion divided into 600 million incomes lost to post stroke morbidity, 480 million incomes lost to stroke mortality, and 690 million as benefits costs to support survivors (45).

Research to understand stroke risk factors thus becomes pivotal issue in primary and secondary prevention of stroke. Equally important is to develop an understanding of how to improve stroke outcomes by how best to monitor stroke complications and manage them appropriately. One of the major complications following stroke is malnutrition. Understanding the nutritional status and its prognosis on stroke outcomes is very important if successful intervention strategies are to be integrated in stroke management. In the next section I will briefly discuss the association between stroke and malnutrition to introduce you to the focus of this research.

1.1.4 Malnutrition and Stroke

Evidence suggests that at the time of stroke, the malnutrition already exists (46, 47). The deterioration in nutritional status in people with stroke during hospital stay is also common (48, 49). Malnutrition prevalence in UK hospitals is not to be underestimated. Edington and colleagues estimated the prevalence of malnutrition to be at 20% on admission at four UK hospitals as estimated with a body mass index (BMI) <19 kg/m^2 (50). These finding were further confirmed by Lamb and colleagues who reported malnutrition, assessed using Malnutrition Universal Assessment Tool (MUST), prevalence at 37% and 24% in women and men patients respectively admitted to a UK hospital; 328 patients were included from all in patients medical, surgical, orthopaedic, and critical care in an acute hospital in North East England (51). An earlier study suggested that the prevalence of malnutrition in acute setting is a concern that continue to persist till today. The study suggested that of the 500 patients included in the study with 100 patient from each of general surgery, orthopaedic surgery, medicine for the elderly, general medicine, and respiratory medicine who have their nutritional status assessed on admission. Forty percent (40%) were diagnosed as experiencing malnutrition (52). Assessing malnutrition using "Malnutrition Universal Screening Tool" in elderly patients (n=150), Stratton et al 2005 reported the prevalence of malnutrition to be at 58% (53).

In a prospective observational study that included 131 patients with stroke, under nutrition 24 hours post-admission was diagnosed in 12.2% of patients compared to 19.8% of patients at one week post admission; p=0.03 (54). In this study malnutrition was diagnosed if one or more of the following criteria were met including a 10% weight loss in the past 3 months and/or 6% weight loss one week post admission, weight index (actual weight compared to reference weight) less than 80%, serum albumin <3.0g/dL, prealbumin <10.0 mg/dL, or transferrin < 150mg/dL (54). Gariballa et al reported a decline in average weight in stroke patients at 2 and 4 weeks post admission to an acute stroke unit were 48% (96/201) and 25%(51/201); p=0.002 (55).

Another study involving 104 patients with acute stroke reported that malnutrition prevalence changed from 16.4% at admission to 26.4% of surviving patients (n=91) and 35% of patients who remained in hospital (n=43) at one and two weeks post admission respectively (see below for implication of malnutrition in this study). Malnutrition was assessed using three measurements of MAC, TSF, and serum albumin (56).

Fluctuations in nutritional status is usually reflected by changes in body composition, such as volume and proportion of fat mass and fat free mass (57, 58). Other body composition indices are also affected with changes in nutritional status (57, 59, 60). Therefore, body composition measurements may be useful in monitoring nutritional status, and evaluating nutrition intervention in management in acute stroke care. There is also existing evidence to suggest that body composition measurements can also be used to predict relevant clinical outcomes. For example, in older people change in body composition such as increased fat mass is associated with functional limitation (61).

Assessment of body composition can be done using simple, cheap low technology methods as well as, costly and complex, and advanced methods. Established methods that are used to assess body composition include skin fold thickness, underwater weighing, dilution method, neutron activation analysis, determination of total body potassium, magnetic resonance imaging (MRI), and dual x ray absorptiometry (DEXA); Chapter 5 discusses each methods in detail. Multifrequency Bioelectrical Impedance Analysis (MF-BIA) used in this study is a relatively new method that can also be used to assess body composition.

MF-BIA estimates the body components based on the difference in conductivity that body tissues imposes on the flow of an electrical current. This difference in the conductivity in different body tissue is due to the impedance imposed by body tissue on the flow of that electrical current. The difference in impedance is used to calculate the volume of body compartments using validated equations programmed in the MF-BIA equipment taking into account of factors such as gender, height, weight, and age (62). Changes in body composition measured by MF-BIA such as FFM and FM can provide information regarding the nutritional adequacy of stroke patients in acute phase. Further body composition components such as total body water may provide

information on a patient’s hydration status. MF-BIA can be a swift method to aid in monitoring patients nutritional and hydration status to aid in developing personalized nutrition intervention strategies and to improve strictly management in acute phase of the stroke.

1.2 Study objectives

In depth understanding of the prognosis of malnutrition on cardiovascular diseases is important. Therefore the aim of the systematic review and meta-analysis presented in Chapter two was to investigate the relationship between nutrition markers of high and low energy intake, low protein intake, and low fluid intake on subsequent outcomes after a cardiovascular event. The nutrition markers examined included high and low body mass index (BMI), weight loss, skinfold thickness, low serum albumin, high serum creatinine, increased serum osmolality, and malnutrition assessed by nutrition assessment tools such as the Subjective Global Assessment tool (SGA). The main outcome assessed was mortality with other secondary outcomes such as morbidity (re-infarction, complications), readmission, disability or functional status, length of hospital stay, and discharge destination.

Chapter 3 presents an observational longitudinal study that describes body composition after ischaemic stroke and their prognosis on outcomes. The primary objective of the longitudinal study was to describe fat free mass and body composition changes during acute stroke phase while considering the extent of these changes by type of feeding regimen, ischaemic stroke subtype, and the stroke severity. The study also examined if body composition changes were correlated with subjective and objective outcomes in both short and longer terms.

Chapter 4 presents the study which examines whether it is possible to diagnose dehydration using bioelectrical impedance analysis. The aim was to assess the levels of dehydration after stroke using the reference standard of serum osmolality, and to explore whether MF-BIA can be substituted for serum osmolality in diagnosing dehydration after stroke.

In the final chapter, Chapter 5 presents the validation studies of MF-BIA. The objective was to validate MF-BIA against reference standard dual x-ray absorptiometry (DEXA) in patients with recent stroke/TIA. The validation of MF-MF-BIA against DEXA can

provide information on the level of agreement between major components of interest, fat mass and fat free mass, measured using MF-BIA and their corresponding values estimated by DEXA for the same study participant. In addition, the internal consistency of MF-BIA measurements, internal validation, was also examined.

I conducted above series of validation studies because MF-BIA method is a relatively new method and it is not considered as the gold standard method in estimating body composition. It requires internal validation to examine it reliability in terms of its consistency in reproducing results. It also requires external validation to understand the level of variation or agreement in MF-BIA estimates compared to that of a reference standard method. I used Dual X-ray Absorptiometry, which is considered a reference standard method with a low margin of error, to externally validate the MF-BIA machine I used in the observational longitudinal study presented in Chapter 3 (63). In addition, because DEXA does not evaluate fluid components such as total body water I carried out a separate study in diagnosing dehydration in stroke patients using reference standard of serum osmolality (64). Upon discharge from hospital I followed up study participants to assess their clinical outcomes as well as quality of life and functional capacity using self reported validated questionnaires to understand the association between body composition changes during acute hospital stay and longer term outcomes such as functional health assessed using the Short Form Survey 36 version 2 (SF36v2), stroke impact using Stroke Impact Scale (SIS) and activities of daily living using Barthel Index.

I hope this study will add new knowledge to the possible utility of MF-BIA in acute stroke care, and inspire future research to further build on this knowledge with the ultimate goal of improving nutritional care in stroke.

Chapter 2: The relationship between nutrition markers and outcomes following a cardiovascular event: A systematic review and meta-analysis of prospective cohort studies

Study Summary

Objective: to systematically investigate the relationship between nutrition markers of high and low energy intake, low protein intake, and low fluid intake on outcomes post cardiovascular event. The nutrition markers examined included high and low Body Mass Index (BMI), weight loss, triceps skinfold thickness, low serum albumin, high serum creatinine, increased serum osmolality, and under nutrition assessed using nutrition assessment tools. Primary outcome was mortality and the secondary outcomes included morbidity (recurrent event, complications), readmission, disability or functional status, length of hospital stay, and discharge destination.

Data sources: MEDLINE, EMBASE, and Web of Science were searched from inception to October 2010.

Study Selection: Two investigators assessed the titles, abstracts, and full text of each study for inclusion into the systematic review. The two assessors were independent and used an inclusion/exclusion form.

Inclusion Criteria: to be included in this systematic review the following criteria must be fulfilled. 1) Prospective cohort studies, 2) People diagnosed with transient ischemic attack (TIA), myocardial infarction (MI), or stroke, 3) Assessing the effect of at least one of serum albumin, serum osmolality, serum creatinine, BMI, weight loss, or TSF, and 4) At least one of these outcomes was reported: primary outcome mortality, secondary outcomes including cardiovascular morbidity (reinfarction, complications), readmission, disability or functional status, length of hospital stay, and discharge destination.

Data Extraction: A data extraction form was designed to collect variables of interest. Two data extractors, the primary author and a clinician, carried out data extraction independently. Data extraction included collecting information on study characteristics,

subject characteristics. For each study the nutrition marker cut-offs that defined extreme nutritional status were recorded. Specified review outcomes described in the protocol were recorded and outcome estimate (odds ratios, relative risks, or hazard ratios) with confidence intervals (or other measure of variance) were recorded for the unadjusted and most adjusted model. Validity of each study was assessed by each data extractor. At the end of the data extraction process, data extractors compared their data collection outcomes; variations were solved through discussion until a consensus was reached.

Data analysis: The main analysis was to compare relationship between each nutrition marker signifying extreme value to its corresponding normal values on outcomes. Meta-analysis for secondary subgrouping was carried out for the nutrition marker with the largest data set. All studies were pooled using an inverse variance method using random effects methodology. Data were primarily sub-grouped by type of risk estimate (hazard ratio, risk ratio, or odds ratio). Secondary subgrouping if possible by age, baseline cardiovascular event, and gender was carried out. Secondary outcomes morbidity (as defined per study), disability, discharge destination, readmission, and length of hospital stay were compared between extreme nutrition marker values and their normal values (for example obese BMI vs., normal BMI) and were always sub-grouped by risk estimate (hazard ratio, risk ratio, or odds ratio) if enough number of studies were present to render such subgrouping possible.

Results: Of the 2000 studies of the search outcome, 23 met the inclusion criteria. 13 studies examined BMI, one weight loss, four on serum albumin and one of which included serum creatinine, one serum osmolality, and four nutrition assessment tools. All studies examined the risk of extreme measures of nutrition markers compared to its normal measure on the primary outcome mortality and secondary outcome morbidity (recurrent event, complications). The risk of obesity compared to normal weight on mortality suggested no association among obese patients RR 1.02 (0.84 to 1.24; p=0.83) as opposed to hazard risk of 0.79 (0.48 to 1.32; p=0.37). No association was also observed when examining the risk of overweight compared to normal weight on mortality RR 0.90 (95% CI 0.76 to 1.96) and HR 1.09 (0.99 to 1.20; p=0.06). Underweight compared to normal weight risk on mortality suggested a 41% increased risk RR 1.41 (95% CI 1.17 to 1.70) in the relative risk of underweight compared to

normal weight on mortality in CVD patient s ($p<0.05$) and absence of heterogeneity. For the risk of high serum albumin compared to low serum album suggested a reduced risk of mortality HR 0.91 (95% CI 0.84 to 0.98; p=0.01), and meta-analysis for the risk of under nutrition assessed using nutrition assessment tool compared to normal nutrition suggested increased risk of mortality OR 1.88 (95% CI 1.40 to 2.53; p=0.0001). Of the 23 studies two had missing data, 22 adjusted for age and one did not adjust for age, 19 studies adjusted for gender and 4 did not, only two studies adjusted for socioeconomic status, six out o the 23 studies did not adjust for comorbidities, nine out of the 23 included studies did not adjust for smoking, and author/funder affiliation was clear for most studies except one study was deemed unclear.

Conclusion: Undernutrition diagnosed using nutrition assessment tool provide evidence that the risk of mortality is higher in undernourished patients compared to well nourished patients. Obesity and overweight were not associated with increased mortality. Underweight, low serum albumin, raised serum osmolality, raised serum creatinine all increase risk of mortality. Prospective observational cohort studies confirm these finding and generate a larger systematic review are required.

2.1 Background

2.1.1 Effect of Malnutrition on metabolism and body composition integrity

The European Society of Parenteral and Enteral Nutrition (ESPEN) (also known as the European Society of Clinical Nutrition and Metabolism) defines malnutrition as "*a state in which a deficiency or excess (or imbalance) of energy, protein, and other nutrients causes measurable adverse effect on tissue/body form (body shape, size, and composition) and function, and clinical outcome*"(65). Malnutrition can take two dimensions, over nutrition and under nutrition. Over nutrition can be caused by excessive macronutrient intake resulting in obesity, and under nutrition can be caused by inadequate macronutrient and fluid intake resulting in weight loss and dehydration respectively. There are other types of malnutrition such as fat and water soluble vitamin deficiencies and toxicities as well as mineral deficiencies and toxicities, but these are beyond the scope of this systematic review. Over nutrition and under nutrition can be assessed by evaluating anthropometric indices, such as weight and body mass index, or serum markers such as serum albumin, and serum osmolality. The next sections present the nutrition markers examined in this chapter and summarised in Table 2.1 that may reflect a type of malnutrition that may influence body composition changes; the main topic of this dissertation.

2.1.2 Anthropometric markers in evaluating over nutrition and under nutrition

2..1.2.1 Body Mass Index, Weight loss, and Upper Arm Anthropometrics

Over nutrition can cause obesity. Obesity can be influenced by many factors including environmental and genetic factors. Environmental factors include lifestyle and cultural values that dictate who we are within our society, and genetic factors that are innate and can determine our metabolism and how the body utilises energy (58). The main component of body composition that increases with obesity is fat mass or adipose tissue (58). Increased adiposity is associated with increased risk of co morbidities including but not limited to type II diabetes (66) , coronary heart disease (67), hypertension (68)

dyslipidaemia (69), and increased risk of mortality (70). On the other hand, under nutrition can affect energy storage. Redman and colleagues demonstrated in a clinical trial that both fat mass and fat free mass loss occurred after six months of 25% calorie restriction in healthy volunteers (n=36) of their study (59). Changes in body composition because of over nutrition or under nutrition are measurable. Energy related under and over nutrition can be measured mainly by anthropometric indices including body mass index (BMI), weight, triceps skinfold thickness (TSF), and mid arm circumference (MAC).

BMI measurement is a swift and non-invasive method to identify both under nutrition (<19kg/m^2) and over nutrition (overweight 25-29.9 kg/m^2; obese ≥30 kg/m^2) (71). Although a low BMI suggesting underweight secondary to underweight is worrying, a single body mass index measurement may not reflect the clinical risk of mortality or poor outcomes in people experiencing body mass index reduction but who are still not classified as underweight. Cook and colleagues provided an example to describe how BMI may not reflect clinical risk of mortality or poor outcomes. They provided an example suggesting that if a patient height was 1.58 m and weight was 67 kg with a BMI of 27 kg/m^2 experiencing 10% weight loss, this patient would not be at risk of mortality based on BMI as the BMI would then be 24 kg/m^2 and within the normal range (72). Nevertheless, a single BMI measure outside the normal range can still provide useful information on health risk. In the case of low BMI (BMI <19 kg/m^2), it may reflect those at risk of negative prognosis outcomes including mortality (73). In the case of over nutrition a high BMI may indicate risk of poor outcomes (74) as body mass index mirrors changes in adiposity (58) which is associated with co morbidities as well as increased risk of mortality.

Body mass index may mirror changes in body composition mainly adiposity, but is not a specific measure unlike triceps skin fold thickness. Triceps skin fold thickness (TSF) is traditionally used to measure adiposity or body fat (75). It uses percentiles values to evaluate the level of adiposity with <5^{th} percentile indicating frailty suggesting severe under nutrition due to very low body fat. When evaluating TSF in patients with liver cirrhosis, those with a TSF <5^{th} percentile had lower survival rate compared to those with a higher TSF percentiles at six and 12 months post discharge ($p<0.001$) and at 24

months ($p<0.002$) (76). In-hospital outcomes are also affected by low TSF measurements; TSF was lower in stable patients with chronic obstructive pulmonary disease (n=39) requiring rehospitalisation compared to similar patients those who did not require rehospitalisation ($p<0.05$) (77).

Both single measurement of BMI and TSF provide information on the effect of energy balance on body composition, but they do not provide information on the deterioration of nutritional status over time. Generally, weight loss can provide information on nutritional status deterioration in a certain time period providing information on the extent of nutritional status change. McWhirter and Pennington 1994 evaluated the nutritional status of 500 patients admitted to five different specialties in an acute teaching hospital. They evaluated the nutritional status for those patients who had a hospital stay greater than 7 days and found that of 112 patients who had their nutritional status evaluated on discharge by weight loss, weight loss made two of the overweight patients (n=29) become moderately undernourished (7%), five (26%) of the mildly undernourished patients became moderately undernourished, and seven (37%) of the moderately undernourished patients became severely undernourished (52). Involuntary weight loss can have negative prognostic impact. Malnutrition assessed by weight loss was associated with increased incidence of stomatitis in post cancer chemotherapy treatment ($p<0.0001$) (78). Wallace carried out a study to understand the consequences of weight loss on older patients. They found that a 4% involuntary weight loss increased the risk on mortality by more than two fold compared to non weight losers over a period of 2 years with a relative risk ratio of 2.43 (95% CI = 1.34 to 4.41) (79).

Lean tissues loss can occur when energy is insufficient. When fuel is insufficient, the body uses its own energy substrates. Fatty acids, from adipose tissue, and amino acids, from body protein (muscles, intestinal lining, etc.), become the main fuel. This metabolic change results in body composition changes that affect lean tissues. One method that can be used to assess change in lean tissue is mid upper arm circumference (MAC) measurement. Changes in MAC can be used to evaluate the extent of muscle wasting due to energy deficiency. It provides information on the extent of muscle mass

loss, which is an important predictor of negative prognosis in acutely unwell patients. Liver cirrhosis patients diagnosed with moderate or severe muscle mass loss with muscle mass of $<5^{th}$ percentile (severely malnourished) and $<10^{th}$ percentile (moderately malnourished) had a lower survival rate compared to those with a 10^{th}-75^{th} and $>75^{th}$ percentile values indicating normal nutrition or over nutrition respectively; evaluated using MAC at 6, 12, and 24 months ($p<0.001$) (76).

2.1.3 Biochemical Markers in evaluating over nutrition and under nutrition

2.1.3.1 Serum Albumin

Serum albumin has been used as a marker to diagnose protein malnutrition. Serum albumin synthesis appears to rise with an increase in protein intake (80). Sullivan and colleagues examined serum albumin in 102 patients with an average nutrient intake <50% of their caloric requirement. Patients with reduced nutrient intake had lower serum albumin levels (mean= 29.1±6.7) g/L) compared to those with normal nutrient intake (n=395; mean=33.2±6.1) g/L) (81). Mitchell and colleagues compared nutrition markers of 150 malnourished hospitalized patients (elderly n=44, age range 62-85 years; and young adults n= 65, age range 19-58 years) with 80 healthy control subjects of the same age range (40 young adults and 40 elderly); judging malnutrition based on a 10% or more weight loss in the past six months. Serum albumin was clearly affected in malnourished patients. Malnourished elderly males (n=15) had serum albumin level of 25.0±1.00 g/L compared to 43.0±1.00 g/L in the elderly well nourished males group (n=20); $p<0.001$. Malnourished elderly females (n=25) serum albumin was 2l.6 ± l1.0 g/L compared to 4l1.0±l.001 g/L in well nourished (n=20); $p<0.01$(82). Low serum albumin was significantly associated with increased length of hospital stay (LOS) ($p<0.001$) (83).

2.1.3.2 Serum Creatinine

It has been suggested that serum creatinine levels may be related to body composition in general (84) and lean body mass specifically (85). Elevated serum creatinine may be related to negative energy balance resulting from muscle breakdown to supplement the necessary energy in cases of inadequate glucose intake and depleted glycogen stores in liver and muscle. Increased serum creatinine levels may reflect a state of muscle metabolism suggesting negative energy balance.

2.1.3.3 Serum Osmolality

Serum osmolality reflects the concentration of solutes, such as minerals and glucose, dissolved in the water content of serum; therefore high serum osmolality means that the blood is more concentrated (higher proportion of solutes to water). Therefore, serum osmolality increases when the fluid intake is inadequate. A study on healthy elderly men documented an increase in serum osmolality after a 24 hour water deprivation (86). Increased serum osmolality is associated with poor clinical outcomes. In critically ill patients mean serum osmolality was 297.0±16.7 mOsm/kg for survivors compared to 312±22.1 mOsm/kg in non-survivors; $p_{correlation}<0.05$ (87). This retrospective observational study compared 16 different laboratory and clinical parameters, acute physiologic and chronic health parameters (APACHE), and sequential organ failure assessment scores in predicting in-hospital mortality. The area under the receiver operating curve (ROC) value for serum osmolality was 0.732 (95%CI:0.692-0.772) second to APACHE in its mortality prediction, but when examined in its predictive ability for mortality at > 5days hospital stay suggested it had the best predictive ability with ROC value of 0.711 (95%CI: 0.661-0.761) (87).

2.1.4 Nutrition assessment tools in evaluating nutritional status

2.1.4.1 Subjective Global Assessment Tool, Mini Nutrition Assessment Tool, and combination of Nutrition Markers

There are several nutrition assessment tools used to evaluate the nutritional status of patients. No method is used universally; nutritional status assessment ranges from complex nutrition assessment tools to a combination of individual anthropometric and biochemical markers. The Subjective Global Assessment Tool (SGA) assesses nutrition based on weight change, dietary intake change, gastrointestinal symptoms, functional capacity changes, and disease in relation to nutrition requirements (88). The final nutritional status is classified as Grade A, B, or C corresponding to well nourished, malnourished, and severely malnourished status respectively (89).

As the name implies the SGA allows subjective evaluation of the nutritional status of patients based on historically used subjective assessment of physical examination and medical history evaluation (89). A validation study of SGA was performed on 59 hospitalized patients, who underwent major gastrointestinal surgery, in whom the classification of nutritional status by SGA was compared with measurements of body composition (subcutaneous fat measured by triceps skinfold and midaxillary line at the level of lower ribs, and muscle wasting at quadriceps and deltoid muscle detected by palpations), serum hepatic protein concentrations, total lymphocyte count, and delayed hypersensitivity skin testing. The outcomes of the comparison suggested a strong correlation between SGA assessment and all measures except total lymphocyte count, transferrin and total body nitrogen. In addition, clinical outcomes correlated to SGA assessment classification with 69% categorized as severely malnourished, 43% as mild/moderately malnourished, and 16% as well-nourished of the 18 individuals who developed infectious complications (90). Few years later a follow up study compared SGA classification with six traditional measurements of nutritional status, including serum albumin, serum transferrin, delayed cutaneous hypersensitivity, anthropometry,

creatinine-height index, and the prognostic nutritional index suggested that the sensitivity and specificity of SGA in assessing malnutrition were 0.82 and 0.72 respectively (91).

Another tool used is the Mini Nutrition Assessment (MNA) developed specifically for older people ≥65 years old to be assessed in various settings including hospital, care home, and in the community. The MNA is an 18 item assessment integrating lifestyle, anthropometric, dietary intake, medical, and psychosocial factors (92) and it considered three major areas (93). It consists of three main areas of assessments with each containing of sub items. The first area is anthropometrics evaluating the four sub items of weight loss, calf circumference, mid arm circumference, and BMI. The second area consists of the six dietary sub items of recent change in appetite, meal per day, fruit, vegetable, protein, fluid intake, and independence in feeding. The third area is the global item consisting of 6 sub items and these include mobility, lifestyle, medication, presence of sore or pressure ulcer, neuron psychosocial health and psychological health (93).

In a validation study of MNA, 105 frail elderly patients were recruited from a geriatric evaluation unit of the University of Toulouse hospital and 50 healthy elderly subjects were recruited from the University of the Third Age in Toulouse. Two physicians trained in nutrition carried out participant's clinical assessment without prior knowledge of MNA results. The physicians also assessed participants comprehensive nutrition status which was considered as a gold standard by evaluating subject's anthropometrics (weight, height, knee height, triceps skin fold, mid arm and calf circumference), biochemical markers (albumin, prealbumin, Creatinine, ceruloplasmin, C-reactive protein, α_1-glycoprotein, cholesterol, triglycerides, vitamins A, D, E, B_1, B_2, B_6, B_{12}, copper, zinc, haemoglobin, blood cell count and differential, and dietary intake using 3-day food record and food frequency questionnaire. When carrying out discriminate analysis to compare MNA results with the physician clinical and comprehensive analysis, MNA identified the nutrition status 92% and 98% correctly based on physicians' clinical and comprehensive analysis respectively (93). For each item (global, anthropometric, subjective, and dietary) a validation study was carried out in 1993 with 90 participants recruited from the geriatric evaluation unit at the University of Toulouse

and 30 from the University of the Third Age. Participants MNA, biochemical measures (albumin, prealbumin, Creatinine, C-reactive protein, α_1-glycoprotein) and clinical assessment were evaluated. MNA identified nutrition status in 89% with identical clinical status assessment and 88% with identical biochemical markers (93) .

The MNA diagnostic accuracy compared to BMI in assessing malnutrition was examined in sub-acute care patients; patients with a known course of treatment requiring comprehensive but not intensive care program or procedure designed for individuals with an illness, injury, or deteriorating disease state after an acute event (94). The highest sensitivity for diagnosing malnutrition by the MNA was correlated with a BMI <22 kg/m^2 (sensitivity 0.70, specificity 0.71) in sub-acute patients (n=837, mean age 76.1±12.1 years) (95).

Both SGA and MNA use a combination of anthropometric, biochemical and other components to evaluate the state of nutrition. In some studies malnutrition was assessed using a combination of different anthropometric and biochemical indicators but not necessarily using validated assessment tool. For example, Yoo et al (54) diagnosed malnutrition if one or more of the following criteria were met including a 10% weight loss in the past 3 months and/or 6% weight loss one week post admission, weight index (actual weight compared to reference weight) less than 80%, serum albumin <3.0g/dl, prealbumin <10.0 mg/dl, or transferrin < 150mg/dl in the evaluation of their study participants' nutritional status (54). As described above studies evaluating nutritional status in clinical care used either validated tools or a combination of nutrition markers mainly, but it was also assessed using individual nutrition markers (either anthropometric or biochemical).

Studies discussed so far provide some data on the prevalence of malnutrition in hospital setting giving an idea of the magnitude of the problem. It is important to understand how malnutrition can impact on outcomes regardless of the method of nutritional status assessment to make recommendations on the best method/s that are associated with poor outcomes (i.e. best prognostic indicators or nutrition markers) to develop strategies in prevention of poor outcomes. Concrete evidence is in dire need. This systematic

review compile evidence of prospective observational cohort studies to aid clinicians in prioritizing nutrition assessment and intervention in patients with CVD. Table 2.1 below presents aforementioned nutrition makers in tabular format and provide information what their extreme cut offs values indicate.

	Measure	Indicator
Anthropometric	BMI $\geq$ 30 kg/m^2	Obesity
	BMI 25-29.9 kg/m^2	Overweight
	BMI<19 kg/m^2	Underweight
	Weight Loss	negative energy balance
	Weight gain	positive energy balance
	Triceps Skin Fold	increase/decrease in fat mass
	Mid Arm Circumference	increase/decrease in lean mass
Biochemical	High Serum Albumin	adequate protein intake
	Low Serum Albumin	inadequate protein intake
	High Serum creatinine	lean tissue breakdown
	High serum osmolality	low fluid intake
Nutrition Assessment Tools	Subjective Global Assessment	under nutrition
	Mini Nutritional Assessment	under nutrition
	Nutrition Marker Combination	under nutrition

Table 2.1. The nutrition markers examined in this systematic review by type, their cut offs, and what each measure indicates

2.1.5 Malnutrition in hospital

The prevalence of under nutrition in hospital setting has been reported to be >20% depending on the measure used and the population studied (50, 96, 97). In another study of patients in German teaching, community, and university hospitals recruited from general surgery, rheumatology, gynaecology, oncology, cardiovascular, urogenital/renal, neurological/dementia, and trauma/orthopaedics surgery reported that 27% were malnourished using SGA (96). The prevalence of malnutrition was reported as high as 50% in adult patients older than 18 years old recruited from several hospitals and specialities, a multicentre study conducted in south and central American, and Caribbean countries using SGA (97).

The prevalence of malnutrition is unsurprisingly high in conditions associated with swallowing difficulty such as stroke. Up to 71% (10/14) of Australian stroke unit dysphagic patients were suffering from malnutrition assessed by SGA within 48 hours of admission compared to 32% of non-dysphagic patients (19/59), p=0.007 (46). Similarly, during the first week of hospitalisation in an acute stroke unit, dysphagic patients were more likely to be malnourished (16/24, 67%) compared to non-dysphagic patients (15/67, 24%) as diagnosed by SGA; p<0.001(46). Dehydration assessed by serum osmolality was also prevalent in stroke patients as 30% of the patients had raised serum osmolality (>296 mOsm/kg) in a study including 167 stroke patients (98).

Although studies documenting the prevalence of malnutrition in coronary heart disease are scarce, obesity as a form of malnutrition or more specifically over nutrition is well documented to increase the risk of coronary heart disease (CHD) (99). In the Honolulu study (n=7,692 men) participants with the highest tertile of subscapular skinfold thickness indicating increased adiposity experienced higher rates of coronary heart disease during a 12 year follow up of men compared to those with lower skinfold tertile (100). CHD risk increased by more than three fold in obese women (BMI $\geq$30 kg/m^2) compared to normal weight women 3.44 (95% CI, 2.81 to 4.21) in a 20 year follow up of 88, 393 women (age range 34 to 59 years of age) who participated in the Nurses' Health Study and did not have previous CVD at baseline (101). Considering that

malnutrition in the form of increased adiposity increases the risk of CHD, it is important to understand further if malnutrition in CHD does have an impact on outcomes after a CVD event. Malnutrition diagnosed by serum albumin suggest that 629 acute myocardial infarction patients (40%) had serum albumin <35 g/L (102).

The proportion of stroke patients with under nutrition increases during acute hospital care (49, 56). Axelsson and colleagues assessed nutritional status by evaluating anthropometric (weight, triceps skinfold thickness and arm muscle circumference) and biochemical (albumin, transferrin and prealbumin) nutrition markers to evaluate nutrition status (49) and found that under nutrition increased from 16% to 22% between admission and discharge.

As can be seen from the literature the prevalence of malnutrition in hospital settings is evident. To understand the impact of malnutrition after a CVD event requires systematic approach. As malnutrition can be diagnosed using variable methods, the measure of malnutrition that can best predict outcomes after a CVD event is unclear. In this systematic review I tried to address these questions.

2.2 Validity of evidence

Given that malnutrition appears to be prevalent after a CVD event, it is important to understand its impact on the final outcomes. A systematic review of the available evidence is essential if such evidence is to be accumulated to aid clinicians in decision making. When carrying out a systematic review and meta-analysis there is always the risk of accumulating biased evidence leading to a final biased effect estimate. In other words, bias in each study included based on the inclusion criteria may accumulate if not controlled for leading to a biased effect estimate outcome concluded from the meta-analysis. This systematic review gathered evidence and presented its outcomes according to the preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement (103) while monitoring closely if each study reported its outcomes following STROBE statement; The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for Reporting Observational Studies (104).

The Cochrane handbook for systematic reviews and meta-analysis, version 5.1 (105) classification of non-randomized controlled trials (NRS) include but is not limited to case-control studies, case series, cross sectional, controlled before-and-after study, and historically controlled studies (106). Observational studies are not clinical trials. However, as in randomized clinical trials the risk of bias must be assessed, but here it is important to evaluate factors that may influence the effect size I am reporting. In order to have an effect size that reflect what I examined and in this case the relationship between malnutrition assessed by the nutrition marker of interest and health outcomes, I must make sure that the effect size I reported is based on adjusted models that controlled for confounders. Therefore as in section 13.5.2.2 in the Cochrane handbook many factors were considered in assessing validity of studies. At the stage of writing the systematic review protocol, I considered what can be a confounder for the effect I am trying to asses in CVD patients. Considering patients with CVD may have other chronic condition that lead to the CVD event I considered factors or conditions such as age, diabetes, kidney diseases, hypertension, and socioeconomic status as confounders that can also influence the health outcomes examined in this work (discussed details in the risk of bias section in methodology section).

The other bias considered was attrition bias. Attrition bias considers completeness of a sample follow-up, and data. In a way this approach tries to understand the sample and how many drop outs were there and due to what reasons, if the sample collected at the beginning of the study was all included in the final analysis and if not what are the reasons (why are there any missing data), and if the follow up was complete (if the study was terminated). This type of bias assessment ensures that the quality of the study is considered. In this systematic review the extent of sample drop out (missing data) was also evaluated to shed light on the quality of each study.

2.3 Aim

The aim of this systematic review was to investigate the relationship between nutrition markers of high and low energy intake, low protein intake, and low fluid intake on relevant clinical outcomes after a cardiovascular event. The nutrition markers examined included high and low BMI, weight loss, skinfold thickness, low serum albumin, high serum creatinine, increased serum osmolality, and malnutrition assessed by different nutrition assessment tools. The primary outcome was all cause mortality and secondary outcomes included morbidity (reinfarction, complications), readmission, disability or functional status, length of hospital stay, and discharge destination.

2.4 Methodology

Protocol and registration: no published protocol exists for this study. The protocol was formulated to aid the author and investigators in carrying out the steps of this systematic review (Appendix I).

2.4.1 Eligibility criteria and study selection

Two investigators assessed the titles, abstracts, and full text of each study for inclusion into the systematic review. The two assessors were independent and used an inclusion/exclusion form. Inclusion criteria included

- Prospective cohort studies
- People diagnosed with transient ischemic attack (TIA), myocardial infarction (MI), or stroke
- Assessing the effect of at least one of serum albumin, serum osmolality, serum creatinine, BMI, weight loss, or TSF
- At least one of these outcomes was reported: primary outcome mortality, secondary outcomes including cardiovascular morbidity (reinfarction, complications), readmission, disability or functional status, length of hospital stay, and discharge destination.

2.4.2 Information Source

MEDLINE, EMBASE, and Web of Science were searched from inception to October 2010. I carried out the search and it was duplicated by another independent investigator with clinical knowledge. All selected studies were available as full text in the used search engines MEDLINE, EMBASE, and Web of Science. Search terms included cohort studies, nutrition markers including serum albumin, waist circumference, total

body water, other measures of hydration, body mass index, body fat, triceps skin fold, and serum Creatinine, and terms for the cardiovascular events stroke, myocardial infarction and transient ischemic attack. Appendix II presents the search strategy with the indexing terms used on MEDLINE (similar search strategy was used in other search engines).

2.4.3 Data items and extraction

A data extraction form (Appendix III) was designed to collect relevant variables of interest. Two data extractors, the primary author and a clinician, carried out data extraction independently. Data extraction included collecting information on study characteristics including study location, period of participant enrolment, and follow up duration. Number of drop outs and the reasons were recorded whenever available. Study characteristics were collected and these included total population eligible for each study, number of males and females, actual number of the population completed in the study (after drop outs), and study inclusion criteria. Baseline event (myocardial infarction, stroke, or transient ischaemic attack) that was examined in each study was recorded, exposure as the nutrition marker including anthropometric (body mass index, weight loss), biochemical (serum albumin, serum osmolality, serum sodium, serum creatinine), or nutrition assessment tools that use a combination of anthropometric and biochemical nutrition markers were all recorded. For each study the nutrition marker cut-offs that defined malnutrition were recorded.

Specified review outcomes described were recorded and outcome estimate (odds ratios, relative risks, or hazard ratios) with 95% confidence intervals (or other measure of variance) were recorded for the unadjusted and most adjusted model. At the end of the data extraction process, data extractors compared their data collection outcomes; variations were solved through discussion until a consensus was reached.

2.4.4 Risk of bias

Three components were evaluated to assess the risk of bias in each included study. These three components were missing data, adjustment for relevant confounders, and source of funding/author affiliation if funded by an interested industry. Information on each component was recorded using a validity tool designed by the investigator (Appendix III) to assess the risk of bias of non-randomized studies as described in Cochrane handbook of systematic reviews and interventions (105).

2.4.4.1 Missing data

To reduce the risk of bias in observational cohort studies, the STROBE statement was formulated indicating how data must be reported in observational cohort studies (104). When reporting results each observational study must present numbers of total population from which sample is drawn, potentially eligible participants for the study, participants included in the actual study based on the inclusion criteria and deemed eligible, those completed follow-up, and included in the final analysis. A study must report the sample size from the beginning to termination of the study during the course of the cohort. In addition, studies must report reasons behind changes in the sample size throughout the cohort.

I reported the actual number of participants included in the study, initially meeting the inclusion criteria, and the actual number that were included in the final analysis using data extraction form and validity tool (Appendix III). If the sample size changed between inclusion and final analysis then I recorded reasons behind changes in the sample size. While prospective cohort studies are expected to lose participants through death/refusal/moving out of areas, it was important to report changes in the sample size as it can contribute to missing data. In the Cochrane handbook for systematic review and meta-analysis (version 5.1), sources of bias presented in chapter 8 (section 4) refer to missing data as attrition bias (106). Missing data suggest that data concerning outcome analysis were unavailable or incomplete shedding light and raising concerns on

the data collection process, data management, and overall quality of study design. Data collection maybe incomplete, data management may not have been of highest standards, and the study design may not have been set to meet realistic objectives. I recorded YES for missing data if a study had >5% of its data missing. I recorded NO for missing data if a study had <5% of its data missing. I recorded UNCLEAR if a study may have had missing data (not clear if a study had >5% missing or not). If missing data was recorded as NO then I considered the study to have a low risk of bias, and if a study missing data was recorded as YES or UNCLEAR then the risk of bias was considered high. I recorded unknown if not information on missing data was provided and the risk of bias was considered high (Table 2.2).

2.4.4.2 Adjustment for Confounders

Adjustment for confounders is an important component to make sure that the risk estimates we extracted reflect the true risk estimate of interest. Therefore the extent to which a model adjusted for confounders was considered. The main confounders considered were age, sex, socioeconomic status, smoking status and co morbidities. Although it is impossible to have each study adjusting for the same confounders, scientific evidence suggests that these certain factors are common confounder for the outcomes of interest in my study.

Age and sex adjustment are both important as differences in their characteristics may contribute to variation in the effect size and interpretation. The probability of stroke is directly correlated with age and differs for men and women. Wolf and colleagues examined the 10 year average probability of stroke incidence in men and women of the Framingham study. After sub grouping men and women into age categories (55-59, 60 64, 65-69, 70-74, 75-79, and 80-84 years old) respectively, their findings suggested that the probability of stroke increased with each age category and was not similar for men and women. For example the probability of stroke for those aged 55-59 was 5.9% and 3.0% for men and women respectively, and the probability of stroke for men and women aged 70-74 was 13.7% and 10.9% respectively (from 11.0% in men and 7.2% in women in the preceding age category of 65-69 years old) (11). A more recent study

examined lifetime risk of CVD for men (n=3564) and women (n=4362) of the Framingham study, both at age 50 year old and with no previous CVD event, up to 95 year old. The risk estimate for CVD event for men and women was 51.7% (95% CI, 49.3 to 54.2) and 39.2% (95% CI, 37.0 to 41.4) respectively. The median survival time for men was 30 (22-27) years while that for women was 36 (28-42) years (107). Available evidence suggests that age and gender are confounders for the risk of CVD event. Age and gender can clearly confound the risk estimate and therefore adjusting for age and gender (or not) can influence the level of bias in the selected studies.

Another confounder assessed was socioeconomic status defined as annual earning or achieved level of education. Socioeconomic status indicating poverty or low/no education may increase the risk of CVD (108). Next in assessing risk of bias was co morbidities adjustment. Co morbidities adjustment included adjusting for diabetes, hypertension, and kidney diseases. The risk of CVD was three times higher in people with diabetes compared to those who do not have diabetes ($p<0.0001$) (109), doubled in the presence of hypertension compared to its absence (110, 111), and kidney diseases increased the risk of CVD between 20%-50% (112, 113). Smoking status adjustment was also examined in the risk of bias assessment. The risk of CVD almost doubled in smokers and those with history of smoking compared to those that do not smoke (24, 114).

Making sure that studies adjusted for age, gender, socioeconomic status, co morbidities (diabetes, hypertension, or renal diseases), and smoking status were very important to ensuring that the risk estimates extracted from each study reflect the risk of extreme vs. normal nutrition marker after a CVD event and not masked by such confounders. This is the rationale why risk estimates of the most adjusted models were extracted whenever possible. In addition, results were sub-grouped by risk estimate type (relative risk, odds, and hazard risk ratios) in order to understand the size of the effect per type of risk estimate. If a study adjusted for all confounders then the risk of bias was considered low. If a study adjusted for all but one (for example for age, gender, socioeconomic status, and co morbidities I recorded YES but recorded NO for smoking status) then the risk of bias was considered medium. If a study adjusted for three or fewer of five confounders (for example, I recorded YES for age, gender, and socioeconomic status,

but recorded NO for co morbidities and smoking status) then the risk of bias was considered high.

2.4.4.3 Funding/author affiliation

Sources of funding and author affiliation were examined. Funding affiliation may mean that the funder might have participated in some form in the study execution or data analysis especially for industry funded studies. For example, if the study funding was received from a pharmaceutical company which hired its own researchers to carry out the study, not independent researchers, this may suggest that funder may have an innate interest in certain outcomes. Author affiliation may mean that the author may have inherent interest in the study giving biased interpretation. If funding and author affiliation with study was recorded as YES or recorded unknown then the risk of bias was considered high. If funding and author affiliation were recorded as NO, then the risk of bias was considered low.

2.4.5 Statistical Analysis

2.4.5.1 Risk Estimates

Quantitative measures of the relationship between a nutrition marker and an outcome measure as they were provided in the publication (relative risk, hazard ratio, or odds ratio) were abstracted. Most adjusted and unadjusted risk estimates were recorded along with any measure of variance reported, and standard errors calculated where possible. Standard errors were calculated from 95% confidence intervals by subtracting the lower limit from upper limit divided by 3.92 (105). The natural log of the effect was entered as required by REVMAN 5.1 software (115) and indicated in section 9.4.3.2 in the Cochrane handbook for systematic review of interventions titled "The generic inverse variance outcome type in RevMan"(105). Heterogeneity was assessed using I^2, an I^2 of 0%, 25%, 50%, and 75% corresponded to a no, low, moderate, and high level of

heterogeneity respectively as suggested by Higgins et al 2003 (116). The higher the heterogeneity the more variation between studies included in the meta-analysis. It will not be of any meaning to take the combined estimate of a meta-analysis if heterogeneity was high. In such circumstance, the combined estimate cannot provide a meaningful interpretation. Heterogeneity indicates that the studies that were included in the meta-analysis to generate the combined effect differed to give clear cut evidence and cannot provide a confident answer for the research question being investigated.

2.4.5.2 Analysis Plan

Main or primary analysis was to compare relationship between each nutrition marker signifying extreme value to its corresponding normal values on primary outcome, mortality, sub grouped by the type of risk estimate statistics type (hazard ratio, odds ratio, or relative risk). The prevalence of malnutrition was common and not rare in all studies examining the prognosis of malnutrition after a CVD. It is not appropriate to pool all risk estimates regardless of type in one meta-analysis. I cannot consider odds ratio and relative risk similar as they can only be considered similar if the prevalence of exposure is rare (106). This is not the case for malnutrition in CVD event as reported earlier in the introduction of this chapter. Secondary analysis was further carried out to examine the risk of extreme nutrition marker compared to its normal parameters (for example obese BMI vs., normal BMI) on secondary outcomes, morbidity (as defined per study), disability, discharge destination, readmission, and length of hospital stay was further sub grouped by the type of risk estimate (hazard ratio, risk ratio, or odds ratio). Subgrouping by risk estimate type (hazard ratio, odds ratio, or relative risk) was classed as primary subgrouping.

In addition to primary subgrouping described above, secondary subgrouping by baselines CVD event, age, or sex was carried out to examine the risk of extreme nutrition marker compared to its normal parameters on primary outcome mortality and only if enough studies were available. The Cochrane handbook for systematic review and meta-analysis, version 5.1, suggests that to carry out subgrouping, at least ten studies must be present to render such sub grouping possible and meaningful (106).

Obesity had the largest number of studies and I carried out secondary subgrouping for obesity only (as I do not have at least 10 studies with the right comparison group for each nutrition marker). All studies were pooled using an inverse variance method using random effects methodology.

Studies included in the systematic review were categorized into studies that evaluated nutritional status using anthropometric, biochemical, and nutrition assessment tools. For each nutrition marker category, the specific nutrition markers were identified and if possible a meta-analysis was carried out. For example, studies using BMI as a nutrition marker were all identified and the relationship between BMI and each outcome was examined in the meta-analysis. Underweight, overweight, and obese body mass index were each compared to normal weight BMI to understand their prognostic value in predicting chosen outcomes on post a CVD event. The same approach was used for all studies for the biochemical and variable nutrition assessment tool categories. For biochemical low and high serum albumin were compared to normal serum albumin respectively. Undernutrition diagnosed by nutrition assessment tools such as SGA was compared with well nourished patients.

2.5 Results

2.5.1 Study Selection

The initial search yielded 2000 titles and abstracts. Of the computer search outcomes, sixty eight articles passed the initial screening and full texts were retrieved. After further scouting a total of 24 studies from the 68 full text papers that passed the initial screening met the inclusion criteria and were included in the systematic review for data extraction (Figure 2.1).

Reasons for exclusion of full text papers included, use of non-human subjects (excluded by default), and use of a nutrition marker that does not meet the study inclusion criteria (e.g. urinary creatinine), the studies in which the participants with cardiovascular disease were analysed but with people with other sorts of illnesses (so the population of interest could not be separated out), and/or the outcomes of interest were not assessed.

2.5.2 Study Characteristics

The total number of participant included in this systematic review was 69,919 (women: 16,201, 23.2%). The median follow up period ranged from 1 month (117) to 35 years (118). There were 14 studies assessing the risk of extreme anthropometry nutrition marker compared to its normal measure on mortality and secondary outcomes with 13 using BMI and one study using weight loss. In the Biochemical nutrition marker analysis there were five studies evaluating the risk of extreme serum biochemistry compared to their corresponding normal range values on mortality and secondary outcomes. Four were on serum albumin, with one of them including serum Creatinine, and one on serum osmolality.

There were four studies that considered the risk of under nutrition compared to normal nutrition on mortality and secondary outcomes using nutrition assessment tools as a nutrition marker. Baseline cardiovascular events included 10 studies on Myocardial Infarction (MI), nine studies on stroke, and four on coronary heart diseases (CHD). Table 2.2 a-b presents a brief description of the characteristics of included studies. Appendix IVa & IVb present detailed description of all studies included in this systematic review and meta-analysis.

2.5.3 Validity of studies

Only two studies had missing data as defined in the methodology (i.e. >5% of the baselines sample recruited were excluded due to missing data necessary for analysis). Of the 24 studies three had missing data (119-121), 23 adjusted for age and one did not (54), 19 studies adjusted for gender and 5 did not (102, 117, 121-123), only two studies adjusted for socioeconomic status (118, 124), seven out of the 24 studies did not adjust for comorbidities (55, 56, 117, 121, 125-127), 10 out of the 24 included studies did not adjust for smoking (55, 56, 98, 102, 117, 120-122, 126-128), and author/funder affiliation was clear for most studies except one study was deemed unclear (122). Tables 2.3a-b presented the validity (assessment of bias) of each study including missing data, adjustment and author/funder affiliation (A/F).

The selected studies mainly examined the risk associated with the extreme nutrition marker compared to its normal measure on mortality (primary outcome). Tables 2.4 a-b present the results of the studies that examine the risk of extreme nutrition marker compared to its normal value on the primary outcome, mortality. Table 2.3c presents the results of studies that the risk of extreme nutrition marker compared to normal nutrition marker on secondary outcomes

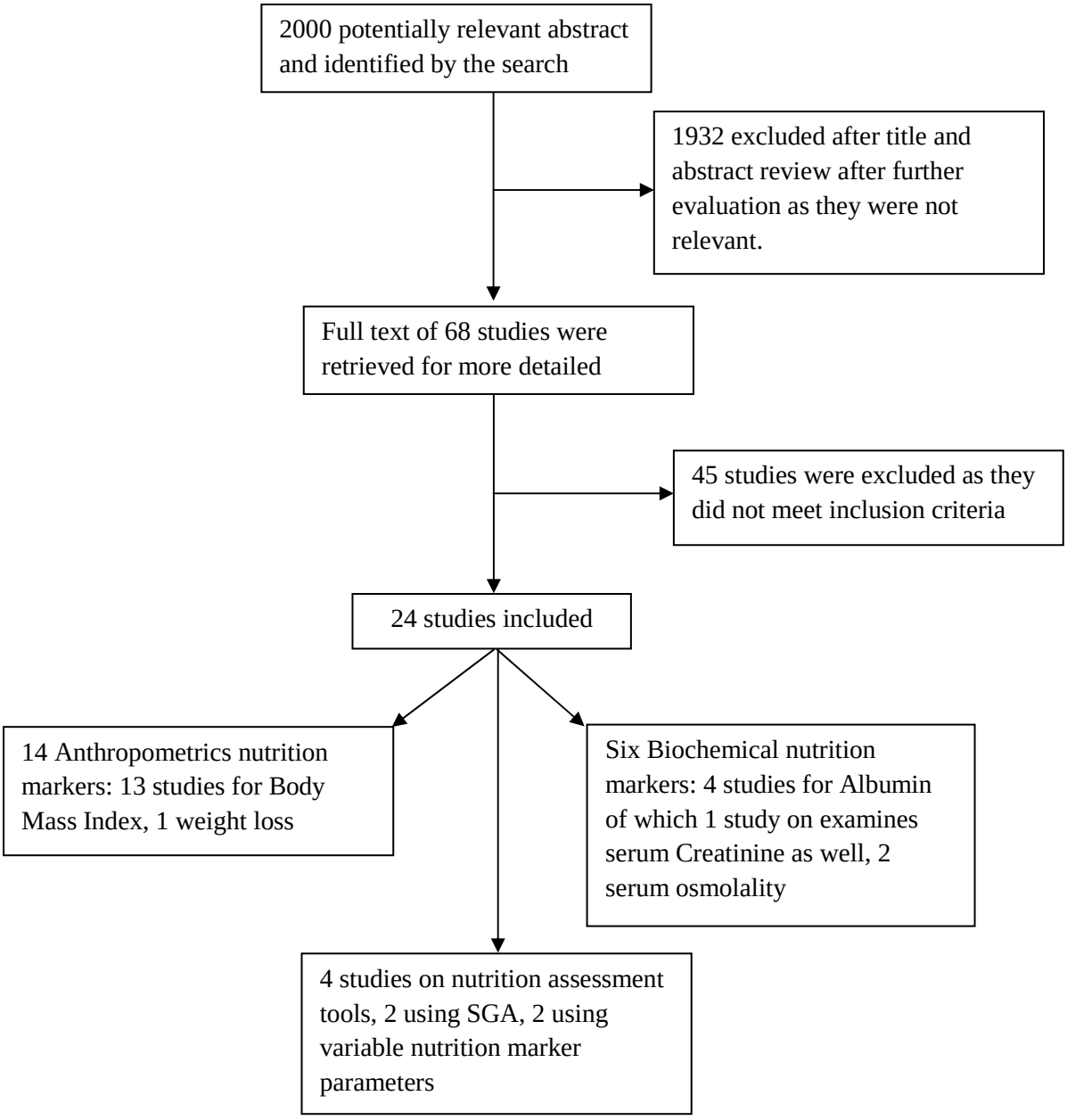

Figure 2.1. The process of filtering electronic search outcomes until reaching the articles included in the systematic review and meta-analysis that met the inclusion criteria.

Study	Follow up (months)	Event	Females/Males	Exposure	Comparison	Outcome Assessed
Anthropometric Nutrition Markers						
Batty 2006 [118]	42	CHD	18403 men*	BMI≥30,25-29.9 kg/m^2	20-25 kg/m^2	Mortality, recurrent event
Buettner 2007 [122]	17	Stroke	480/1196	BMI≥30,25-29.9 kg/m^2	20-25 kg/m^2	Mortality, recurrent event
Dagenaise 2005 [129]	54	CHD	2182/6620	BMI≥30 kg/m^2	< 25 kg/m^2	Mortality, recurrent event
Domanski 2006[130]	57.6	CHD	1171/5693	BMI≥30 kg/m^2	< 25 kg/m^2	Mortality, recurrent event
Kragelund 2005 [119]	120	MI	2172/4502	BMI≥30, 25-29.9, <19 kg/m^2	20-25 kg/m^2	Mortality, recurrent event
Lopez-Jimenez 2008 [120]	6.2	MI	1022/1296	BMI≥30, 25-29.9, <19 kg/m^2	20-25 kg/m^2	Mortality, recurrent event
Mehta 2007 [131]	12	CHD	606/1719	BMI≥30 kg/m^2	< 25 kg/m^2	Mortality
Nigam 2006 [132]	12	MI	278/616	BMI≥30 kg/m^2	< 25 kg/m^2	Mortality, recurrent event
Nikolsky 2006 [123]	12	MI	542/1493	BMI≥30 kg/m^2	< 25 kg/m^2	Mortality
Rana 2004 (124)	45	MI	1317/581	BMI≥30, 25-29.9 kg/m^2	20-25 kg/m^2	Mortality

Table 2.2a. Characteristics of included studies utilizing anthropometric nutrition markers included in the systematic review continued.

Study	Follow up (months)	Event	Females/Males	Exposure	Comparison	Outcome Assessed
Rea 2001 [125]	36	MI	968/1573	BMI≥30 kg/m^2	< 25 kg/m^2	Recurrent events
Sierra-Johnsson 2007 [133]	76.8	MI	79/298	weight loss		Mortality, recurrent event
Wu 2010 [128]	16	MI	1885/4675	BMI≥30, 25-29.9 kg/m^2	< 25 kg/m^2	Mortality
Zeller 2008 [134]	12	MI	593/1636	BMI≥30, 25-29.9 kg/m^2	< 25 kg/m^2	Mortality

Table 2.2a. Characteristics of included studies utilizing anthropometric nutrition markers included in the systematic review. Not all studies were included in the meta-analysis.

*men only

Study	Follow up (months)	Event	Females/Males	Exposure	Comparison	Outcome Assessed
Serum biochemical Nutrition Markers						
Bhalla 2000 [98]	3	Stroke	87/80	>296 mOsm/kg	<296 mOsm/kg	Mortality, disability
Carter 2007 [135]	88.8	Stroke	271/274	>38 g/L	<38 g/L	Mortality
Gariballa 1998 [126]	3	Stroke	180/81	<35 g/L	≥35 g/L	Mortality
Gariballa 1998 [55]	3	Stroke	129/96	≥35 g/L	<35 g/L	Mortality
Hirakawa 1998 [102]	LHS	MI	521/1070	<35 g/L	≥35 g/L	LHS*
Kelly 2004 [121]	21	Stroke	55/47	> 297 mOsm/kg	<297 mOsm/kg	thromboembolism
Nutrition Assessment Tools*						
Davalos 1996 [56]	3	Stroke	37/67	Undernutrition*	Well nourished*	Disability
Davis 2004 [117]	1	Stroke	87/98	Undernutrition~	Well nourished*	Disability
Food Trial 2003 [127]	6	Stroke	1492/1520	Undernutrition^	Well nourished*	disability/Mortality
Yoo 2008 [54]	3	Stroke	47/84	Undernutrition$	Well nourished*	Complications**

Table 2.2b. Characteristics of studies utilizing biochemical nutrition markers and nutrition assessment tools included in the systematic review.

*LHS: Length of Hospital Stay

*under nourished definition in Davalos 1996: TSF >59.5% and 62.5% and MAMC (mid arm muscle circumference) below 85% and 86.4% and <34 g/l serum albumin

~under nourished by SGA: rate B or C by the SGA for nutritional status

^under nourished by Food Trial Collaboration: by clinician judgement

$ Undernourished definition in Yoo 2008: at least two parameter (described in result section for Nutrition Assessment tools) are below normal of the one assessed in the study.

** Complication in Yoo 2008: Pneumonia, Myocardial Infarction (MI), urinary tract infection, pressure sore, deep vein thrombosis, extra cranial haemorrhage

Study	Missing data	Age	Gender	SES	comorbidities	smoking	A/F
Anthropometric Nutrition Markers							
Batty 2006	No	Yes	Yes	Yes	Yes	Yes	No
Buettner 2007	No	Yes	No	No	Yes	No	unclear
Dagenaise 2005	No	Yes	Yes	No	Yes	Yes	No
Domanski 2006	No	Yes	Yes	No	Yes	Yes	No
Kragelund 2005	Yes	Yes	Yes	No	Yes	Yes	No
Lopez-Jimenez 2008	Yes	Yes	Yes	No	Yes	No	No
Mehta 2007	No	Yes	Yes	No	Yes	Yes	No
Nigam 2006	No	Yes	Yes	No	Yes	Yes	No
Nikolsky 2006	No	Yes	No	No	Yes	Yes	No
Rana 2004	No	Yes	Yes	Yes	Yes	Yes	No
Rea 2001	No	Yes	Yes	No	No	Yes	No
Sierra-Johnsson	No	Yes	Yes	No	Yes	Yes	No

Table 2.3a. Validity assessment of studies utilizing anthropometric nutrition markers, continued

Study	Missing data	Age	Gender	SES	comorbidities	smoking	A/F
Wu 2010	No	Yes	Yes	No	Yes	No	No
Zeller 2008	No	Yes	Yes	No	Yes	Yes	No

Table 2.3a. Validity assessment of studies utilizing anthropometric nutrition markers
*SES: Socioeconomic Status, Hyper: Hypertension, RD: Renal Disease, A/F: Author funder Affiliation

For Missing Data: Yes/unclear means high risk of bias, No means low risk of bias, unknown: no information provided

For Age, Gender, SES, comorbidities, and smoking: Yes means low risk of bias, No/Unclear means high risk of bias, For A/F affiliation: Yes/unlcear means high risk of bias, No means low risk of bias

Study	Missing data	Age	Gender	SES	comorbidities	smoking	A/F
Serum biochemical Nutrition Markers							
Bhalla 2000	No	Yes	Yes	No	Yes	No	No
Carter 2007	No	Yes	Yes	No	Yes	Yes	No
Gariballa 1998	No	Yes	Yes	No	No	No	No
Gariballa 1998	No	Yes	Yes	No	No	Yes	No
Hirakawa 1998	No	Yes	No	No	Yes	No	No
Kelly 2004	No	Yes	No	No	No	No	No
Nutrition Assessment Tools							
Davalos 1996	No	Yes	Yes	No	No	No	No
Davis 2004	No	Yes	No	No	No	No	No
Food Trial Collaboration 2003	No	Yes	Yes	No	No	No	No
Sung-Hee Yoo 2008	No	No	Yes	No	Yes	Yes	No

Table 2.3b. Validity assessment of studies utilizing biochemical nutrition markers and nutrition assessment tools

For Missing Data: Yes/unclear means high risk of bias, No means low risk of bias, unknown: no information provided

For Age, Gender, SES, comorbidities, and smoking: Yes means low risk of bias, No/Unclear means high risk of bias

For A/F affiliation: Yes/unlcear means high risk of bias, No means low risk of bias.

Study	Effect	Unadjusted (95% CI)	p-value	Adjusted (95% CI)	p-value	Extreme Group (n)	Comparison Group (n)
Anthropometric markers							
Obesity and Mortality						BMI≥30 kg/m^2	20-25 kg/m^2
Kragelund 2004 (Men)	RR	0.85 (0.76 to 0.96)	P<0.01	0.99 (0.85 to 1.16)	0.2	544	1613
Kragelund 2004 (Women)	RR	0.85 (0.72 to 1.01)	P>0.05	0.9 (0.74 to 1.09)	0.3	255	989
Batty 2006	HR	NA		1.13 (0.91 to 1.40)	0.24	128	1336
Buettner 2007	HR	0.37 (0.17 to 0.77)	0.012	0.27 (0.08 to 0.92)	0.036	292	551
Lopez -Jimenez 2008	HR	NA		0.74 (0.51 to 1.08)	0.1	700	528
Rana 2004	RR	2.57 (1.87 to 3.51)	p<0.05	1.46 (0.99 to 2.16)	0.8	459	607
Overweight and Mortality						BMI 25-30 kg/m^2	20-25 kg/m^2
Kragelund 2004 (Men)	RR	0.83 (0.76 to 0.90)	P<0.001	0.93 (0.85 to 1.03)	p>0.05	1996	1613
Kragelund 2004 (Women)	RR	0.86 (0.77 to 0.98)	P<0.05	0.78 (0.68 to 0.89)	p<0.001	610	989
Batty 2006	HR	NA		1.11 (1.00 to 1.22)	0.24	1132	1336
Lopez -Jimenez 2008	HR	NA		0.96 (0.69 to 1.34)	0.8	872	528
Rana 2004	RR	0.54 (0.50 to 0.59)	P<0.05	1.14 (.80 to 1.62)	p<0.05	832	607

Table 2.4a. Extreme anthropometric nutrition markers risk on mortality continued

Study	Effect	Unadjusted (95% CI)	p-value	Adjusted (95% CI)	p-value	Extreme Group (n)	Comparison Group (n)
Underweight and Mortality						BMI<20 kg/m^2	20-25 kg/m^2
Kragelund 2004 (Men)	RR	1.73 (1.23 to 2.44)	P<0.01	1.28 (0.87 to 1.90)	p>0.05	41	1613
Kragelund 2004 (Women)	RR	1.70 (1.37 to 2.06)	P<0.001	1.45 (1.17 to 1.80)	p<0.001	120	989
Lopez -Jimenez 2008	HR	NA		1.77 (1.00 to 3.12)	0.05	84	528
Weight Loss and mortality						weight loss	No weight loss
Sierra Johnson 2008	HR	0.59 (0.31 to 1.10)	0.101	0.63 (0.33 to 1.20)	0.17	220	157

Table 2.4a. Extreme anthropometric nutrition markers risk on mortality

Study	Effect	Unadjusted (95% CI)	p-value	Adjusted (95% CI)	Adjusted p-value	Extreme group (n)	Comparison Group (n)
Biochemical markers & Mortality							
Low Serum Albumin							
Gariballa 1998	OR	NA		1.13 (1.01 to 1.27)	0.035	38	163
High Serum Albumin							
Gariballa 1998 (≥ 35g/l)	HR	NA		0.91 (0.84 to 0.99)	0.03	38	163
Carter 2007 (38-40 g/l)*	HR	0.78 (0.59 to 1.09)	0.15	0.79 [0.57 to 1.11]	0.144	174	330
Carter 2007 (>43 g/l)*	HR	0.45 (0.32 to 0.65)	<0.001	0.65 [0.44, 0.96]	0.031	267	330
High Serum Creatinine							
Carter 2007 (82-97 mmol/l)	HR	1.39 (0.94 to 2.05)	0.096	1.60 (1.05 to 2.45)	0.03	240	330
Carter 2007 (98-117 mmol/l)	HR	1.62 (1.12 to 2.34)	0.010	1.51 (1.01 to 2.27)	0.045	196	330
Carter 2007 (>117 mmol/l)	HR	2.26 (1.58 to 3.24)	<0.001	1.85 (1.25 to 2.73)	0.002	109	330
High Serum Osmolality							
Bhalla 2000 (>296 mmol/kg)	OR	NA	NA	2.40 (1.00 to 5.9	0.05		

Table 2.4b. Extreme biochemical nutrition marker and nutrition assessment tools on mortality continued

Study	Effect	Unadjusted (95% CI)	p-value	Adjusted (95% CI)	Adjusted p-value	Extreme group (n)	Comparison Group (n)
Nutrition Assessment tools							
Davis 2004	OR	3.1 (1.3 to 7.7)		3.2 (1.0 to 10.4)		30	155
Food Trial Coll. 2003	OR	2.32 (1.78 to 3.02)	<0.0001	1.82 (1.34 to 2.47)	0.0001	275	2149

Table 2.4b. Extreme biochemical nutrition marker and nutrition assessment tools on mortality
*Target: extreme of the nutrition marker examined (Obesity, overweight, underweight, and weight loss in anthropometric markers, low and high serum albumin and high serum creatinine in biochemical markers, under nutrition in nutrition assessment tools). *comparison: normal range of nutrition marker in question (normal weight for anthropometrics or no weight loss), serum albumin ≤34 g/L.

Study	Effect	unadjusted (95% CI)	p-value	adjusted	p-value	extreme	normal
Obesity & recurrent events							
Buettner 2009	HR	NA		0.66 (0.26 to 1.66)*	0.012	292	551
Weight loss and recurrent events							
Sierra Johnsson 2008	HR	0.60 (0.40 to 0.89)	0.013	0.59 (0.39 to 0.90)	0.015	220	157
Biochemical Nutrition Markers							
Low Serum Albumin and length of hospital stay							
Hirakawa 2006	HR	NA		1.01 (1.00 to 1.01)	P>0.05	629	962
High serum osmolality and disability							
Bhalla 2000	OR	NA		2.34 (0.65 to 8.44)	0.2	50	117
High Serum osmolality and thromboembolism							
Kelly 2004	OR	2.7 (1.1 to 7.0)	0.04	4.7 (1.4 to 16.3)	0.02	24	78
Nutrition assessment tools							
Undernutrition and complications							
Yoo 2008	OR	NA		4.49 (1.07 to 18.94)	0.04	26	105

Table 2.4c. Extreme anthropometric, biochemical nutrition markers and nutrition assessment tool risk on secondary outcomes continued

Study	Effect	unadjusted (95% CI)	p-value	adjusted	p-value	extreme	normal
Undernutrition and disability							
Davis 2004	OR	3.4 (1.3 to 8.7)	0.01	2.7 (0.7 to 9.0)	0.18	30	155
Davalos 1996	OR	NA		3.5 (1.2 to 10.2)	p<0.05	24	67

Table 2.4c. Extreme anthropometric, biochemical nutrition markers and nutrition assessment tool risk on secondary outcomes.

2.5.4 Anthropometric nutrition markers studies description

Nine studies (Table 2.2 a) examined the prognosis of anthropometric nutrition markers in cardiac patients with myocardial infarction, four studies in cardiac patients with coronary heart disease, and one study in cardiac patients with stroke. The 14 studies total participant population was 63,476 of which 13,295 (20.9%) were women. Nine studies came from the USA, one from each Spain, Germany, Canada, United Kingdom, and France.

Validity: Two studies had missing data (Table 2.2). None of the studies adjusted for all factors I considered in the validity tool at once. Eleven studies adjusted for gender, three did not adjust (122, 123), and gender adjustment was not applicable for one study (118) as all were men. One study adjusted for socioeconomic status (124). All studies adjusted for baseline co-morbidities. Of the 14 studies only three did not adjust for smoking (120, 122, 128). Table 2.2 presents details on the validity of all studies included in this systematic review.

Authors also adjusted for other confounder that were presented in their sample baselines characteristics, but were not specified in my data form. These included lifestyle related behaviours (118, 119, 123, 124, 130-132, 134, 136) such as tea and alcohol consumption (124) and physical activity (118). Other studies adjusted for blood pressure either systolic or diastolic blood pressure or both (118, 123, 128, 130-132, 134, 136). Some studies also adjusted for biochemical parameters such as total cholesterol (118, 123, 130), hyperlipidaemia (131), C-reactive protein (134), and hyperhemocystenemia (136), and haematological parameters (122). Some studies adjusted for invasive treatment (119, 123, 124, 130, 131, 134) and medications (119, 124, 130, 132, 136) additionally.

2.5.4.1 Risk of obesity compared to normal weight on mortality and secondary outcomes

Individual study results examining the risk of obesity compared to normal weight are presented in Tables 2.3 a & b. Only five studies used normal weight (20-25 kg/m^2) as the comparison group category. Other studies used a comparison group of BMI<25 kg/m^2 including underweight and normal weight subjects in the same category therefore were not included in the meta-analysis. Of the five studies in the meta-analysis two used relative risk ratio (RR), and three used hazard ratio (HR). The forest plot in Figure 2.2 shows the meta-analysis sub grouped by risk estimate type for the risk of obesity compared to normal weight on mortality. There were no studies which reported odds ratio. Heterogeneity was assessed by I^2 and it was 75% in studies reporting hazard ratio and 58% in studies reporting the effect as a relative risk. This suggested a moderate to high level of variation between studies. This level of heterogeneity makes it difficult to interpret the overall effect of the relationship between obesity and mortality compared to normal weight. There are clear variations between studies included in the meta-analysis. Due to moderately high level of heterogeneity, there is no confidence in providing an evidence to aid in decision making that can be withdrawn from this meta-analysis.

I did not have at least 10 studies to carry out secondary subgrouping. The largest set of data for single forest plot was available from examining the risk of obesity compared to normal weight on mortality (presented above). Only secondary sub grouping by baseline CVD event (myocardial infarction) and age examining the risk of obesity compared to normal weight on mortality was possible. The relative risk suggested a reduced risk of mortality with no statistical significance. Heterogeneity was moderate at 67%. The risk of obesity compared to normal BMI decreased with increasing age. Table 2.5 presents the results of primary and secondary subgrouping for the risk of obesity compared to normal weight on mortality.

Not enough studies examined the risk of extreme anthropometric nutrition marker on secondary outcomes (no more than one study) to allow meta-analysis subgrouping by risk estimate (Table 2.3c).

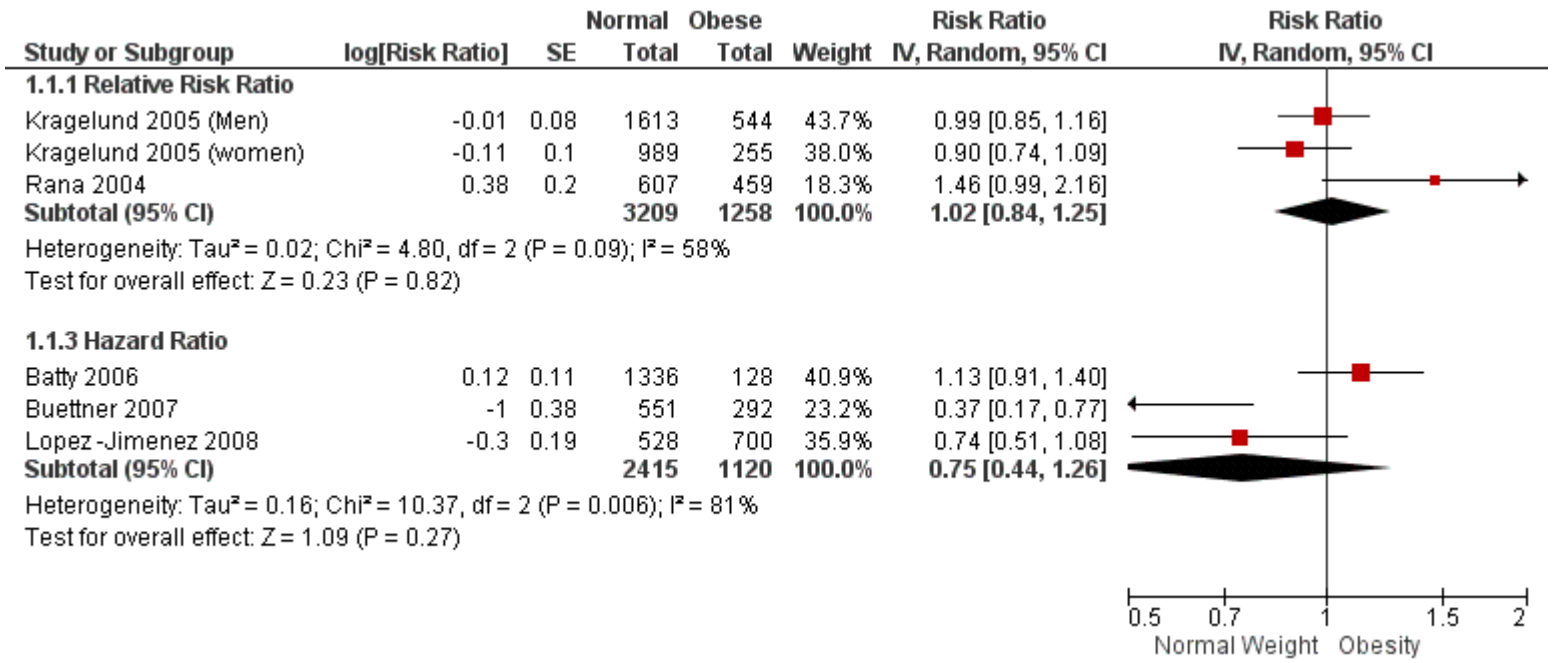

Figure 2.2. Forest plot showing the risk of obesity compared to normal weight on mortality post-CVD sub grouped by relative risk ratio, and odds ratio for the most adjusted risk estimates of studies included in the meta-analysis. In the relative risk subgrouping the diamond can be seen on the right side of the forest plot axis suggesting increased risk of obesity on mortality, while in the hazard ratio subgrouping the diamond is on the left size side of the axis suggesting reduced risk of obesity on mortality; both compared to normal weight.

Mortality	No. studies	Effect size	p-value	Obese	Normal weight	Heterogeneity
Type of Risk estimate						
Relative Risk Ratio	2	1.02 (0.84 to 1.24)	0.83	1258	3209	58%
Odds Ratio		NA				
Hazard Ratio	3	0.79 (0.48 to 1.32)	0.37	1120	2415	75%
Age						
50-59 years	3	1.09 (0.92 to 1.28)	0.34	1287	2471	69%
60-69 years	2	0.95 (0.81 to 1.11)	0.51	836	2164	85%
70-79 years	NA					
Gender						
Men	NA					
Women	NA					
Baseline CVD event						
Myocardial Infarction (MI)	5	0.98 (0.89 to 1.08)	0.67	2378	5624	67%

Table 2.5. Meta-analysis result for studies that examined the risk of obesity on mortality post CVD event sub grouped by type of risk estimate, age, gender, baselines CVD event (only MI), and the risk of obesity on morbidity relationship between obesity and mortality post-CVD event sub group by morbidity defined as recurrent event (secondary outcomes); no other secondary outcomes were examined.

2.5.4.1.1 Sensitivity analysis

Studies that reported unadjusted risk estimates were entered into a meta-analysis sub grouped by effect type (relative risk ratio, hazard ratio, and odds ratio). The sensitivity analysis (entering unadjusted risk estimates only) results for the risk of obesity compared to normal weight on mortality decreased by 6% suggesting but confidence intervals were wide to suggest that obesity (n=1258) may reduce the risk of mortality compared to normal weight (n=3209); RR 0.94 (95% 0. 86 to 1.93; p=0.19). Obesity lost its protective effect once other confounders were considered. The contribution of other confounders to the effect size may have outweighed that of obesity resulting in a 2% increase in the risk mortality in obese participants compared to participants with normal weight (in adjusted analysis). However, it cannot be said that obesity reduces the risk of mortality as this does not hold any statistical significance (as in adjusted meta-analysis). Furthermore, the level of heterogeneity was high at 95%, and therefore it was impossible to draw any conclusion from these findings.

2.5.4.2 Risk of mortality in overweight patients compared to normal weight patients post CVD event

Only four studies examined the risk of mortality in overweight patients (25-29.9 kg/m^2) compared to the comparison group of interest, normal weight (20-25 kg/m^2) post CVD event (myocardial infarction). One study presented both unadjusted and adjusted relative risk ratios. Meta-analysis for the risk of mortality in overweight patients compared to normal weight patients sub grouped by type of risk estimate is shown in Figure 2.4. Studies reporting the effect as RR suggested a 10% reduced risk of mortality in overweight patients compared to normal weight patients. A high level of heterogeneity was observed. Studies reporting the risk as hazards ratio suggested increased risk by 9% with a 0% heterogeneity. Not enough studies were available for secondary subgrouping to be possible as indicated in the analysis plan.

2.5.4.2.1 Sensitivity analysis for the risk of overweight compared to normal weight on mortality post CVD event

I carried out a sensitivity analysis for the risk of overweight compared to normal weight on mortality post CVD event by including only unadjusted risk estimates. The result showed reduced risk but heterogeneity was high (97%) suggesting that evidence cannot be drawn despite statistical significance; RR 0.71 (95% CI 0.67 to 0.74; $p<0.05$). Total overweight was 832 and normal weight was 607. Despite statistical significance the high level of heterogeneity makes such risk estimate not one that can provide evidence on the reduced risk of mortality in overweight patients.

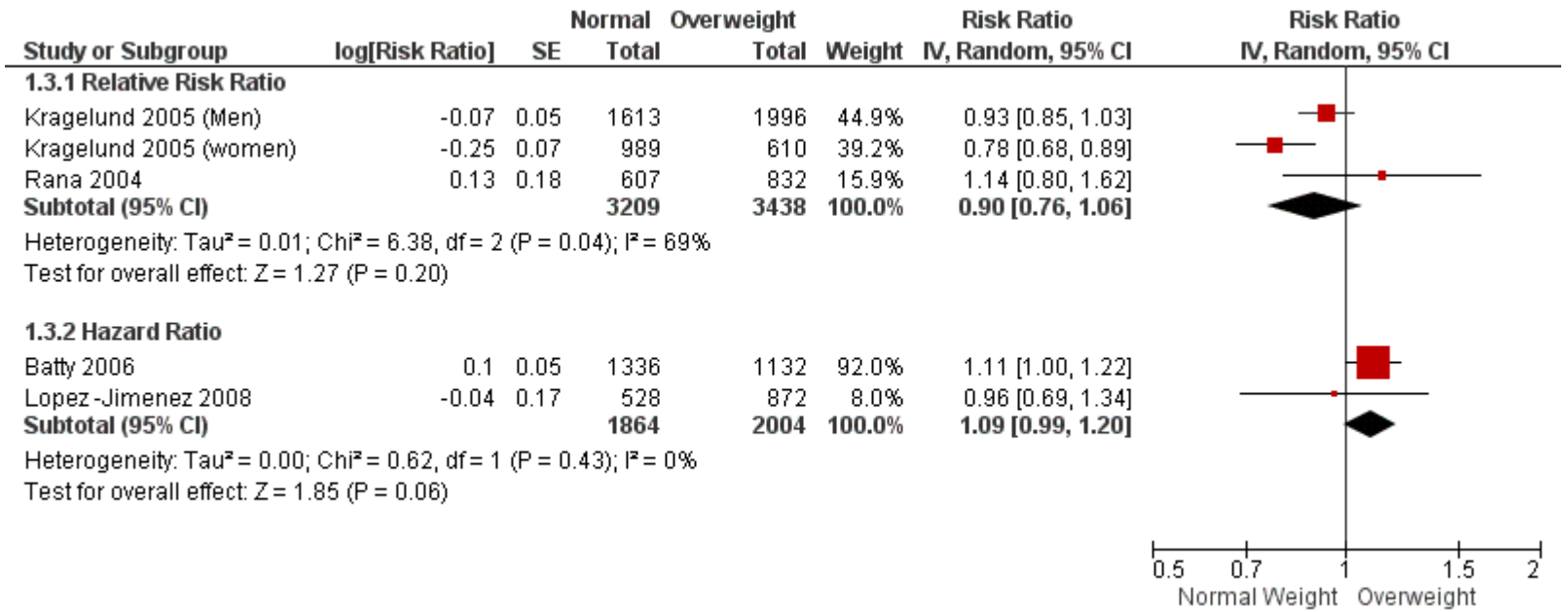

Figure 02.3. Meta-analysis forest plot for risk overweight (25-29.9 kg/m^2) compared to normal weight (20-25 kg/m^2) mortality. In the relative risk subgrouping you can see the effect (diamond) going to the left of the forest plot suggesting reduced risk of overweight on mortality, while in the hazard ratio subgrouping diamond can be seen on the right side of the axis suggesting increased risk of overweight on mortality; both compared to normal weight.

2.5.4.3 Risk of mortality in underweight patients compared to normal weight patients post CVD event

Two studies examined the risk of mortality in underweight patients (<19 kg/m^2) compared to normal weight (20-25 kg/m^2) patients post CVD event. All studies suggested increased risk of mortality in underweight patients. Both studies examined the risk of mortality in underweight patients compared to normal weight patients post myocardial infarction. Figure 2.4 presents the meta-analysis results of studies examining the risk of mortality in underweight patients compared to normal weight patients post CVD event sub-grouped by risk estimate type, no studies reported odds ratio or relative risk; only hazard ratio. Heterogeneity was low suggesting that they provide the same outcome which was increased risk of mortality.

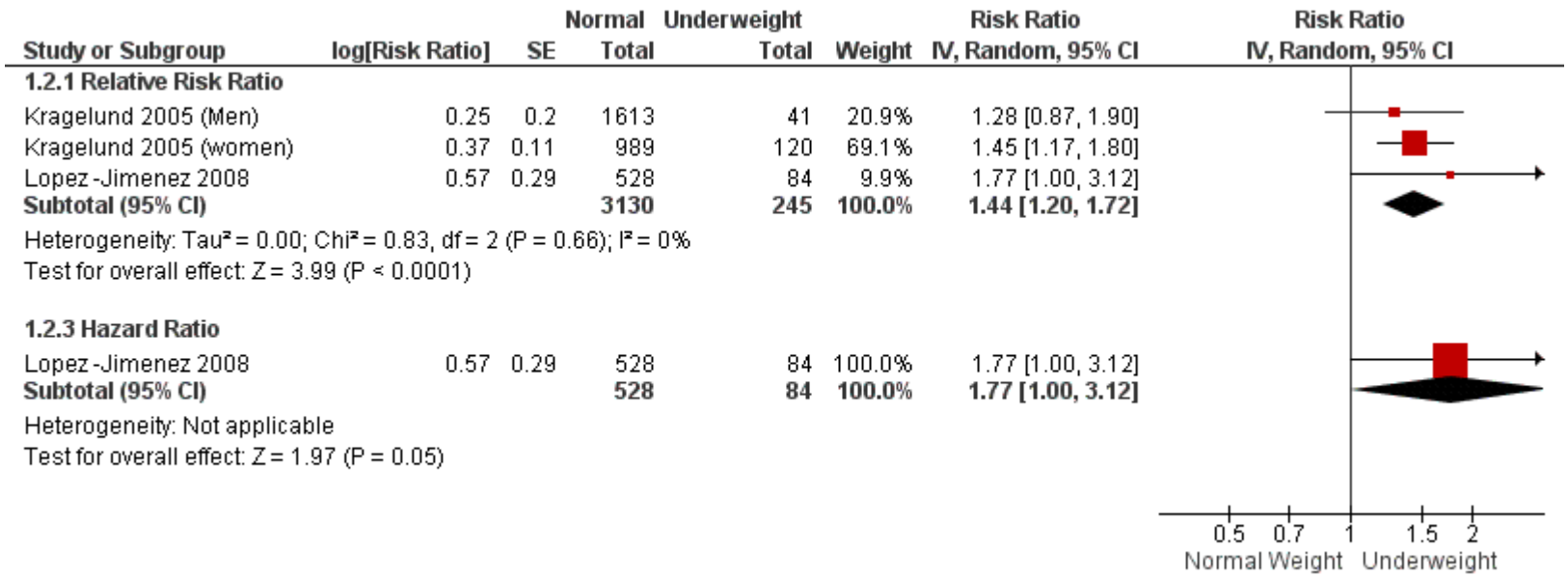

Figure 2.4. meta-analysis forest plot of studies examining the risk of underweight (BMI<19 kg/m^2) compared to normal weight (BMI 20-25 kg/m^2) on mortality compared to normal weight patients post CVD event. In the relative risk and hazard ratio subgrouping you can see the diamond on the right size of the forest plot axis suggesting increased risk of underweight on mortality; both compared to normal weight.

2.5.4.4 Risk of weight loss compared to weigh loss absence on mortality and morbidity post CVD event

Only one study (103) examined the risk of weight loss during acute hospital stay (Table 2.4 a) compared to no weight loss on mortality and recurrent CVD event (Table 2.4 c). The result suggested no association with mortality in patients experiencing weight loss post CVD event compared to those with no weight loss HR 0.63 (0.33 to 1.20; p=0.116) and reduced risk of recurrent CVD event 0.59 (0.39 to 0.90; p=0.015).

2.5.5 Biochemical Studies description

There were six studies examining the effect of malnutrition assessed by biochemical nutrition marker on outcome. The total number of participants was 2911 participants (42.7%, n=1188 women. Two studies examined the risk of high serum albumin compared to its normal range on mortality (55, 135) and one of which also examined the risk of high serum creatinine on mortality compared to its normal value on mortality (135). One study examined the risk of low serum albumin compared to normal serum albumin on secondary outcome length of hospital stay (102), and one examined the risk of high serum osmolality compared to its normal value on mortality (98). Of those studies one was on baseline myocardial infarction (102). Four studies came from the United Kingdom and one from Japan (102). The median follow up period ranged 3 months (55, 98, 126) to 7.4 years(135).

Validity assessment: no study had missing data. Only one study did not adjust for gender (102) and none of them adjusted for socioeconomic status. Of the six studies, two did not adjust for co morbidities (55, 126) and three did not adjust for smoking (98, 102, 126). Funding and author affiliation was all assessed as NO suggesting low risk of bias.

2.5.5.1 Risk of high or low biochemical nutrition marker compared to normal values on mortality and secondary outcomes post CVD event

Gariballa e al 1998 (55) and Carter et al 2007 (135) examined the risk of high serum albumin compared to low serum albumin on mortality post CVD event (stroke). Meta-analysis results suggested a reduced risk of mortality HR 0.91 (0.84 to 0.98); p=0.01. The result of the meta-analysis is presented in Figure 2.5. The heterogeneity was absent. Not enough studies were available to carry out secondary sub grouping by baselines CVD event, gender, or age. All high serum albumin studies were presented risk estimates as hazard risk and none presented odds ratio or relative risk. Only study examined the risk of low serum compared to normal serum albumin values and suggested an increased risk of mortality OR 1.13 (1.01 to 1.27; p=0.035). Only one study Hirakawa 2006 examined the risk of low serum albumin (<35 g/L) compared to higher serum albumin (≥35 g/L) on secondary outcome length of hospital stay. The outcome suggested no increased or decreased risk in length of hospital stay in those with low serum albumin compared to those with higher serum albumin OR 1.01 (1.00 to 1.01) p>0.05.

Not enough studies were available to carry out a meta-analysis sub grouped by risk estimate type (primary subgrouping) for the risk of low serum albumin, high serum osmolality, or high serum creatinine compared to normal values on mortality. One study for each of those nutrition makers was available. Risk of low serum albumin on mortality compared to high serum albumin suggested an increased risk by 13% (OR 1.13, 95% CI 1.01 to 1.27; p=0.035). The risk of increased serum osmolality (>296 mOsm/kg) compared to normal serum osmolality on mortality resulted in an increased risk of death by more than two fold OR 2.40 (95%CI 1.00 to 1.59; p=0.05). Bhalla 2000 examined the risk of high serum osmolality (>296 mOsm/kg) compared to low serum osmolality on disability and found no risk OR 2.34 (0.65 to 8.44); p=0.2. Rowat and colleagues examined the risk of high serum osmolality compared to its normal values on thromboembolism and found an almost five fold increased risk OR 4.7 (95% CI 1.4 to 16.3; p=0.02).

The risk of high serum creatinine (82-97 mmol/L) compared to low serum creatinine (<82 mmol/L) suggested a statistically insignificant (with wide confidence interval range) increased risk of mortality by at least 30% HR 1.39 (95% CI 0.94 to 2.05; p=0.096). The same study examined higher parameters of serum creatinine at 98-117 mmol/L and >117 mmol/L compared to low serum creatinine and showed an increased risk of mortality with a HR of 1.62 (95% CI 1.12 to 2.34; p=0.01) and 2.26 (95% CI 1.58 to 2.24; p=<0.001), respectively.

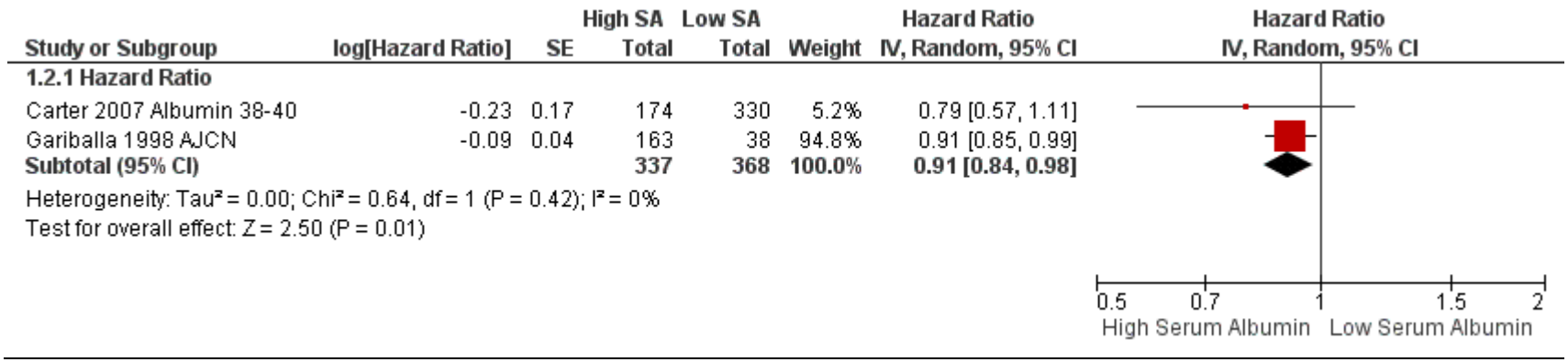

Figure 2.5. Forest plot for the adjusted risk of high serum albumin (≥35 g/L) compared to low serum albumin (<35 g/L) on mortality post baselines CVD sub grouped by risk estimate type, for studies examining the event for adjusted risk estimate. The diamond is moving toward the left of the forest plot axis suggesting reduced risk of high serum albumin on mortality.

2.5.6 Nutrition assessment tools study description:

Studies which reported the association between the nutritional assessment tools and outcome used a combined biochemical and anthropometrics nutrition markers or a validated nutrition assessment tool (e.g. MNA and SGA). The total number of participant in this category was 3,432 of whom 1663 (48.5%) were women. The follow up period ranged from 30 days (117) to 3 months (55, 126). There were four studies, one each from Spain, Australia, and South Korea, and one was a multi-centre global study.

Validity assessment: no missing data were reported. Of the four studies only one study did not adjust for age (54) and one did not adjust for gender (117). None of the four studies adjusted for socioeconomic status. Only one study adjusted for co morbidities and one for smoking (54).

2.5.6.1 Risk of under nutrition compared to normal nutritional status on mortality and secondary outcomes post-CVD event:

Two studies examined the risk of under nutrition compared to the normal nutrition on mortality in patients with stroke. Both unadjusted and the adjusted risk estimates suggested the increased odds of mortality in patients diagnosed with under nutrition compared to those without the diagnosis of under nutrition. The meta-analysis results suggested 89% relative increase in odds; OR 1.89 (95% CI 1.40 to 2.56). The I^2 value was "0" suggesting that the two studies did not differ in the interpretation of their findings. Figure 2.6 presents the meta-analysis result of the two studies examining the risk of under nutrition compared to normal nutrition on mortality after stroke. No studies reported hazard or relative risk estimates for the risk of under nutrition compared to normal nutrition on mortality. Secondary subgrouping was not possible. There were not enough studies to carry out subgrouping by baseline CVD event, age, or sex.

Two studies were possible to include in a meta-analysis examining the risk of under nutrition on disability. The meta-analysis of Davis 2004 and Davalos 1996, suggested an increased risk of disability associated with under nutrition compared with patients with no under nutrition OR 2.83 (95% CI 1.59 to 2.03). I^2 was '0' suggesting the absence heterogeneity. The results of the meta-analysis are presented in Figure 2.7. One study examined the risk of under nutrition compared to normal nutrition on complications and suggested increased risk OR 4.49 (1.07 to 18.94; p=0.04). No studies reported hazard or relative risk.

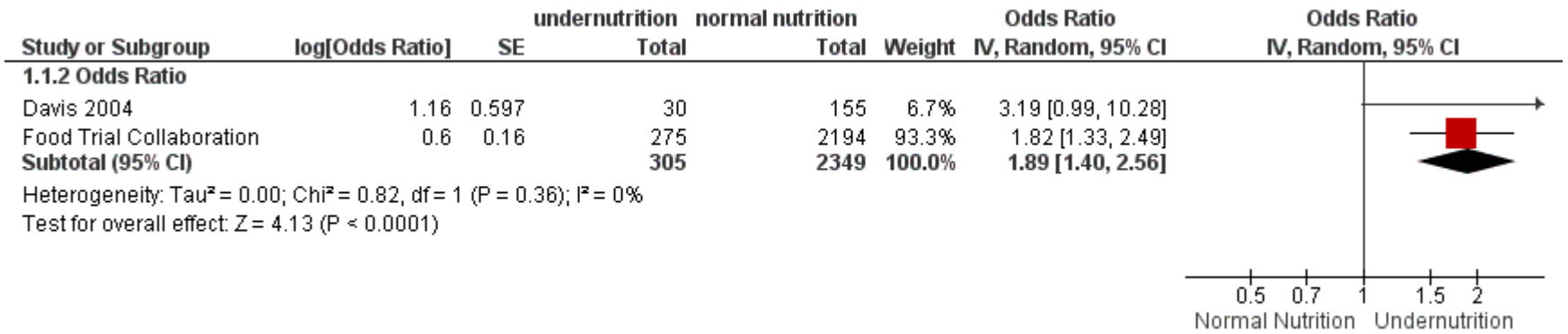

Figure 2.6. Meta-analysis forest plot for adjusted risk of under nutrition compared to normal nutritional status on mortality post CVD event. The diamond is on the right of the forest plot axis suggesting increased risk of mortality.

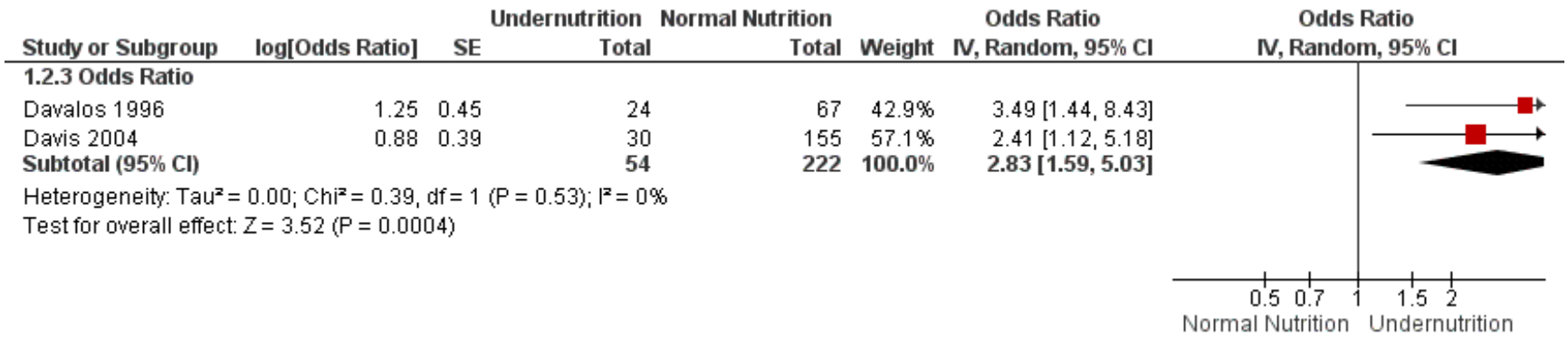

Figure 2.7. Forest plot of the studies that examining the risk of under nutrition compared normal nutritional status on disability. The diamond is on the right side of the axis suggesting increased risk.

2.5.6.1.1 Sensitivity analysis

I carried out a sensitivity analysis, meta-analysis using unadjusted risk estimate, for studies examining the risk of under nutrition (n=305) compared to normal nutrition (n=2,349) on mortality post CVD event sub-grouped by risk estimate type. The result suggested an increased risk of mortality with no heterogeneity observed; OR 2.38 (1.84 to 3.06) $p<0.05$. This result is coherent with adjusted risk estimate examined earlier. Undernutrition is an independent predictor of mortality post CVD event.

2.6 Discussion

2.6.1 Summary of Study finding

There were a total of 23 studies with a total population of 69,817 (women: 16,146, 23.1%). Fourteen studies examined extreme anthropometric nutrition marker risk, five examined extreme serum biochemistry risk, and four examined under nutrition assessed by nutrition assessment tool risk, compared to their normal corresponding values on primary and secondary outcomes.

Meta-analysis results for the risk of obesity compared to normal weight on mortality suggested no risk on mortality among obese patients RR 1.02 (0.84 to 1.24; p=0.83) as opposed to hazard risk of 0.79 (0.48 to 1.32; p=0.37). None of the risk estimates were statically significant and heterogeneity was moderate when subgrouping by RR (58%) and high when subgrouping by HR (75%) suggesting variability among studies entered in the meta-analysis to lead similar finding.

In secondary subgrouping by age no risk of obesity compared to normal weight on mortality was observed. The risk of mortality in the 50-59 years old age 1.09 (0.92 to 1.28; p=0.34) and the 60-69 years old was 0.95 (95% CI 0.81 to 1.11; p=0.51) (Table 2.4). In both of the meta-analysis by age heterogeneity was moderate for the 50-59 years old subgrouping (69%) and high for the 60-69 years old subgrouping (75%) suggesting variability among studies making it difficult to draw a coherent conclusion. Further none of these studies risk estimates were statically significant.

In myocardial infarction patients on baseline obesity compared to normal weight did not show any risk on mortality 0.98 (95% CI 0.89 to 1.08; p=0.67) n obese myocardial infarction patients compared to normal weight myocardial infarction patients with moderate heterogeneity of 67%.

In studies that examined the risk of overweight compared to normal weight on mortality no effect was observed when subgrouping by relative risk of hazard risk ratio RR 0.90 (95% CI 0.76 to 1.96; p=0.20) and HR 1.09 (0.99 to 1.20; p=0.06). For studies examining the risk of overweight compared to normal weight on mortality sub grouped by relative risk heterogeneity was 69% and for those examining the risk of overweight compared to normal weight sub grouped by hazard risk heterogeneity was 0%, but no statically significant effect was observed.

There was an increase by 41% (RR 1.41, 95% CI 1.17 to 1.70) in the relative risk of underweight compared to normal weight on mortality in CVD patient s ($p<0.05$) and absence of heterogeneity. The risk of underweight on mortality increased by 41% post myocardial infarction RR 1.44 (95% CI 1.20 to 1.72; $p<0.0001$) and heterogeneity was absent suggesting coherence in studies risk estimate direction (increase) in included studies.

Meta-analysis of studies examining the risk of high serum albumin compared to normal serum albumin suggested that there was a statistically significant reduced risk of mortality HR 0.91 (95% CI 0.84 to 0.98; p=0.01) with both studies providing the same conclusion (risk reduction) with heterogeneity being absent (0%). For the nutrition markers low serum albumin, high serum osmolality, and high serum creatinine compared to their normal values one study was available for each making it not enough evidence to base a conclusion upon. Only study examined the risk of low serum compared to normal serum albumin values and suggested an increased risk of mortality OR 1.13 (1.01 to 1.27; p=0.035). The risk of increased serum osmolality (>296 mOsm/kg) compared to normal serum osmolality on mortality resulted in an increased risk of death by more than two fold OR 2.40 (1.00 to 1.59; p=0.05). The risk of high serum creatinine (82-97 mmol/L) compared to low serum creatinine (<82 mmol/L) suggested a statistically insignificant (with wide confidence interval range) increased risk of mortality by at least 30% HR 1.39 (95% CI 0.94 to 2.05; p=0.096). The same study examined higher parameters of serum creatinine at 98-117 mmol/L and >117 mmol/L compared to low serum creatinine and suggested an increased risk of mortality with a HR of 1.62 (95% CI 1.12 to 2.34; p=0.01) and 2.26 (95% CI 1.58 to 2.24; p=<0.001) respectively.

The final nutrition markers examined was nutrition assessment tool (a combination of biochemical and anthropometric nutrition markers) or a validated nutrition assessment tools such as SGA and MNA (see introduction). Meta-analysis risk of under nutrition compared to normal nutrition on mortality post CVD event suggested a statistically significant increased risk OR 1.88 (95% CI 1.40 to 2.53, p=0.0001) with no heterogeneity observed. The risk of under nutrition assessed using nutrition assessment tools on disability suggest an increased risk with no heterogeneity observed and statistical significance.

2.6.2 Interpretation

Obesity and overweight can be associated with pro-inflammatory and pro- thrombotic states (137) increasing the risk for conditions such as diabetes, hypertension, high systolic and/or diastolic blood pressure, and glucose intolerance. Abdominal obesity is related to CVD events (138). The results of the meta-analysis suggest that underweight patients are at increased risk of mortality compared to normal weight patients post CVD event.

Underweight is a form of under nutrition and may serve as a marker of frailty. Evidence presented earlier in the introduction suggests it can increase the risk of poor outcomes. Edington et al 1999 examined the relationship between BMI as a nutrition marker in community strictly, among patients with cardiovascular diseases, including coronary heart disease, angina, myocardial infarction, stroke, transient ischaemic attack on selected outcomes including hospital admissions and mortality. This study was not included in this systematic review as it did not specify the effect of BMI on each individual CVD condition individually. They found that CVD patients with a BMI of <20 kg/m^2 had the highest hospital admission rates ($p<0.001$) and had their risk of death increased by two fold ($p<0.001$) compared to those with a BMI of >25 kg/m^2 (139).

Weight loss as suggested in the one study seemed to have no association with mortality, but reduces the risk of recurrent event. If weight loss occurred in obese or overweight

patients it can improve their health and post-CVD event outcomes as it can place them within the healthy weight range. On the contrary, if patients were already malnourished weight loss could cause further deterioration in their nutritional status increasing the risk of poor outcomes.

No meta-analysis was possible for the relationship between low serum albumin and mortality. However, one study (126) suggested that low serum albumin increases the risk of mortality. One study cannot provide conclusive evidence. Low serum albumin may not be related all the time to deteriorating nutritional status (140). On the contrary, high serum albumin compared to normal serum albumin suggested reduced risk of mortality regardless of subgrouping with absence of heterogeneity. In-vivo studies suggest that albumin synthesis ceased when nutritional intake decreased or was inadequate (141).

High serum creatinine compared to normal serum creatinine suggested increased risk on mortality. Serum creatinine is suggested to be related to lean body mass (142, 143). Therefore such elevation in serum creatinine may be related to muscle breakdown as fuel substrates due to under nutrition, or such elevation in serum creatinine could be due to an increase in lean body mass. Evidence to date suggest that the relationship between lean body composition and health outcome is limited and studies have shown that the contribution of lean body mass to serum creatinine is minimal (84) to suggest that serum creatinine reflect nutritional status. It may be that the elevation in serum creatinine is related to glomerular filtration rate which also decreases with age (143) and the included study by Carter et al (135) was conducted in an ageing population (mean age 76 years, range 69-82 years) making it difficult to draw conclusion based on one study.

Increased serum osmolality reflects hydration status and suggests dehydration. In the one study included in this systematic review high serum osmolality compared to its normal value increased the risk of mortality as well as disability. Dehydration seems to be a potential marker for poor outcomes including mortality. The result of the serum osmolality study examined was coherent with other studies that suggest a strong

association between dehydration and poor outcomes. A study by Rowat et al suggested that from the 2,549 stroke patients included in the study, of the 43% (687/1580) diagnosed as dehydrated on admission died in hospital or were discharged to institutional care compared with 177 of 969 of patients without dehydration (χ^2=170.5; *P*<0.0001 (144). This study was not included in this review as it uses a serum urea to creatinine ratio and I am interested in individual nutrition markers and their normal values as a comparison group and the study did not report any risk estimate.

When evaluating studies assessing the prognosis of under nutrition assessed by nutrition assessment tool, all studies provided coherent outcomes. In all of the studies the risk of under nutrition compared to normal nutrition was associated with mortality and poor outcomes. These finding are consistent with findings from other studies. Martineau et al examined the malnutrition diagnosed using SGA in 73 stroke patients and found that 19.2% of patients were malnourished further malnourished patients had longer length of stay of 13 days (compared to 8 days in well nourished patients; p<0.001) and higher rates of complications (infections, tachycardia, pressure ulcers and falls) at 50% (compared to 14% in well nourished patients; p=0.003) (46). Another study examined the length of hospital stay in malnourished stroke patients compared to stroke patients with no malnutrition in a rehabilitation unit (n=49) and reported that length of hospital stay was significantly lower in patients with no malnutrition (44.9±14.4 days; n=10) compared to malnourished patients (58.9±14.9 days; n=18); p=0.011 (145). Malnutrition was diagnosed by the presence of at least two of the following assessed parameter on admission, body weight ≤90% of reference weight or ≤95% of usual weight or BMI < 20 kg/m^2, or the total mean of four skinfold thickness < 5th percentile, or mid arm muscle circumference < 5th percentile, or serum albumin <35 g/L, or serum transferrin <2.0 g/L, or total lymphocyte counts <1,800 n/mm^3. The result of meta-analysis for studies assessing the risk of under nutrition compared to normal nutrition on mortality and secondary outcomes included in my systematic review were coherent with these result and similar in their assessment of malnutrition.

2.6.3 Strengths and Limitations

The main strengths of this study is that it examined prospective cohort studies only, allowing for better homogeneity in study designs and quality and clearer assessment of the validity of each study, by focusing on assessing the validity of one of study design. The other strengths include using the same comparison groups (normal nutrition marker value), which makes sure that extracted risk estimates included in the meta-analysis for each predictor (nutrition marker) share the same comparison group characteristics.

The main limitation of this study was that there were not a large number of studies for primary and secondary subgrouping of each nutrition marker examined. In addition there were not enough studies examining secondary outcomes of interest. Many studies assessed in this review selected to be included in the systematic review were excluded from final meta-analysis due to not using the comparison group of interest. Sometimes there was only one study for specific nutrition marker of interest that examines primary or secondary outcomes making it not applicable for subgrouping in a meta-analysis. These limitations make it difficult to reach firm conclusions and thus this review could not provide conclusive evidence based on systematic review of existing evidence.

Other limitations and weaknesses of the studies included in this systematic review are related to the differences in confounders adjusted in individual studies. As discussed in the risk of bias section in the methodology I chose age, gender, socioeconomic status, co morbidities (diabetes, hypertension, or renal diseases), and smoking status as factors that might influence the outcomes of studies. One study did not adjust for age (54), four studies did not adjust for gender (102, 117, 122, 123), seven studies did not adjust for smoking (56, 98, 117, 120, 122, 126-128), only one study adjusted for socioeconomic status (124), five studies did not adjust for co morbidities (diabetes, hypertension, and Kidney disease) (55, 56, 117, 126, 127). Adjustment for other possible confounders also varied between studies (see Appendix IVa & IVb) making the risk estimates being affected by the level of adjustment.

The length of follow up varied between studies (Appendix IVa & IVb); one month (117), 3 months ((55, 98, 126), and some more than a year (119, 124). This variability in follow up may attenuate the risk estimates. Prognosis of post-CVD event may not be related to nutritional status diagnosed during hospital stay, but can be related to many other factors that occurred after hospital discharge which were not considered.

In summary, limited number of studies to allow primary and secondary subgrouping, not enough studies examining secondary outcomes, not all studies using the same reference group (i.e. normal nutrition), differences in confounder adjustment, between studies and variability in the length of follow up periods contributed to the limitations and weaknesses.

2.6.4 Relevance to Clinicians

Malnutrition is prevalent among patients with CVD events. Diagnosing malnutrition in patients with CVD is important as evidence suggest malnutrition is a prognostic indicator for outcomes. In this systematic review morbidity (functional status, length of hospital stay, hospital readmission) and mortality were selected to assess the prognostic value of malnutrition assessed using specific nutrition markers. Malnutrition contributes to impaired immunity (146) and increases the risk of morbidity. Malnutrition also affects physical strength (147). Weight loss experienced in malnutrition contributes to weakness resulting in increase in dependency and decline in functional status. The loss of functional capacity contributes to patient inability to perform their previous activities affecting daily life. Malnutrition also affects mental health (148). Malnutrition thus increases the costs on the health system (149). Based on this systematic review finding diagnosing malnutrition should be based on a comprehensive assessment of different nutrition markers ranging from anthropometric, biochemical and others makers such as dietary intake to detect any abnormal nutrition markers parameters that can indicate nutritional status deterioration. If malnutrition is diagnosed nutrition intervention followed by nutritional status monitoring must be a priority.

2.6.5 Conclusion

The risk of obesity compared to normal weight on mortality resulted in variable results with no statistical significance and moderate to high heterogeneity, which was also apparent when carrying out secondary subgrouping. There was no risk associated for obesity or overweight compared to normal weight on mortality but, I cannot draw a firm conclusion on obesity or overweight risk on mortality considering that heterogeneity was high suggesting variability in study's findings and no statistical significance.

The risk of underweight (compared to normal weight) and under nutrition (assessed using nutrition assessment tools compared to normal nutrition) on mortality suggested an increased risk while the risk of high serum albumin (compared to normal serum albumin) decreases the risk of mortality. There were two studies for each of the mentioned nutrition markers. Despite the absence of heterogeneity and statistical significance there are not enough studies to draw firm conclusion that can suggest that was systematic evidence. Similarly the result of the meta-analysis assessing the risk of under nutrition compared to normal nutrition on disability which suggested an increased risk is based on two studies not enough to draw on concrete evidence.

For low serum albumin, high serum osmolality, and high serum creatinine their risk on mortality compared to their normal parameter suggested increased risk on mortality. These were individual studies and systematic evidence cannot be drawn from them therefore confirmatory studies are required and future systematic review is recommended.

Main limitation was that there were not enough studies to carry out subgrouping for each nutrition marker resulting in carrying only subgrouping for the nutrition marker (obesity) with the large set of data. Most studies that met the inclusion criteria did not have the right comparison group resulting in excluding them from any meta-analysis. Due to the limitations and the fact that there are not enough studies to draw firm conclusion, clinicians must rely on diagnosing malnutrition through monitoring

different nutrition markers ranging from anthropometric and biochemical nutrition markers. Further prospective cohort studies to understand association between nutritional status and outcomes after acute CVD event are required to allow for the generation of evidence through the synthesis of larger systematic review and meta-analysis.

CHAPTER 3: Body composition changes after stroke and their relationship with short and longer term outcomes

Abstract

Background: Malnutrition after stroke is common and can lead to tissue catabolism and body composition changes and may have impact on stroke recovery. This study seeks to evaluate these relationships using multi-frequency bioelectrical impedance analysis (MF-BIA).

Methodology: Ischaemic stroke patients admitted to an acute unit were prospectively recruited between January-July 2011. Patients' demographics, anthropometric measures, biochemistry and body composition variables (BioScan 920-2, Maltron International Ltd, Essex, United Kingdom) were measured on admission and discharge. Mean fat free mass (FFM), fat mass (FM), and protein mass change and mean changes/day between admission and discharge were compared between (soft mashed/pureed and Nil-By-Mouth (NBM)) vs. normal feeding and between soft mashed/pureed vs. NBM. They were followed up at 6 months after discharge using Patient Administrative System (PAS) and by postal questionnaires for mortality, discharge destination and other functional outcomes including Barthel Index, Health Related Quality of Life using Short-Form-36 version 2.0 (SF-36v2), and Stroke Impact Scale (SIS).

Results: Total number of participant was 40, men=22(55%), mean age 69.8(±10.5) years, range 50-89 years, mean length of stay=4±4.1) days, range 2-24 days. There were 17 Lacunar, 12 posterior circulation, 5 partial anterior circulation, and 6 total anterior circulation infarcts. Average NIHSS score was 5.0 (range 1-22). Noticeable differences included higher protein mass loss for patients on modified diets (soft mashed/pureed) or nil by mouth -1.0 (-2.0 to 0.1) kg, compared to patients on normal oral diet -0.3 (-0.9 to 0.3) kg. Lager fat free mass loss was observed in patients prescribed nil-by-mouth (NBM) feeding regimen -1.9 (-4.3 to 0.5) kg compared to non-NBM (normal oral/soft mashed/pureed) (-0.3 (-1.1 to 0.5) kg. NBM group experienced higher fat mass gains 1.4 (-1.8 to 4.6) kg compared to non-NBM 0.1 (-0.64 to 0.9) kg. Further stratification by stroke subtype did not result in any statistically significant differences between or within groups. Eighteen participants responded to follow up questionnaire (45%). Those with fat free mass, protein mass, muscle mass, and body cell mass losses and fat

mass gain follow up questionnaire result was no statistically significantly different from those with fat free mass, protein mass, muscle mass, and body cell mass gains and fat mass loss.

Conclusion: While the body composition changes observed in acute stroke were not statistically significant due to relatively small sample size, understanding these changes may, however, help designing targeted interventions in post-stroke nutritional care.

3.1 Background

Stroke is a condition associated with several complications ranging from inability to swallow, to becoming completely dependent. Of 1,259 stroke patients in the South London register assessed one week and 3 months post stroke, a wide range of disabilities were reported. They reported 1-2 impairments in 6% of patients, 3-5 impairments in 31% of patients, 6-10 impairment in 51% of patients, and ≥10 impairments in 11% of patients with dysphagia and upper limb weakness being the most frequent impairments in 44% and 77% of patients, respectively (150). The physical limitations that stroke incurs on its survivors may affect their activities of daily living and hence the quality of life. While initial neurological damage can relate to these limitations, it is also important to note that recovery from stroke may be influenced by the body composition changes such as fat free mass loss, muscles mass loss, and other tissue losses, during acute stroke phase, resulting in reduced functional capacity in longer term.

Understanding the extent of the occurrence of these body composition changes early after stroke may therefore help to understand the relationship between these changes and stroke outcomes including functional health. In this part of my investigation, I used multi-frequency bioelectrical impedance analysis (MF-BIA) to examine these changes. MF-BIA method is a swift, simple, and non-invasive method that can provide an evaluation of different body compartments. Body composition measurement can be carried out on stroke patients on admission and discharge to evaluate the extent of changes occurring during their acute hospital stay using MF-BIA machine. This technology can be used in clinical setting to understand body composition changes immediately after stroke if it is practical to so in acute setting. Possible relationship between body composition changes and outcomes such as morbidity and mortality, and outcomes reported by patients, such as quality of life, can then be investigated.

In this Chapter, I present the results of a prospective longitudinal cohort study which examined the extent of body composition changes in patients with an acute ischaemic stroke during their hospital admission and explored if any association existed between

these changes during acute hospital stay and short (at discharge) and longer term outcomes at six month post discharge.

3.1.3 Stroke Complications and dietary intake

Stroke can have various effects on the body including daily dietary intake. Reduced dietary intake can result in weight loss and can further affect body composition in stroke patients. Hence body composition can provide information on the nutritional status and adequacy. The focus of this dissertation is on examining body composition changes after stroke as to date no studies has examined which body component is most affected after stroke.

Dietary intake in acute stroke is often inadequate, which is usually attributed to high incidence of dysphagia after stroke, and a range of other secondary complications such as cognitive problems affecting eating behaviours, reduced ability to feed oneself independently, disorientation, paralysis, and depression (151, 152). Reduced dietary intake can lead to weight loss, which is well documented after stroke (153, 154).

Dysphagia is one of the commonest complications after stroke. In a recent review, Martino and colleagues (155), reported the incidence of dysphagia as varying from 37% to 78%; using different dysphagia diagnostic criteria including cursory (water swallowing test), clinical (clinical scores), and instrumental (video fluoroscopy) methods. The authors concluded that dysphagia after stroke is common regardless of diagnostic method used. Dysphagia is considered as the primary cause of reduced dietary and fluid intake in stroke patients (151, 152).

There is also a direct association between dysphagia and malnutrition in stroke patients. The proportion of dysphagic patients suffering from malnutrition, assessed using the patient's self-reported Subjective Global Assessment (SGA) tool was 71% (10/14) compared to non-dysphagic patients (19/59; 32%) in acute stroke, p=0.007 (46). One

week after admission to an acute stroke unit, dysphagic patients were more likely to be malnourished (16/24; 67%) compared to non-dysphagic patients (15/67; 24%; p<0.001) (56). The association between dysphagia and malnutrition is prevalent not only in acute settings, but also in care home settings. A study carried out in stroke patients residing in a care home reported a significantly higher prevalence of malnutrition in dysphagic patients (4/20; 20%) compared to non-dysphagic patients (4/40; 10%); p=0.044 (156). The prevalence of malnutrition was also significantly higher in dysphagic compared to non-dysphagic patients (62.5% vs. 32.0% respectively) on admission to a rehabilitation unit; p<0.032) (157).

There are other reasons why stroke patients may have reduced dietary intake in longer term. The physical and mental impairments associated with disabilities in stroke patients can alter dietary intake; making the eating process physically, socially, and mentally difficult. Hoarding and leakage of food from the mouth, and chewing problems contributed to eating difficulties after stroke in 44% of patients with eating problems (154). Other problems contributing to eating difficulty include food spills, difficulty to sit appropriately for eating, inability to concentrate, prolonged eating time, and inability to control foods in the plate (158).

The eating difficulties that stroke patients experience could make the whole process an unpleasant experience for them. There is some evidence to suggest that their new disability and limitations may put stroke patients into a state of depression. In an observational study by Axelssen et al. (154) the authors reported that 65% of the patients in their study entered into a denial phase not accepting their new condition i.e. inability to eat as before. The authors postulated that the denial phase caused patients to enter into depression and increased the risk of anorexia (up to 50% in their series) (154). A mean weight loss of 2.6 kg was reported in the 78% of patients with eating difficulties in their study (154). Gariballa et al reported a statistically significant decline in average weight between week 0 (63.7±13.6 kg), week 2 (62.4±13.7 kg) in 48% (96/201), week 4 (61.6±12.5 kg) in 25%(51/201) of the 225 patients in their study; p=0.002 (55).

Weight loss may still occur long term after stroke. A more recent population based study documented weight loss of ≥3.0 kg in 24% and 26% of stroke patients four months and one year post-stroke respectively (153). If weight loss persists for a long duration it can contribute to severe body mass index (BMI) changes that can be classified as malnutrition; BMI <18.5 Kg/m^2 in <65 years old population and a BMI <22 Kg/m^2 in ≥ 65 years old population (159).

Stroke complications resulting in reduced dietary and fluid intake lead to high incidence and prevalence of malnutrition among stroke patients. In the next section I discuss the prevalence and incidence of malnutrition in stroke patients.

3.1.4 Malnutrition in stroke

The European Society of Parententeral and Enteral Nutrition (ESPEN) which is also known as the European Society of Clinical Nutrition and Metabolism defines malnutrition as "*a state in which a deficiency or excess (or imbalance) of energy, protein, and other nutrients causes measurable adverse effect on tissue/body form (body shape, size, and composition) and function, and clinical outcome*"(65).

Malnutrition is shown to be prevalent among stroke patients on admission to a stroke unit. This may be partly due to the fact that malnutrition is common in older age and the majority of patients with stroke are older people. The reported rates of malnutrition varied between different studies depending on the different methods used to assess malnutrition. Unosson and colleagues reported that 8.0% of their study subjects (≥70 years old) were protein malnourished on admission; based on serum protein concentrations (48). However, they did not use a validated malnutrition assessment tool such as the Subjective Global Assessment (SGA) or the Mini Nutritional Assessment (MNA) used in other studies (46, 47, 160, 161). These studies also reported variable malnutrition prevalence rates on admission to an acute stroke unit. The prevalence of malnutrition using SGA was reported to be 19.0% in one study (46) and 32.1% in another study (47). The two studies that used both SGA and MNA tool reported

malnutrition to be at 16.0% (160) and 26.3% (161) respectively at the time of admission to stroke unit. A consistent finding in all these studies, however, is that malnutrition seems to be prevalent among stroke patients on admission with acute stroke thereby increasing the risk of further deterioration of nutritional status during their hospital stay.

The proportion of stroke patients with malnutrition also appear to increase during acute hospital care (49, 56). One study reported a 6.0% increase in the prevalence of malnutrition from 16.0% at the time of hospital admission to 22.0% at the time of discharge measured anthropometrically using Triceps Skin Fold thickness (TSF), Mid Arm Circumference (MAC), weight and biochemical parameters including albumin (49). Another study involving 104 patients with acute stroke reported that malnutrition prevalence changed from 16.4% at admission to 26.4% of surviving patients (n=91) and 35% of patients who remained in hospital (n=43) at one and two weeks post admission respectively (see below for implication of malnutrition in this study). Malnutrition was assessed using three measurements of MAC, TSF, and serum albumin (56). Another study showed consistent findings reporting a constant decline in BMI ($p=0.006$), Triceps and biceps skin fold thicknesses (both $p<0.0001$ MAC ($p=0.001$), albumin ($p<0.0001$), and transferrin ($p=0.02$) between week 2 and week 4 post admission in stroke (55).

In a more recent prospective observational study that included 131 ischaemic stroke patients, malnutrition 24 hours post-admission was diagnosed in 12.2% of patients compared to 19.8% of patients at one week post admission; $p=0.03$ (54). The study used five criteria including a 10% weight loss in the past 3 months and/or 6% weight loss one week post admission, weight index (actual weight compared to reference weight) less than 80%, serum albumin <3.0g/dL, prealbumin <10.0 mg/dL, or transferrin < 150mg/dL. Malnutrition in the acute phase also increased the risk of malnutrition subsequently for example on discharge to rehabilitation services. The proportion of patients diagnosed with malnutrition on admission to stroke rehabilitation services ranged from 35% to 67% (157, 159, 162).

I have summarised the prevalence of malnutrition in stroke. In the next section I present how the immobility and stress response in stroke can affect body composition.

3.1.5 Immobility, stress response, and body composition

In acute illness bed rest alone can contribute to body composition changes mainly fat free mass loss. One study showed total lean mass loss of 0.84±0.34 kg (-1.7±0.6 %) ($p<0.05$) and fat mass gain of 0.48±0.16 kg (6.6±2.3%) ($p<0.03$) after a 14 days of bed rest in six healthy men (mean age 30± 6) years old) (163). Lean tissue loss is further exacerbated with the stress response instigated in acute illness. Patients with acute stroke have been shown to have a increased stress response; they have high cortisol levels, resulting in the deterioration of their nutritional status (56).

Elevated cortisol levels further induce catabolic process in the body resulting in lean tissue loss. By injecting cortisol in volunteering healthy subjects (n=5) to mimic the stress response in acute condition, Gelfand and colleagues reported muscle breakdown to be evident by the increased appearance of amino acids in blood (164). These finding were further supported by Brillon and colleagues, when hydrocortisone was inject in nine healthy volunteers up to 5-20% in muscle protein breakdown occurred evident by increased appearance of plasma amino acids (Leucine and Phenylalanine) in blood circulation (165). Ferrando and colleagues conducted a study to examine the effect of cortisol on the catabolic processes during a period of bed rest. Hydrocortisone sodium succinate was infused in healthy men (n=6) to mimic the cortisol response in trauma, a level of approximately 31 g/L of cortisol in plasma. Blood samples were withdrawn and muscle biopsy was obtained from the vastus lateralis (largest muscle of the quadriceps femoris) at different times. Participants then entered into a 14 day bed rest. Loss of total leg lean mass was (0.51±0.23 kg; $p= 0.04$), and intracellular glutamine concentration decreased significantly in response to cortisol on day 14 of the bed rest being at 8711±525 μmol/L compared to 9850±783 μmol/L pre bed rest; $p= 0.03$). Amino acid appearance rate in the circulation also increased; amino acid efflux increased from 302 ±60 to 508±180 $nmol \cdot min^{-1} \cdot 100\ ml\ leg^{-1}$ for phenylalanine, 3037±891 to 3716 ±1225 $nmol \cdot min^{-1} \cdot 100\ ml\ leg^{-1}$ for glutamine, and from 2230± 603 to 2876 ± 1038 $nmol \cdot min^{-1} \cdot 100\ ml\ leg^{-1}$ for alanine (166).

In older people with stroke, sarcopenia, another physiological change is taking place exacerbating the body composition changes such as fat free mass loss, muscle loss and fat mass gain, in addition to stress response and immobility due to stroke. Sarcopenia is defined as muscle loss that occurs with the aging process leading to general weakness (61, 167). The resulting changes in body composition due to the stress response, bed rest, and sarcopenia can have negative consequences on stroke outcomes is further compounded by the poor dietary intake discussed above. Hence there is no doubt that the combination of immobility, heightened stress response, and malnutrition all contribute to body composition changes in acute stroke. It was also documented that an increase in fat mass was associated with functional limitations in older people (61, 168). Interventions to prevent loss of tissue in acute condition such as stroke are important to prevent any possible poor prognosis of such changes on outcomes. In the following section I discuss some nutritional interventions which primarily targeted promotion of feeding in people with dysphagia in stroke.

3.1.6 Nutritional intervention studies in stroke

Studies assessing the effects of enhanced nutritional interventions in people who have had an acute stroke have provided variable results to date. Bath and colleagues carried out a review (169) of the available studies to understand the effect of different enteral feeding methods on stroke outcomes and concluded at the time of the review that further studies were required for a solid conclusion.

A randomized controlled trial reported lower treatment failure defined as death at six weeks in the PEG group (0/16, 0%) compared to the NG group (3/14, 21.4%) and reported that six of the 16 patients in PEG group were discharged by six weeks after PEG insertion compared to none in the NG group; $p<0.05$. Six week case fatality in the PEG group was 12.0% compared to 57.0% in the NG group; $p<0.05$ (170). Further the trial reported significant improvement in nutritional status extrapolated from albumin levels in those who received Percutaneous Endoscopic Gastrostomy (PEG) compared to Nasogastric (NG) tube feeding at six weeks after commencement of feeding regimes. Albumin levels improved from 27.1g/L to 30 g/L in the PEG group compared to

reduction from 31.4 g/L to 22.4 g/L in the NG group; p<0.003 (167). Despite these reported favourable outcomes with PEG intervention it was difficult to draw any firm conclusion for several reasons. The sample size was relatively small (n=30) to make it generalizable and the authors indicated that all patients were in stable condition without specifying the extent of the stability of patients' condition before randomizing their patients making it difficult to know if more stable patients were randomized to PEG feeding regimen. There was no clear sample size calculation for the reported outcome as the first 30 patients who fulfilled the study inclusion criteria (cerebrovascular accident with dysphagia for more than 8 days) were recruited.

A recent randomized controlled trial by Hamidon et al compared the effects of PEG and NG feeding on patients' nutritional status up to 4 weeks post intervention. In PEG fed patients (n=10) albumin levels were significantly higher than NG tube fed patients (n=12); p=0.045. Within groups, PEG fed patients' albumin levels rose more than NG fed patients; PEG group (p=0.025) vs. NG group (p=0.047) 4 weeks post intervention indicating better improvement in nutritional status in PEG compared to NG patients (171). Better treatment outcomes were also reported in the PEG group compared to the NG group; the treatment failure frequency was reported to be 50% in the NG group compared to no failure in the PEG group; p<0.036. The authors concluded that PEG feeding improves nutritional status more than NG feeding (171).

The FOOD trial, the largest nutritional intervention trial in stroke patients to date, reported a different outcome. The FOOD trial studied the effect of early vs. none and type of nutritional support (PEG vs. NG feeding) on long term stroke outcomes; up to 6 months post discharge (172). Patients were randomised to either no enteral tube feeding or enteral tube feeding 7 days post-admission to stroke unit, or randomised to PEG vs. NG tube feeding 7 days post admission. Poor outcome (defined as modified Ranking scale (mRs) score of 4-5) and death were evaluated 6 months post discharge. There was no statistically significant difference in effect between early or no tube feeding on the risk of death (42% mortality for early tube feeding vs. 48% mortality rate for no tube feeding; n=429, OR=0.79, CI 95% 0.60-1.03) or combined death or poor outcome (79% and 80%, respectively; n=429, OR=0.93, 95%CI 0.67-1.30) (172). Similarly, no statistically significant differences in the effects of the two nutritional support regimens on death and poor outcome were observed. Six months after admission 89% of patients

who had been randomised to PEG (n=162) compared to 81% of those given NG feeding (n=159) experienced death or poor outcome (OR=1.86, 95% CI 0.99-3.50) (30). The effect on mortality of the different nutritional regimens was not statistically significant either (49% and 48% for the PEG and NG feeding; OR= 1.04, 95% CI 0.67-1.61) (172).

The effect of early nutritional supplementation on death or poor outcome (mRs score of 3-5) at 6 months post discharge were also examined in the FOOD Trial (173). Patients were randomly allocated to normal hospital diet or normal diet with additional oral nutritional supplementation (360 ml oral protein supplement of 6.27 kJ/ml and 62.5 g/L in protein daily) during hospital stay until discharge. There was no effect of supplementation on mortality outcome. Death was reported at 13% and 12% for the non-supplemented (n= 2012) and supplemented (n=2000) groups respectively; OR=0.94, 95% CI 0.78-1.13. As for death or poor outcome it was reported at 58% and 59% for the non-supplemented (n=1995) and supplemented (n=2009) groups respectively indicating no effect of supplementation; OR= 1.03, 95% CI 0.91-1.17 (173). Nutrition interventions as reported by the FOOD Trials did not have any important or significant impact on stroke outcomes up to 6 months post stroke.

The FOOD trial adjusted for several prognostic variables including age, gender, pre-morbid status before stroke (living alone and independence), condition after stroke (ability to talk, lift arms, and walk), and ability to swallow. The FOOD trial while being a multicentre study has its strengths and weaknesses. The strengths as reported by the authors include its large sample size, at least 10 times larger than any previous trial, and the recruitment of patients from various centres; and thus increased generalizability. There are several weaknesses as suggested by the authors. Weaknesses included informal methods in assessing nutritional status, failure to record the total number of eligible subjects in each centre, and inability to have an onsite source to report change in nutritional status and patient nutrient intake. The lack of a universal method in classifying malnourished patients may have contributed to MF-MF-BIAs in categorizing malnourished patients, inability to report nutritional status improvement in malnourished patients assigned to tube feeding (172) or nutritional supplements (173) initially, and inability to record systematically patients nutrient intake that could be mostly met through oral hospital diet masking the benefits of tube feeding (172) or

nutritional supplements (173) initially. Furthermore, being a pragmatic multicentre trial the investigators did not adopt targeted intervention approach i.e. tailoring nutritional management according to needs for example based on monitoring of body composition changes.

It remains unclear which is the preferred type of nutritional intervention. These limitations may have influenced outcomes. The FOOD trial despite being a large multicentre study cannot help in providing evidence to help clinicians in decision making considering the inability to record and follow confounding factors that could have contributed for the reported outcomes.

From the existing literature, it is evident that the prevalence of malnutrition among acute stroke patients is common and may result in poor outcomes. I have presented the impact of malnutrition on health outcomes after stroke and the summary of evidence from the nutritional intervention studies in stroke. In the next section I present methods which are used to assess nutritional status.

3.1.7 Assessing nutritional status and body composition in stroke

Given the prevalence of malnutrition in stroke patients, the stress response associated with the trauma from stroke, and the expected bed rest and their possible influence on body composition changes after stroke, assessing body composition in stroke patients may be useful in guiding nutritional interventions in stroke. It may be argued that we can always calculate BMI or asses weight change, both are relatively easily measurable in clinical setting, but neither of them can provide information on the actual constituent of body composition changes. Despite BMI being normally used to assess malnutrition (BMI< 18.5 kg/m^2 for general population and a BMI<22 kg/m^2 for and older population) (174), BMI as well as weight, cannot predict body composition changes. If an increase in BMI occurs it could be attributed to increased fat mass and extracellular water content due to cellular dehydration (60) and not necessarily due to improved nutritional status. BMI and weight change do not reflect changes in body composition such as fat

mass, fat free mass and total body water changes (dehydration in stroke patients is discussed in details in the Chapter 4).

Different methods of assessing body composition are described in details in the Chapter 5 of the Thesis. In this section I briefly present the standard methods or measurements which can be used in routine clinical practice for monitoring of nutritional status and evaluating of treatment success as well as in clinical trial settings.

Upper arm anthropometric measurements have been used to reflect body composition changes associated with nutritional status (46). Mid Arm Circumference (MAC) and Triceps Skin Fold (TSF) thickness are being suggested to reflect fat free mass and fat mass respectively. However, the accuracy of these anthropometric measures is questionable. Furthermore, there are disadvantage in using upper arm anthropometric measures. The poor reproducibility of TSF due to margin of error between measurements makes the validity of this method questionable (175). Measurement of TSF requires a level of skill and training. In addition, TSF body fat values were biased when compared to reference measurement produced by underwater weighing (176); see Chapter 5 for details of underwater weighing method. On the contrary MAC is a relatively easy procedure making its measures more reproducible (177), but MAC utility in assessing whole body composition of fat free mass is questionable. It is because MAC is more of a localized measure to evaluate arm muscle area and not whole lean mass tissue (178). Its measures did not show a strong correlation with lean-tissue masses measured by dual x ray absorptiometry (DEXA); the correlation was relatively poor ($r = 0.26$-0.34) (178).

It may be argued that biochemical makers of nutrition can also be used to assess nutritional adequacy. Biochemical measures such as albumin are traditionally used to assess nutritional status (179). However, many studies demonstrated that their usefulness in evaluating protein malnutrition is questionable (180-182). Serum albumin synthesis appears to rise with an increase in protein intake (80) hence its serum values does not necessarily reflect the actual composition of lean body tissue or fat free mass. As discussed in the section above on the stress response and body composition, it is the

rise in amino acids such as alanine, phenyl nine, and glutamine in serum that are indicative of lean body tissue catabolism, but such diagnostics tests are not routinely carried out in clinical settings. Therefore, routinely available biochemical tests cannot be used to predict changes in important components of the body such as fat mass or fat free mass (174). The bioelectrical impedance analysis (MF-BIA) may provide an ideal tool in assessing body composition.

3.1.8 Body composition and its assessment using multi-frequency bioelectrical impedance analysis (MF-BIA)

Body composition describes the constituents of the human body from the different types of tissues to water (also see Chapter 4 for details). For the purpose of this Chapter body composition is referred as the proportion of fat and lean tissues in the human body. Lean tissue represents all the non-fat tissue including muscle, body organs, and bone. Fat free mass consists of any tissue other than fat (183). The non-fat tissue or fat free mass is an important component of the body as it is metabolically active and is involved in all the functional and structural characteristic of the human body. On the other hand, fat tissue or fat mass provides energy reserves and cushioning to internal organs. However, obesity characterised by excess amount of fat tissue is a risk factor for many chronic diseases.

Clarys and colleagues dissected 25 cadavers (age range 44-94 years) and compared them to 19th and 20th century cadaver data of similar age range. Mean skin, muscle and bone proportion of current day cadavers were 8.5%, 50.0%, and 20.6% respectively in their Brussels study, similar to that of the 19th century data (mean proportion of skin, muscle and bone were 7.5%, 49.2%, and 21.3% respectively), but slightly different than the 20th century data (mean proportion of skin, muscle and bone were 8.6%. 44.4%, and 18.4% respectively) suggesting that these variations in proportions of body components can be attributed to nutritional state (184).

The approach of viewing body composition as two main components, fat mass and fat free mass, is known as the two component model (2-C model). Viewing the body as compartment allows deciding on which body composition components to measure and what assessment method to be utilized (assessment methods are discussed in details in the Chapter 5). The two component model (2-C model) was first evaluated using under water weighing method (185). The 2-C model is not the only model used to assess body composition. Fat free mass consists of other components such as bone, minerals, water, and proteins. These components can also be measured. When total body water is included in addition to fat mass and fat free mass the resulting model is known as a three component model (3-C model). The 3-C model can be assessed using the dilution method to assess total body water in addition to under water weighting for fat mass and fat free mass (please also refer to the Chapter 5 for details of these methods). Including bone density and body water in addition to fat mass and fat free mass results in the four component model (4-C model). In the 4-C model, dual X-ray absorptiometry (DEXA) is required to assess bone density (it also provides measurement of fat mass allowing calculation of fat free mass dependent on weight) and dilution methods for example is required for total body water assessment (186).

Measuring additional component of fat free mass increases the body component model with additional or different assessment methods required (discussed in the Chapter 5). When more than four components are being measured the model becomes a multi component model and this can be assessed using bioelectrical impedance analysis. Bioelectrical impedance analysis can measure several components without the need for other expensive methods. This method has been previously validated in selected patient populations. I validated the multi-frequency bioelectrical impedance analysis (MF-BIA) machine I used in this study against DEXA and also conducted internal validation studies. The rationale, methods and results of these validation studies are presented in the Chapter 5.

The principle underlying MF-BIA analysis is also described details in the Chapter 5. It is based on the resistance imposed by certain components of the human body; body impedance, to a flowing electrical current. Body fat is non-conductive to the electrical current while lean body mass, consisting of electrolytes and water, is conductive. When

an electrical current passes through the human body it faces resistance from the adipose tissue, impedance, while passing through the non-adipose tissue component to complete its circuit. The difference in conductivity, current input and output, is used to calculate fat mass and fat free mass using a validated formula already programmed in the MF-BIA analysis equipment (43). For this study I chose MF-BIA BioScan 920-2 model by Maltron International; software (MiStat 920 Software; www.maltronint.com).

Using the MF-BIA methodology body composition can be measured using a single frequency current (SF-BIA) or a multi-frequency current (MF-BIA). In SF-BIA a single current of a known quantity, usually 50 kHz, passes through the body tissue and the difference in current input and output is used to calculate fat free mass and total body water (44). In MF-BIA, currents of several frequencies (1, 5, 50, 100, and 200, up to 500 kHz) are passed through the body tissue separately and impedance is generated, currents input and output difference is measured and used in different validated equations already integrated in the equipment to extrapolate body composition variables. MF-BIA gives measurement of fat free mass, total body water, and extracellular and intracellular water (44); fat free mass is then used to calculate fat mass by subtracting it from body weight.

MF-BIA is relatively cheap compared to other methods that can be used to measure body components (please refer to Chapter 5 for the different methods in assessing body composition). It is simple to perform, non-invasive (187), and quick in providing reproducible results with less than 1.0% error (188). Its simplicity lies in the fact that no more than proper operating of the equipment is required and can be performed at bed-side with minimal requirement of the training to use the device. It produces results instantly and time efficient. The MF-MF-BIA method, therefore, is convenient to use in the busy clinical setting.

3.2 Study objectives and rationale

3.2.1 Study Objectives

The primary objective of this study presented in this chapter was to describe changes in fat free mass and the body composition after acute stroke while considering the magnitude of these changes by type of feeding regimen, ischaemic stroke subtype, and the stroke severity. Other study objectives included examining if body composition changes are correlated with or influenced objective outcomes including hospital readmission, discharge destination, morbidity and mortality. This study also examine if body composition changes had a prognostic influence on subjective outcomes such as health related quality of life and functional capacity up to 6 month follow up post hospital discharge.

3.2.2 Rationale

I hypothesized that body composition changes after stroke do occur and the magnitude and proportion of changes occurring in various components of the body (fat mass, fat free mass etc.) are different depending on stroke type and severity. Evidence indicates that a proportion of stroke patients are malnourished on acute admission and their nutritional status deteriorates during acute hospital stay. Malnutrition combined with possible extended bed rest and stress response in acute conditions results in body tissue catabolism. The human body tries to generate energy from the available energy reserves and this result in catabolic process that result in body composition changes.

Second, I hypothesized that negative body composition changes (defined as reduced fat free mass, increased fat mass) occurs after stroke. The body composition changes after stroke are influenced by the timing and methods of feeding independently of stroke severity. The reasoning for such hypothesis stems from the fact that studies on elderly populations, main stroke population, suggested that sarcopenia (loss of lean body mass),

leads to loss of functional capacity compounded by immobility. Additionally, malnutrition of stroke patients and the stress response in acute stroke phase can result in major body composition changes (hypothesis I) with fat free mass being the most affected component.

Third, I hypothesized that fat free mass and body composition changes correlate with increased risk of mortality, readmissions to secondary care settings, admission to care homes, and reduced functional capacity. It would be reasonable to predict that changes in fat free mass correlate with stroke outcome. Fat free mass or lean body mass loss, results in reduced strength and mobility and overall functional capacity. Fat free mass loss, therefore, can result in disability. Fat free mass loss indicates the severity of the illness. I hypothesized that fat free mass loss during acute stroke phase will have long term effect after stroke that can be measured by objective outcome measures of readmission to secondary care after hospital discharge location, mortality outcome and functional limitation measured by Barthel Index (BI) controlling for case mix and prognostic indicators.

Further it was hypothesized that fat free mass loss is associated with reduced functional capacity and quality of life as measured by the Stroke Impact Scale, Short Form Survey 36v2, and Barthel Index Scores at six months post hospital discharge. The catabolic process that results in fat free mass could lead to delayed recovery and may be associated with poor outcomes. The loss of fat free mass in acute stroke is further compounded in older people who constitute the main stroke populations who may be experiencing sarcopenia (loss of lean body mass). Fat free mass or lean body mass loss, results in reduced strength which results in reduced functional capacity. Therefore such body composition changes may be associated with negative on long term outcomes affecting health related quality of life. Three different standard self reported questionnaires were therefore used to assess long term functional capacity and health related quality of life. These included the Barthel Index Score (BI), the Stroke Impact Scale (SIS), and the Short Form Survey 36 version 2 (SF36v2) (see methods for description and references).

The decision to use SF36v2 in the follow up period was because it can provide a detailed assessment of a participant's physical and mental health providing a comprehensive health related quality of life assessment. As for the SIS, it was selected because it can provide information on what current activities are being carried out by participant, and if a participant can perform favoured activities of the past (integral to their life quality) and the instrument was specially designed to be used in stroke patient population (please also refer to methods). Finally and for evaluating minimal daily activities level, I chose the Barthel Index score. The Ability to perform minimal daily activities is essential for daily living. Minimal activities that we cannot perform basic to our living can have a deep impact on our feeling and life quality.

The reason I chose the six month recruitment and six month follow up is for pragmatic reason as my project is limited by the period of PhD study. This follow-up period required amendment of initially submitted protocol (with 9 month follow-up) due to some technical delay in the time period between receiving the ethical approval (end of July 2010) and Research and Development approval which was gained at the end of November of 2011. Therefore, after consultation and suggestion from my thesis supervisors, the follow-up of the study was carried out 6 months later after appropriate approvals were obtained (Appendix IV: Longitudinal Study protocol).

I chose a longitudinal study design as it provides me with the opportunity to monitor the sample population overtime and observe any possible outcomes. A longitudinal study allows reporting the prognosis of body composition changes on long term outcome and simply not a snapshot of their prognosis (as would be the case in cross sectional studies). In addition, studies examining body composition changes in stroke patients and it prognosis were not carried out before. No effect size or conclusion can be drawn without observed associations. Therefore a clinical trial will not be appropriate (for example providing amino acid supplements to one group vs. placebo for control and then examine body composition changes and their prognosis) as such trial will not be based on a concrete evidence. Trial risks on participants are not understood yet, and sample size selection is not possible given that we do not know the estimate of a sample we need with the objective of drawing a conclusion or seeing an effect of statistical significance for clinically meaningful effect size for relevant outcomes.

Carrying out a case-control study may not be appropriate. The purpose is to understand the extent of body composition changes after stroke. My cases would be stroke patients, but controls would be difficult to choose given that it is not possible to determine controls (patients with no body composition changes after stroke). Further if I decide to choose controls with no stroke this simply defeats the purpose of my whole comparison in a case-control study.

Therefore longitudinal study design is the ideal study design given the lack of data on body composition changes after stroke. It allows for monitoring participants over a period of time to understand the prognosis of such changes on the daily lives of stroke patients.

3.3 Methodology

3.3.1 Ethics

The study was approved by Cambridgeshire I research ethics committee. The final protocol submitted to the committee is available in Appendix VI.

3.3.2 Settings

This prospective longitudinal cohort study which form part of my PhD project was conducted in acute hospital setting at the Norfolk and Norwich University Hospital National Health Services (NHS) Foundation Trust (www.nnuh.nhs.uk). The hospital has a catchment population of approximately 750,000. It covers city of Norwich and the surrounding rural areas. The participants were recruited from the Acute Stroke Unit (then Gunthorpe Ward) located at the main hospital site. The unit admits approximately 900- 1000 acute stroke patients annually.

The acute stroke ward is a 36 bedded unit. The average length of acute hospital stay was 13 days (usually ranged between 5 and 20 days at the time of study) with the average length of stay for milder stroke is ~5 days. In-patient mortality rate is ~ 22% (189) with one year mortality rate of ischaemic strokes is 35% (190). At the beginning of the study stroke patients were admitted to the Acute Medical Admission Unit (AMU) via Accident and Emergency Medicine Department (A&E) or referred to AMU by General Practitioners (GP) first before being admitted to the ward. The admission pathways changed halfway through the study and all acute stroke patients were directly admitted from A&E to the acute stroke unit from May 2011.

3.3.2 Study Design

The study design was a longitudinal observational cohort study conducted over a period of 12 months. Patients admitted to Gunthorpe Acute stroke unit at Norfolk and Norwich University Hospital (NNUH) between January and July 2011 and diagnosed with either type of stroke (ischaemic or haemorrhagic stroke) were recruited to the study. Eligibility criteria are detailed below. Patients with transient ischemic attack (TIA) were excluded specifically for the longitudinal prospective cohort study (but included for the MF-BIA external validation study as described in the Chapter 5).

The study participants were recruited over the period of first six months of the study and they were followed up six months post discharge. The following eligibility criteria were used for inclusion in the study

- Age 17 years or over

- Newly diagnosed stroke (either first or recurrent). The objective is to investigate what body composition changes occur after an incident stroke. Patients with only confirmed stroke are included in the study. Stroke diagnosis was confirmed by a specialist in stroke medicine based on history, clinical examination and neuroradiological imaging (computed tomography (CT) or magnetic resonant imaging (MRI).

- Participants were recruited within 48 hours of hospital admission. Forty-eight hours was chosen as a sufficient enough period to allow for the medical team to evaluate patients' state of health and decide their survival chances, carry out all necessary tests such as blood tests (biochemistry measures) and neuroradiological imaging (CT/MRI) to confirm the diagnosis of stroke and type of stroke. The 48 hours period allowed for recruiting participants that meet the eligibility criteria without interrupting the flow of essential routine immediate and urgent health care provision to the participants.

For validation against DEXA scan only, I also recruited patients diagnosed with transient ischaemic attack (TIA) (participants can participate in the DEXA validation part without taking part in the longitudinal study, which examined body composition changes and the relationship between these changes and outcomes at 6 months).

Patients were not approached if they met the study exclusion criteria detailed below:

- Patients with very severe stroke who were appropriate for palliation only (expected survival of less than 48 hours).

- Severe stroke defined as National Institute of Health Stroke Scale (NIHSS) ≥30 (http://www.ninds.nih.gov/doctors/NIH_Stroke_Scale.pdf) whose likelihood of survival ≥7 days is small (<50%) as judged by the stroke physician. If survival chances of a patient are very low and their likelihood of dying within 7 days is high, it was not appropriate to be recruited into this study. Carrying out research in such circumstances was unethical especially the participants of the study were unlikely to be benefited directly and immediately from participating in the study.

- Life expectancy was less than 3 months prior to the event. If life expectancy prior to the onset of stroke is less than 3 months then the longer term outcome at 6 month after stroke would have been biased by this. Furthermore, it may be confounded by the fact that the body composition changes that were unrelated to stroke but to the overall deteriorating health status that resulted in such a short life expectancy might have been already occurring in such patients.

- If they had other potential confounding conditions that might have been masking/exaggerating the effect of post stroke nutrition on body composition changes. These conditions were defined as co-existing terminal illness e.g. advanced cancer, end stage chronic diseases such as end stage renal failure and end stage chronic obstructive pulmonary disease (COPD). Existence of such illnesses may influence the variables of interest, components of the body

composition, long-term outcomes, and can contribute to confounding effect as findings may not be related directly to stroke, but to these conditions and their treatment.

3.3.3 Recruitment procedure

Patients who had confirmed diagnosis of stroke who were potentially eligible to the study were informed about the study by a clinical team member (medical, nursing or therapy staff) and they were specifically asked whether they would agree to speak to the investigator. Those who were interested in talking to the investigator about the study were then approached by the investigator.

The investigator, PhD student, used the information provided by the clinical staff and screened the eligibility of the patient to the study in those who expressed interest to the study. The following information were checked for patient eligibility;-

- Date and time of patient's symptom onset
- Date and time of hospital admission
- The final diagnosis of the patient

At the first contact with the potentially eligible participant I introduced myself, and obtained verbal consent from them to explain the study. Once the patient agreed, I briefly explained the study objectives, relevance and importance of the study for stroke patients specifically stating that the participants themselves might not directly benefit from it. If the patient remained interested in participating in the study, I then went through each of the study procedure using the study Participant Information Sheet (PIS) (Appendix VII), the letter to participant general practitioner (Appendix VIII) and consent form (Appendix IX) both of which a copy was provided to participants upon consent with the a copies as well placed in the consented patient medical notes).

Consenting patients to participate in a study in acute stroke setting is complex. Therefore I followed the inclusion and exclusion criteria strictly. In case of any doubt with regards to the capacity of the patient to participate, I involved an independent third person, usually a nurse who looks after the patient, as a witness.

After going through each measurement procedures, I summarised additional information written in the PIS including the fact that the patient could seek an independent advice from the Patient Advice Liaison Services (PALS) if he/she would like to complain, Ethics approval status, who were research team members, that the refusal of participation would not affect their treatment, and data protection procedures for their identifiable personal and clinical data. The PIS was left with the patient to read and go over for as long as required to them. I returned to them later and asked whether they remained interested in participating in the study.

If the response was positive I provided the patient with a consent form to initial and sign according to NHS ethics committee guidelines. If necessary, I read out and explained the consent form to the patient. Upon receiving patient's written informed consent, patient medical notes were reviewed and I recorded data including admission date and time, onset of symptoms date and time, presence of co-morbid conditions, anthropometric, and blood biochemistry data. In addition I recorded data ascertained from the speech therapists' entry and observation and fluid and food charts which was assessed in <48 hours of patient admission by the speech therapist including presence or absence of dysphagia, initial type of diet (pureed, soft, mashed, NBM) on admission and type of fluid if they were nil by mouth. Once I finished these baseline data recording I carried out anthropometric and body composition measurements as detailed below.

3.3.4 Anthropometric, biochemistry and clinical, and body composition measurements data

3.3.4.1 Anthropometric measurements

All anthropometric measurements were repeated three times (except weight and height) both at the time of admission and on discharge. Averages of these three measurements were used for analyses. Standard Operating Procedures (SOPs) used in this study (as well as hydration study presented in Chapter 4) are presented in Appendix X.

Weight

Participant's weight was measured by the researcher if it was not measured by a nurse on admission upon study recruitment and patient consent. If the participant's weight has been already measured at the time of admission by the nursing staff prior to recruitment, it was taken as baseline weight and was recorded. If the patient was unable to get on the weighing machine due to immobility weight was measured using a hoist (Loco-motor multi-lift hoist, MEDISAVE, WYEMOUTH, UK). Weight was measured while participant was wearing light clothing (hospital gown) with barefoot. If participant was able to get up from their bed a weighting chair (SECA 955 electronic scale, MEDISAVE, WYEMOUTH, UK) was used where the participant was asked to sit on the chair upright and place their legs on designated leg rest position. Weight in kilograms was recorded to the nearest decimal point. Weight measurement was repeated at the time of discharge.

Height

Height in cm was recorded for each participant on admission. If height has not already been recorded by a nurse at the time of recruitment, the investigator carried out height measurement. The participant was asked to remove footwear and stand upright with their back facing stadiometer placed on a wall. Participants were asked to stand with heels, back of the buttock, and back the head touching the stadiometer erect board with

the arms on their side. I made sure that all three points (heels, back of the buttock, and back the head) were touching the stadiometer before moving the head piece of the meter from above until it was comfortably touching the top point of the head; height was recorded to the nearest decimal point; 0.1 cm (191).

In the case of bedridden patients (n=5), height was estimated using forearm length. To measure forearm length participant was asked to tuck their hand to the chest facing inward with arm straight. The distance between the ulna bones, olecranon process, at the elbow and the distal end of the ulna at the styloid process of the ulna was measured using a standard tape measure. Standardized charts available on the ward were used to estimate height based on forearm length for men and women respectively according to their age (under and over 65 years) (BAPEN 1985). Height measurement was not repeated on discharge as it is unlikely to change during the participant's in-patient hospital stay.

Body mass index

Body mass index was calculated using the MF-BIA machine using the formula BMI = weight/ $(\text{height})^2$ with weight measured in kilograms (kg) and height in meters (m) squared. BMI calculation was repeated for discharge using repeated weight measurement on discharge and height measurement on admission (see standard procedure for measuring weight and height above).

Mid Arm Circumference (MAC)

Mid Arm Circumference (MAC) was measured at a centre point of the upper arm mid way between acromion process of the scapula and olecranon process of the ulna using a measuring tape(192). The MAC was measured twice, at baseline and on discharge with each measurement repeated three times. Mean values of MAC at admission and discharge was calculated respectively.

Triceps Skin Fold (TSF) Thickness

A skinfold calliper (Harpenden Skinfold Calliper, Harpenden, UK) was used to measure skinfold thickness of triceps. The midpoint on the posterior aspect of the right upper arm was identified first by the investigator by defining the midpoint at the back of the participant triceps; length of upper arm measured and midpoint located. Then a skinfold was grasped avoiding including any underlying muscle. The calliper was placed at a 90-degree angle and grasping a pinch full of skin with any muscle and the measurement recorded in millimetres(192).

Waist Circumference (WC)

The highest point of the iliac crest of the hip bone was identified and then the midpoint between the highest point of the iliac crest and the lowest point of the rib cage end was identified; Waist circumference was measured around the smallest circumference between the ribs and the iliac crest. When it was not possible to find a natural waistline it was measured at the level of "the navel". The tape was wrapped horizontally around the waist to measure the waist circumference (192).

Hip Circumference

The widest point of the buttocks was located in a standing position(192). The measuring tape was placed on the widest point of the buttock and wrapped horizontally around the hip to measure the hip circumference. Waist and hip circumferences were recorded for patients who were able to stand only.

Waist to hip ratio calculations

The averages of the three waist and hip measurements were calculated. The waist to hip ratio (WHR) was calculated by dividing the average waist circumference by average hip circumference.

Handgrip strength

Handgrip strength of unaffected side was measured using a dynamometer (GRIP-D TKK 540, TAKEI PHYSICAL FITNESS, CHINA). If no arm was affected the grip strength of dominant hand was measured. The dynamometer was set at 0.0 and the patient was asked to squeeze with as much power as possible and the measurement was recorded once the dynamometer showed no further increase in measurement as the participant could no longer increase grip power. The same procedure was repeated three times.

3.3.4.2 Measurement of Body Composition

In this study, body composition measures were assessed using Multi frequency Bioelectrical Impedance Analysis (MF-BIA) equipment (Maltron BioScan 920-2, Maltron International Co. Essex, United Kingdom)). The MF-BIA measures body composition components based on the extent of resistance to a harmless electrical current as it travels through the body. The electrical current travels freely through muscle tissue and body fluids, but experiences resistance from some of the body components such as fat tissue. The amount of resistance with the specification of age, height, weight, and gender of the subject allows the calculation of body composition components using an already programmed built-in formula in the equipment; for more detailed information refer to the validation chapter where MF- MF-MF-BIA measurement was validated against Dual X-ray Absorptiometry (DEXA) in 10 subjects in the Chapter 5. The internal validity of the MF-BIA machine used in this study was also assessed and reported in the Chapter 5.

The electrodes from the MF-BIA equipment were attached to the patient using sticky patches similar to ECG patches. The investigator first placed the patches on the hands, at the wrist and on the knuckles between the middle and ring fingers, and on the feet with one patch on the talus bone and the other horizontally between the third and fourth metatarsals. The cables of the MF-MF-BIA machine were then attached to the patches with the red coloured cable (positive) being closer to the heart and the black coloured

cable (negative) farthest. Patient's characteristic demographic information including study identification number (study ID), age, gender, height, weight, and ethnicity, were all entered prior to measurement and the body composition measurements listed below were recorded. The MF-MF-BIA measurement was repeated twice, < one minute apart for internal validation purposes of MF-MF-BIA (please refer to Chapter 5 for details). The average of the two consecutive measurements was used for the analysis in this study.

From MF-MF-BIA measures data on fat free mass (Kg), fat free mass percentage, fat mass (Kg), fat mass percentage, total body water (L), total body water percentage, extra and intracellular water (L), extra to intracellular water ratio, body cell mass (Kg) and per cent, extracellular mass (Kg) and percentage, estimates of Creatinine clearance rate (ml/min) and glomerular filtration rate (ml/min), protein mass (Kg), mineral mass (Kg), mineral mass percentage, total body calcium and potassium (g), muscles mass (Kg), glycogen mass (g), dry weight (Kg), extracellular fluid (L), plasma fluid-intravascular (L), interstitial fluid-extravascular (L), body volume (L), and body density (Kg/L) were collected and recorded.

MF-BIA measurements were carried out twice at the baseline (within 48 hours of admission) and at the time of discharge (usually within 6-48 hours before discharge) as described above. In addition to baseline (at enrolment) and discharge measurements for each participant, MF-BIA measurement was repeated in participants who received a new feeding regimen within 48 hours of the commencement of the new regimen. There was no published literature on when best to measure body composition changes after a change in feeding regimen in stroke patients, and the selection of this time frame was for pragmatic reasons and based on the advice by the clinicians using consensus approach. Therefore, it was decided that 48 hour duration should be elapsed before carrying out the repeat MF-BIA measurements to allow the participant to adapt changes occurred in body composition due to the new feeding regimen. The average of the consecutive two measurements was used for the analysis in this study.

3.3.4.3 Biochemistry and Clinical Data

Other variables collected at the baseline (at the study enrolment) included routine full blood count including Haemoglobin, Leucocytes (Neutrophils, Basophils, Eosinophil, and Lymphocytes counts), Platelets, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and Erythrocytes sedimentation rate (ESR) (if available). Routine urea and electrolyte test data were also collected including Sodium, Potassium, Urea, Creatinine, and liver function test (albumin, total protein, alkaline phosphatase, alanine transaminases (serum glutamic pyruvic transaminase) and gamma glutamyl transpeptidase (GGT). Serum lipids levels were also recorded whenever available and included total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) cholesterols and triglycerides (TG). In addition, glucose (non-fasting), haemoglobin A1C (Hb_{A1C}) in those with diabetes, and C - reactive protein (CRP) (whenever available) were also collected. All blood test results were collected using the ICE-Desktop system, a software system that records patient information and clinical test reports.

Other relevant clinical data were collected from medical records at the time of enrolment to the study and described briefly below.

Stoke severity as assessed by the National Institute of Health Stroke Scale (NIHSS)

NIHSS (Appendix XI), http://www.ninds.nih.gov/doctors/NIH_Stroke_Scale.pdf, evaluates the severity of stroke using a score which ranges from 0-42 with increasing score indicating an increase in stroke severity. NIHSS evaluates the level of neurological deficit after stoke using 15 items based on neurological examination. Each item is valuated using a 3 to 5 grading with 0 being normal including the levels of consciousness, language, neglect, visual-field loss, extra ocular movement, motor strength, ataxia, dysarthria, and sensory loss.

Premorbid modified Rankin Score (pre stroke mRs) evaluates the extent of disability or dependence before the stroke. A clinician usually carries out the assessment. A number (a rank score) is given depending on clinician judgment. These rank are designated as 0 for no symptoms, 1 for no significant disability (can carry out usual activities), 2 for

slight disability (cannot carry out all usual activities), 3 for moderate disability (can walk without assistance but requires help with most activities), 4 for moderately severe disability (requires assistance including walking), 5 for sever disability (requires continuous assistance, nursing and attention), and 6 for dead. Pre-stroke mRs provides a good understanding of a patient level of mobility before stroke to understand the impact of stroke on their physical functioning. The inter observer agreement of mRs is moderate with 70% agreement (193). Pre-stroke mRs correlation with other measures was varied (spearman rho) showing a strong correlation with the frailty index 0.82 (95% CI, 0.78–0.86) but mild correlation with the Charlson comorbidity index 0.50 (95% CI, 0.40–0.59) (193).

The Malnutrition Universal Screening Tool (MUST) which evaluates nutritional status was collected from clinical notes as recorded by the dietician. MUST identifies patients at risk of malnourishment using a five-domain method, domain one is recording body mass index and giving it a score; BMI of ≥20, 18.5-20, and <18.5 are given scores of 0, 1, and 2, respectively. Unplanned weight loss in past 3-6 months is also considered in the scoring system as step two. A score of 0, 1, or 2 was given for a 5%, 5-10% or ≥10% unintentional weight loss in the past 3-6 months respectively. The third domain is to donate a score of 2 for acute disease if no nutritional intake is likely for more than 5 days due to the illness. In domain four and five scores are all added to give a total score and risk of malnutrition respectively. Zero score suggest low risk of malnutrition, one suggests medium risk of malnutrition, and score of ≥2 corresponds to high risk of malnutrition (Appendix XII).

3.3.6 Data collection at the time of hospital discharge

On participant's discharge, discharge date was recorded. Apart from repeating weight and body composition measurements as indicated earlier, I collected data on discharge destination (early support discharge services, home, or rehabilitation) and discharge status (dead or alive) upon hospital discharge.

3.3.7 Follow up data collection

Final follow up data was collected six months post discharge between August and December 2011. Follow up data consisted of subjective and objective outcomes measures described below.

3.3.7.1 Objective outcomes

I collected objective outcome data from the Patient Administrative System (PAS) and medical records of patients. Objective outcomes at six months included morbidity (recurrence of stroke, incidence of other cardiovascular events), hospital readmissions (and reason for re-admissions), and mortality during the follow up.

3.3.7.2 Subjective Outcomes

I collected subjective outcomes of self-reported functional health measured using patient reported outcome measure (PROM) using Stroke Impact Scale (SIS) (MAPI research incorporation, Lyon, France), and disability index using Barthel Index (Mahoney 1965), and Health Related Quality of Life (HRQL) questionnaire, using Short Form -36 survey version 2 (SF-36v2) (Quality Metric International Corporation, Lincoln, Rhode Island, USA).

3.3.7.1 Short Form Survey 36v2 (SF36v2)

To evaluate the quality of life I used the SF36v2 questionnaire (Appendix XIII). The SF36v2 evaluates eight dimensions of the respondent's health that reflect health related quality of life. These eight dimensions are summarised as two summary scores (physical health component and mental health component summary (PCS and MCS) scores). Each dimension assessed in the SF36v2 carries a different weight. These weights are

calculated to provide the overall PCS and MCS summary scores (194). In the next sections I will discuss the PCS and MCS components of SF-36 and describe the scoring respectively.

The Physical Health Component Summary (PCS) is the product of the total weights of four components. The four components are Physical Functioning (PF), Role Physical (RP), Bodily Pain (BP), and General Health (GH). The first component is Physical functioning (Question 3). It is assessed by the total weight aggregated from ten items evaluating the extent of physical limitations. Each item can be given a value from 1-3. One reflects maximum of physical limitation while three no physical limitations at all. Items evaluated concern ability to carry out vigorous activities, moderate activities, carry grocery items, and be able to climb stairs, walk a certain distance, and carry out personal care activities such as bathing and dressing. The next item is Role Physical (Question 4). Role Physical component weight is aggregated by four items examining the extent at which daily physical activities are being limited after the onset of stroke symptoms. Each item is given a value of 1-5 with 1 being the worst possible value and 5 being the best possible value indicating that daily physical activities are not affected. Items evaluated activities carried before stroke are being limited and to what extent, and difficulties experienced carrying previous activities after the onset of stroke. Bodily Pain (BP) is assessed as in other components but in two questions (question 7 and 8). Question 7 asks about the extent of pain experienced in the past month giving a value from 1-6 with one being no pain and six being severe pain, and question 8 asks about the interference of pain with daily activities given values 1-5 with one being no interference and 5 being all the time. The last component to provide an input into the PCS summary component is the General Health component (GH). GH weight is aggregated through values donated to question 1 and 11 (consisting of four items). In question one general health is evaluated by being given values 1-5 with one being excellent health and five being poor health. In question 11 values are given on a scale of 1-5 donated to each statement about health with one being completely false and five being completely true statement. The four items (statements) are if the patient feels they are ill more than anyone else, feel they are healthy as anybody else, expect health to get worse by time, and if they feel their health is excellent.

The Mental Component Summary (MCS) is evaluated through four different components these include Vitality (VT), Social functioning (SF), Mental Health (MH), and Role-Emotional (RE). Each component as in the PCS component carries different weight. Four items in question nine evaluate the vitality component regarding how the respondent feel. Each item is can be given a value of 1-5 with the value of 1 indicating the highest frequency and 5 indicating no frequency in experiencing the concerned feeling inquired about in the item. The four items ask about the frequency of feeling full of life, full of energy, worn out, and tired in the past four weeks. Question six and ten evaluate social functioning. There is one item per question. Both questions evaluate how emotional feeling and physical health interfere with everyday social interaction and social time. Items are can have values of 1-5 with one being highest frequency meaning all the time and 5 being lowest frequency or none of the time. Role emotional is another component that solely evaluates the influence of mental health on daily activities. The three items are given values between 1-5 with 1 being the worst and five being no interference at all. The three items evaluate how mental health affect the frequency of doing daily activities, accomplishing tasks, and interference with ability to carry out such activities. The final component is more specific to the actual state of mental health. It is evaluated in question 9. Evaluated components are given values from 1-5 with one being highest frequency and five being the least frequency of the event occurring. Items evaluated the extent of feeling nervous, down, peaceful, depressed, and happy respectively.

Calculating the final score is not a simple procedure and is a complex mathematical process. First, items number one and eleven scores must be recoded. The purpose of recoding is to allow universal scale across all items in which increased score per item means better health. In item one and 11 increased score means poorer health; as opposed to other items. Once recoded according to the scoring guide (the guide is provided by quality metric upon purchase of the SF36v2), each health domain, mental and physical health, and raw score is calculated. For MCS the raw scores for each Vitality (VT), Social functioning (SF), Mental Health (MH), and Role-Emotional (RE) items are determined. For PCS the raw scores for General Health (GH), Role Physical (RP), Physical Functioning (PF), and Bodily Pain (BP) items are determined. After the determination of each component raw scores each component raw score is converted to a 0-100 score using the following formulae

$$\frac{\text{(Actual Raw Score - Lowest possible Raw Score)}}{\text{Raw score range}} \times 100$$

The resultant scores (on a scale of 0-100) are further converted to z-scores. The conversion to z-scores is to allow an understanding of the extent of deviation of the component score from the reference group score mean. The mean score of the 1998 United States general population is used as the reference. Mathematically this is done by dividing the standard deviation of 0-100 scores of the 1998 United States general population mean by the difference between my study population component score mean and the reference (1998 US general population) mean.

$$\text{Z-score component} = \frac{\text{(component (0-100) mean score - reference mean score)}}{\text{SD of the general population}}$$

To calculate the Physical Component summary and the Mental component summary T-scores, the z-score of each item is multiplied by 10 (standard deviation of the reference group) and the sum of each multiplication for each item in the PCS and MCS are added to 50 (mean of the reference group) respectively. The reasoning for converting z-scores to t-scores is to allow the comparison between the studied group mean and standard deviation with the mean and standard deviation of the US general population or the reference group (194).

$$\text{PCS T-score} = 50 + ((GHzx10) + (RPzx10) + (BPzx10) + (PFx10))$$

$$\text{MCS T-score} = 50 + ((VTzx10) + (SFzx10) + (MHzx10) + (Rezx10))$$

The same procedure can also be used to calculate the T-score of each item alone, but instead of aggregating the scores it is required to take each item z score, then multiply it

by reference SD of 10 and add 50 (reference group mean). The use of T-scores came to provide a standard interpretation in which the T-score for an item or summary score can provide information on how different a studied group differ in their SF36v2 scores than the reference group (the 1998 US general population normative score). A T-score allows for comparing the deviation in a studied population from the norm (194).

The SF36v2 can be used to assess the quality of life post illness or condition to evaluate the efficacy of a treatment (195, 196) or even how a population is coping with certain living environment (197). The scoring of the SF36v2 using T-scores while using a comparison group in the 1998 US general population as a normal disease free population makes it a very useful tool for my study. Using the SF36v2 allows me to draw conclusions on the extent to which it has been affected after a condition that can cause substantial changes on the health related quality of life.

3.3.7.2 The Stroke Impact Scale

The stroke impact scale (Appendix XIV) includes questions which ask the respondent to evaluate how stroke have impacted on their health and life. It consists of nine questions that include several items in each. Question one through 8 ask the patient about their post stroke physical and mental status. The first question asks the respondents to evaluate the strength of the most affected side from stroke. The strength question have four components with a possible values of 1-5 with the lower score indicating that the impact was high (score of 1 denotes no strength). The next areas assessed in question two are memory and thinking capacity. There are seven items with each having a possible value from 1-5 with the lowest suggesting the greatest impact. Items assess a respondent's ability to remembering chores such as medication time or appointments, remembering past day events and things being told, problem solving, concentrating, and thinking quickly. Emotions are another domain evaluated through using nine items. Items in the emotion domain evaluate feeling of being happy, sad, nervous, and self-worth. Scoring in the emotion domain differ slightly as three of the nine items scores must be recoded (described below) while others follow the same rule having a score of 1-5 with the lowest score suggesting the highest impact. Question four evaluates

communication skills. There are seven items assessing ability to engage in conversations, communicate via telephone, listen, and understand what was being said. The daily activities question consists of ten items. The items in the question evaluate the extent and the ability of the respondent to engage in daily activities such as bathing, dressing, grooming, shopping, toilet use, handling money, carrying house chores, and eating. Each item of daily activities response can have one possible value from 1-5 that can be given with a score of one being the highest impact on daily living and five no impact. The mobility is assessed through nine items. Items in the question concerning mobility assess basics activities including ability to maintain balance while sitting and walking, getting into and out of the car, climbing stairs and walking. Question seven assesses ability to use affected hand in daily activities such as picking up money, turning door knob, tying a shoe lace, opening a jar of food. The final question evaluated social participation. It consists of eight items and evaluates the ability of the respondent in participating in activities which he/she could participate in before the stroke. The range of activities includes work, sports, family, social, and spiritual activities. All of the components can be given a score of 1-5 with the lowest score i.e. a score of one suggest the highest impact on the respondent.

Before scoring the SIS, and to make the scoring universal across all domains, three items in the emotion domain have to be recoded. As mentioned previously the scoring from 1-5 is possible for each item and with the lowest score of one suggesting highest impact, this is not the case for three items for emotion, the lowest score suggest lowest impact therefore they need to be recoded. A score of 1 is recoded to 5, 2 to 4, 3 to 3, 4 to 2, and 5 to 1, respectively. To calculate each dimension score the following formulae is used to have each dimension scored out of 100 or 100% (198).

$$(\text{Raw score} - \text{minimum score}) / (\text{Maximum} - \text{minimum score}) \times 100$$

Row scores each question are the total sum of the item scores. So for strength (a four item dimension the minimum score is 4 (four items being scored as 1) and maximum score is 20 (four items being scored as 5). For missing data and if <50% of the items score are missing then mean of the scores is used in the following formulae

(Mean score - minimum score)/ (Maximum-minimum score) x 100

If ≥50% of item scores are missing then the whole dimension scoring is considered missing (198).

The stroke impact scale (SIS) is a measure that allows to see if the impact of stroke is still apparent in stroke patients even after recovery (199). It is a very reliable score in which high degree of internal consistency (α-coefficient=0.9) was observed when testing and re-testing the same patients again (200). The SIS is a very useful tool for my study that allows me to evaluate the extent of recovery in patients with different extent of body composition changes while taking into account of the stroke severity.

3.3.7.3 Barthel Index

The Barthel Index (Appendix XV) is the most widely used measure of physical disability in carrying out activities of daily living (ADL). The Barthel Index can be used in clinical and rehabilitation settings (201) and for research purposes, (202, 203). The inter-ratter agreement was shown to be reasonable (n=94 elderly patients) (204) and good (n=25) (205). Review of previous studies suggest that the Barthel Index scores can reasonably predict physical disability level post stroke (206).

The self-reported or observer rated ten specific areas of assessment include feeding, bathing, grooming, dressing, bowel control, bladder control, self-toilet use, and transfer to bed, mobility, and stair use. For each item a score of 0, 5, or 10 was given for each activity of daily living with 0 indicating complete dependence or inability, 5 when assistance is needed or occasional accidents (in case of bladder and bowel function), and 10 refers to the independence or complete control. Barthel index scoring is straight forward and unlike SF36v2 or SIS described above. Scores are added up for each item

assessed to give a final maximum score out of 100. The higher the score the more independent a patient is in carrying out daily activities (207).

I sent the follow-up questionnaire to all study participants by post at six month after discharge. The follow up questionnaire package included a newsletter, the SF36v2, SIS questionnaires and Barthel Index. The newsletter included a brief introduction about my background and a reminder about the study and its objectives in lay language understandable to the general public. My work contact details as the contact information of the investigator was also included in the postal package in case if they wished to receive any further information. A guidance note for participants on how to complete each questionnaire was also included in the postal package. A pre-paid envelope was also included for the questionnaires to be returned to the investigator.

On receipt of the completed questionnaires, the responses were scored according to the scoring algorithm as per developer for each questionnaire. Results of each component of individual scale and the summary scores (e.g. PCS and MCS for SF-36v2) were recorded in the database. If a participant did not respond to initial mailing within two weeks, they were contacted by telephone on two occasions, two and four weeks after initial postage, to find out if any help was required and also to encourage their response, and record reasons for not responding.

Statistical analysis

Baseline data were presented descriptively. Body composition changes between admission and discharge were calculated for each participant by subtracting admission values from the discharge values to understand if an increase or a decrease in these body components had occurred during the acute hospital stay. The difference was divided by duration between admission and discharge MF-BIA measurements in days to calculate rate of change per day.

Further sensitivity analyses were conducted after excluding all participants in whom the duration between MF-BIA measurements was <48 hours. These sensitivity analyses were carried out using changes in the body components as percentages of body weight.

Descriptive statistics for fat free mass, fat mass, body cell mass, protein mass, muscle mass on admission and discharge were calculated stratified by type of feeding regimen (Normal Oral Diet, Soft mashed/Pureed diet, and Nil-by-Mouth (NBM), stroke severity by NIHSS scores of <10 vs. ≥10 (not enough data to stratify by higher NIHSS score), and type of ischaemic stroke (Total Anterior Circulation Infarct (most severe form of stroke) vs. other types).

Univariate logistic regression analysis was carried out to examine if there was an association between fat free mass loss, fat mass gain, muscle mass loss, body cell mass loss, and protein mass loss with the predictor variables Nil-by-Mouth (reference category being other types of diet; normal oral and soft mashed/pureed), modified diet(reference category being normal oral diet), total anterior circulation infarct (reference group non-TACI stroke subtypes), and more severe strokes with the an NIHSS ≥10 (reference category NIHSS <10).

Linear regression analysis was also carried out to examine if there is an association between predictors fat free mass loss, fat mass gain, muscle mass loss, body cell mass loss, and protein mass loss and outcomes length of hospital stay. Basically I was trying to examine if such body composition changes have influence on length of hospital stay. If any results were of significance multivariate logistic and linear regression analysis was carried out.

The individual component summary score of SF36v2 was calculated using the program provided by the supplier (see above section for calculation details and supplier information). Average scores for each component, PCS and MCS of the SF36v2 were all presented descriptively separately for those who gained and those who lose fat free mass, fat mass, muscle mass, body cell mass, and protein mass respectively. Mean

difference was calculated for each body composition measures and p-values are presented to examine any statistically significant differences between those who gained and lose these components of body for MCS and PCS respectively. For the Stroke Impact Scale (SIS) each dimension score was calculated. Average scores for each component of the SIS (PCS and MCS) and patient reported overall stroke recovery (question 11 of the SIS) were presented for those with fat free mass, fat mass, muscle mass, body cell mass, and protein mass gains and losses in respectively.

Mean difference of fat free mass, fat mass, protein mass were calculated for each body composition measures and p-values presented to examine any statistically significant differences between those with body composition gain and losses for each dimension of the SIS and the patient reported overall stroke recovery (question 11 of the SIS). Barthel Index scores were calculated and overall average scores were presented for those with fat free mass, fat mass, muscle mass, body cell mass, and protein mass gains and losses in respectively.

3.4 Results

A total of 40 participants were recruited to this study. Their mean age was 70.3±9.9 years (range 50-89 years); 55.0% of them were men. All study participants had ischaemic stroke. The majority of the study population experienced Lacunar infarct (42.5%). Majority of strokes according to the National Institute of Health Stroke Severity (NIHSS) scale were mild strokes with NIHSS <10 (85.7%). For fat free mass (FFM), fat mass (FM), and body cell mass (BCM) data were available in 40 patients. For protein mass (PM) and muscle mass (MM) data were available for 39 patients. Table 3.1 present the baselines characteristics.

Eighteen study participants responded to follow up questionnaire of which 10 were men and eight were women. Mean age was 69.1±9.7) years (range 50-89 years). Their average length of hospital stay was 3.2 day (range 1-8 days), and average NIHSS score was 5.9 (range 1-21). Six of these participants had Lacunar Infarct (LACI), one participant Partial Anterior Circulation Infarct (PACI), seven had Posterior Circulation Infarct (POCI), and four total anterior Circulation Infarct (TACI). One participant was prescribed nil-by-mouth (NBM) during the acute hospital stay, 16 received normal oral feeding, and one was on pureed diet. On discharge 14 were discharged to home, three to rehabilitation, and one was initially transferred to another hospital. At six month post discharge they all resided at their respective home addresses.

There were no statistically significant differences between each of the anthropometric measurements recorded on admission and discharge. Table 3.2 shows mean anthropometric measurements on admission and discharge and their differences.

	All	Men	Women
Number	40	22	18
Mean age (std) years	70.3 (9.9)	69.7 (10.6)	71.1 (9.2)
Age Range (years)	50-89	50-89	59-89
Weight (kg)	77.4 (13.9)	79.5 (14.5)	74.7 (13.1)
Height (m)	1.7 (0.1)	1.8 (0.08)	1.6 (0.06)
Body Mass Index (kg/m2)	26.8 (4.7)	25.7 (4.2)	28.2 (5.0)
Triceps Skin Fold thickness (mm)	11.2 (3.9)	10.9 (3.8)	11.5 (4.0)
Mid Arm Circumference (cm)	28.8 (4.2)	28.4 (3.5)	29.1 (5.0)
Handgrip Strength (kg)	20.1 (10.8)	24.4 (12)	15.1 (6.7)
Average length of Hospital say (range) days	4.1 (1-24)	4.8 (1-24)	3.1 (1-7)
Premorbid Rankin Score*			
0 =No symptoms	20	8	12
1 =No significant disability	14	9	5
2 = Slight disability.	2	1	1
3 = Moderate disability.	1	1	0
4 = Moderately severe disability		-	-
5 =Severe disability	-	-	-
Total Anterior Circulation Infarct	6	4	2
Left Side	4	2	2
Right Side	2	2	0
Partial Anterior Circulation Infarct	5	2	3
Left Side	2	1	1
Right Side	3	1	2
Lacunar Infarct	17	9	8
Left Side	10	3	7
Right Side	7	6	1

Table 3.1. Baselines (admission) characteristics of the study population including demographic, anthropometric, and clinical data, continued

	All	Men	Women
Posterior Circulation Infarct	12	7	2
Left Side	5	3	1
Right Side	7	4	1
NIHSS Score (n=37) categories			
1 to 9 (mild stroke)	30	15	14
10 to 20 (moderate stroke)	4	2	2
≥20 (severe stroke)	1	1	1

Table 3.1. Baselines (admission) characteristics of the study population including demographic, anthropometric, and clinical data.

Anthropometric Measure	Admission	Discharge	Mean difference (95% CI)	p-value
Weight (kg)				
All	77.4 (13.9)	77.1 (13.7)	0.29 (-0.23 to 0.81)	0.26
Men	79.5 (14.5)	79 (14.3)	0.55 (-0.41 to 1.51)	0.25
Women	74.7 (13.1)	74.7 (13.1)	-0.02 (-0.09 to 0.03)	0.33
Body Mass Index (kg/m2)				
All	26.8 (4.7)	26.6 (4.7)	0.22 (-0.1 to 0.6)	0.20
Men	25.7 (4.2)	25.3 (4.2)	0.4 (-0.3 to 1.0)	0.24
Women	28.2 (5.0)	28.1 (5.0)	0.04 (-0.01 to 0.08)	0.10
Triceps Skinfold Thickness (mm)				
All	11.2 (3.9)	11.2 (3.9)	0.01 (-0.02 to 0.03)	0.55
Men	10.9 (3.8)	10.9 (3.8)	-0.01 (-0.03 to 0.02)	0.70
Women	11.5 (4.0)	11.5 (4.0)	0.02 (-0.02 to 0.06)	0.31
Mid Arm Muscle Circumference (cm)				
All	28.8 (4.2)	28.7 (4.3)	-0.04 (0.002 to 0.07)	0.04
Men	28.4 (3.5)	28.4 (3.6)	0.03 (-0.02 to 0.1)	0.24
Women	29.1 (5.0)	29.1 (5.0)	0.04 (-0.002 to 0.08)	0.06

Table 3.2. Admission and discharge anthropometric measurements by sex-specific analysis continued

Anthropometric Measure	Admission	Discharge	Mean difference (95% CI)	p-value
Handgrip Strength (kg)				
All	20.1 (10.8)	20.4 (11.7)	0.24 (-1.4 to 1.0)	0.69
Men	24.4 (12)	24.7 (13.4)	0.4 (-2.6 to 1.9)	0.74
Women	15.1 (6.7)	15.3 (6.5)	-0.1 (-0.7 to 0.5)	0.75

Table 3.2. Admission and discharge anthropometric measurements by sex-specific analysis.

There were no statistically significant changes in any of the body composition indices during the acute hospital stay between the admission and discharge in all as well as for men and women separately. Fat free mass decreased in the whole population, with men showing an increase and women showing a decrease as a group. All population regardless of gender showed an increase in fat mass, a decrease in protein mass and body cell mass. Muscle mass increased in men but decrease in women, but the whole study population overall average change suggested muscle mass increase. Table 3.3 describes the body composition changes between admission and discharge and their average change during hospital stay for the whole recruited study population, and then men and women separately.

Body Composition	Admission	Discharge	Mean difference (95% CI)	p-value
Fat Free Mass (kg)				
All	51.6 (9.6)	51.1(9.2)	-0.5 (-1.22 to 0.23)	0.18
Men	56.6 (8.7)	55.9 (8.8)	0.7 (-0.3 to 1.6)	0.16
Women	45.6 (6.9)	45.3 (5.7)	-0.3 (-1.6 to 1.0)	0.62
Fat Mass (kg)				
All	25.7(10.2)	26 (10.3)	0.3 (-1.01-0.44)	0.43
Men	22.9 (8.3)	23.2 (8.8)	0.3 (-1.2 to 0.6)	0.49
Women	29.2 (11.3)	29.4 (11.3)	0.2 (-1.1 to 1.6)	0.68
Protein Mass (kg)				
All	7.5 (2.9)	7.0 (2.9)	-0.5 (-0.97 to 0.01)	0.06
Men	9.1 (2.6)	8.5 (2.7)	-0.6 (-1.3 to 0.23)	0.16
Women	5.4 (1.8)	5.0 (1.7)	-0.4 (-1.1 to 0.25)	0.20
Body Cell Mass (kg)				
All	28.7 (7.6)	27.7 (6.2)	-1.0 (-3.2 to 1.2)	0.36
Men	30.3 (4.9)	30.2 (6.7)	-0.1 (-1.8 to 1.7)	0.94
Women	26.8 (9.8)	24.7 (4.0)	-2.1 (-6.7 to 2.4)	0.34

Table 3.3. Body composition values on admission and discharge, continued

Body Composition	Admission	Discharge	Mean difference (95% CI)	p-value
Muscle Mass (kg)				
All	23.0 (5.7)	24.4 (13.0)	1.4 (-5.55-2.75)	0.50
Men	26.6 (4.9)	26.3 (6.1)	-0.3 (-1.8 to 1.1)	0.63
Women	18.4 (2.4)	22.1 (18.4)	3.7 (-13.5 to 6.1)	0.44

Table 3.3. Body composition values on admission and discharge and their average change during hospital stay for the whole study population and men, and women separately.

3.4.1 Extent of fat free mass and other body composition changes by type of feeding regimen

Fat free mass losses with both groups (normal oral diet and modified diet) were not statistically significant. Fat mass gain was observed in the majority of the normal oral diet group (55%) and modified diet group (64%) with higher gains in the modified diet group; $p>0.05$. Larger proportions of patients in each the normal oral diet (62%) and modified diet (82%) groups experienced protein mass loss with more pronounced losses seen in the modified diet group. On the contrary, the more pronounced losses were observed in the normal diet group with regards to body cell mass losses with higher proportion in both groups experiencing such losses. Extent of muscle mass loss was higher in the modified diet group ($p=0.05$) with the normal oral diet group experiencing muscle mass gains. Table 3.4 describes body composition changes of the study population stratified by normal oral diet and modified diet groups showing average changes between admission and discharge within groups.

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day (between MF-BIA tests)
Fat Free Mass (kg)							
Normal oral	16 (55%)	13 (45%)	52.1 (9.7)	51.6 (8.9)	-0.5 (-1.1 to 0.3)	0.23	-0.4 (1.4)
Modified diet	8 (73%)	3 (27%)	50.3 (9.7)	49.8 (10.2)	-0.4 (-2.0 to 1.2)	0.57	-0.4 (0.9)
Fat mass (kg)							
Normal oral	13 (45%)	16(55%)	26.1 (10.1)	26.3 (10.2)	0.2 (-0.7 to 1.1)	0.66	0.3 (1.6)
Modified diet	4 (36%)	7 (64%)	24.8 (10.9)	25.3 (11.1)	0.5 (-0.9 to 1.9)	0.44	0.4 (0.9)
Protein mass (kg)*							
Normal oral	18 (64%)	10 (36%)	7.5 (2.8)	7.3 (3.0)	-0.3 (-0.9 to 0.3)	0.32	-0.3 (1.2)
Modified diet	9 (82%)	2 (18%)	7.3 (3.1)	6.3 (2.7)	-1.0 (-2.0 to 0.1)	0.07	-0.5 (0.6)
Body Cell Mass (kg)							
Normal oral	17 (59%)	12 (41%)	29.3 (8.3)	28.1 (6.3)	-1.2 (-4.3 to 1.8)	0.40	-1.8 (6.4)
Modified diet	7 (64%)	4 (36%)	27.2 (5.5)	26.9 (6.2)	-0.3 (-1.5 to 1.0)	0.64	-0.1 (1.4)

Table 3.4. Body composition changes between admission and discharge for patient on normal oral diet and modified diet, continued

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day (between MF-BIA tests)
Muscle Mass (kg)*							
Normal oral	16 (55%)	12 (45%)	23.2 (5.7)	25.6 (14.7)	2.4 (-3.4 to 8.2)	0.40	0.4 (5.2)
Modified diet	8 (73%)	3 (27%)	22.7 (5.9)	21.5 (6.3)	-1.2 (-2.3 to 0.0)	0.05	-0.5 (1.1)

Table 3.4. Body composition changes between admission and discharge for patients on normal oral diet and modified type of diet. Modified type of diet includes soft mashed and pureed diets, and nil-by-mouth NBM.

Body cell mass admission (normal oral diet) not normally distributed (Shapiro-Wilk: $p<0.05$); Median=27.8 g, Interquartile range=22.7 to 33.1 g

Body cell mass discharge (modified diet) not normally distributed (Shapiro-Wilk: $p=0.05$); Median=26.1 g, Interquartile range=22.3 to 28.9 g

Muscle mass admission (normal oral diet) not normally distributed (Shapiro-Wilk: $p=0.04$) Median 23.4 g, Interquartile range = 23.9 to 31.2 g

Muscle mass discharge (normal oral diet) not normally distributed (Shapiro-Wilk: $p<0.05$) Interquartile range = 17.6 to 27.9 g

Muscle mass discharge (modified diet) Not Normally distributed (Shapiro-Wilk: $p=0.01$); Median=19.6 g, Interquartile range= 17.4 to 22.7 g

When analyses was stratified by non-NBM and NBM, majority of participants experienced fat free mass, protein mass, body cell mass, and muscle mass losses and fat mass gains. All participant in the NBM group, and majority of non-NBM experienced fat free mass losses (54%). The Extent of fat free mass losses was higher in the NBM group compared to non-NBM groups. Majority of participant in the non-NBM group (54%) and 80% of participants in the NBM group experienced fat mass gains. The extent of fat mass gain was higher in NBM compared to non-NBM. Both groups experienced protein mass loss with a proportion of 68% 80% in the non-NBM and NBM respectively; the extent of protein mass losses higher in the NBM group. Only the non-NBM group experienced body cell mass losses with 62% of the group experiencing loss. The non-NBM group experienced body cell mass losses while such losses were almost absent in the NBM group. The non-NBM group experienced muscle mass gains and the NBM group experienced loss. None of the body composition changes between admission and discharge were statistically significant within groups. Table 3.5 describes body composition changes of the study population stratified by non NBM and NBM types of feeding regimen showing p-values of change within groups.

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day (between MF-BIA tests)
Fat Free Mass (kg)							
non-NBM	19 (54%)	16 (46%)	52.1 (9.9)	51.8 (9.4)	-0.3 (-1.1 to 0.5)	0.45	-0.4 (1.4)
NBM	100% (5)	0 (0%)	48.5 (7.5)	46.6 (7.3)	-1.9 (-4.3 to 0.5)	0.09	-0.9 (1.0)
Fat mass (kg)							
non-NBM	16 (46%)	19 (54%)	26.6 (10.2)	26.7 (7.6)	0.1 (-0.6 to 0.9)	0.74	0.3 (1.5)
NBM	1 (20%)	4 (80%)	19.4 (7.8)	20.8 (8.2)	1.4 (-1.8 to 4.6)	0.29	0.8 (1.1)
Protein mass (kg)*							
non-NBM	23(68%)	11 (32%)	7.6 (2.9)	7.2 (2.9)	-0.4 (-1.0 to 0.1)	0.13	-0.3(1.1)
NBM	4 (80%)	1 (20%)	6.5 (3.0)	5.6 (2.8)	-0.9 (-2.1 to 0.4)	0.12	-0.6 (0.8)
Body Cell Mass (kg)							
non-NBM	21 (62%)	14 (38%)	29.1 (7.9)	28.0 (6.4)	-1.1 (-3.6 to 1.3)	0. 35	-1.5 (6.3)
NBM	3 (60%)	2 (40%)	26.0 (4.9)	26 (5.5)	0.01 (-2.8 to 3.0)	0.93	-0.1 (1.5)

Table 3.5. Body composition changes between admission and discharge stratified by non-NBM vs. NBM, continued

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day (between MF-BIA tests)
Muscle Mass (kg)*							
non-NBM	20 (59%)	14 (41%)	23.3 (5.8)	25.1 (13.7)	1.8 (-2.9 to 6.6)	0.44	0.3 (4.8)
NBM	4 (80%)	1 (20%)	21.5 (5.1)	20.1 (5.2)	-1.4 (-3.4 to 0.6)	0.12	0.9 (1.2)

Table 3.5. Body composition changes between admission and discharge for patients on non nil-by-mouth feeding regimen and those on nil-by-mouth (NBM) feeding regimen; non-NBM includes normal oral diet, soft-mashed, and pureed diets.

Body cell mass admission (non-NBM) not normally distributed (Shapiro-Wilk: $p<0.05$); Median=27.5 g, interquartile range=23.0 to 30.0 g

Body cell mass discharge (non-NBM) not normally distributed (Shapiro Wilk: $p=0.03$); Median=26.8 g, interquartile range=23.8 to 30.6 g

Muscle mass discharge (non-NBM) not normally distributed (Shapiro-Wilk: $p<0.05$); Median=22.1 g; Interquartile range= 17.7 to 27.8 g

3.4.2 Extent of body composition changes by type of stroke

When analyses were stratified by TACI and non-TACI subtype of stroke, majority of participants experienced fat free mass, protein mass, body cell mass, and muscle mass losses and fat mass gains. More than half of participant with TACI (67%), and non-TACI (59%) experienced fat free mass losses. The Extent of fat free mass losses was higher in the TACI group compared to non-TACI groups. The majority of patients in each group experienced fat mass gain with the extent of fat mass gains being more pronounced in the TACI group. All participants in the TACI group and 64% in the non-TACI group experienced protein mass loss, with statistically significant protein mass losses ($p=0.05$) seen in the TACI group between admission and discharge. Similarly body cell mass loss extent was higher in the TACI compared to non-TACI study participants with majority in both groups experiencing muscle mass (56% in non-TACI vs. 83% in TACI). Muscle mass loss was experienced in 83% of patients with TACI ($p=0.05$) as opposed to non-TACI patients who had muscle mass gains. Table 3.6 shows body composition changes between admission and discharge in patients with Total Anterior Circulation infarct (TACI) and those with other types of infarct. .

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day (between MF-BIA tests)
Fat Free Mass (kg)							
non-TACI	20 (59%)	14 (41%)	50.8 (9.7)	50.4 (9.3)	-0.4 (-2.1 to 0.4)	0.34	-0.4 (1.4)
TACI	4 (67%)	2 (33%)	56.2 (7.8)	55.1 (8.2)	-1.1 (-3.1 to 1.0)	0.23	-0.3 (1.0)
Fat mass (kg)							
non-TACI	15 (44%)	19 (56%)	25.7 (10.9)	25.9 (11.1)	-0.2 (-0.7 to 1.0)	0.68	0.4 (1.5)
TACI	2 (33%)	4 (67%)	25.9 (3.8)	26.9 (4.1)	1.0 (-1.2 to 3.1)	0.30	0.2 (1.0)
Protein mass (kg)							
non-TACI	21(64%)	12 (36%)	7.2 (3.9)	6.8 (3.1)	-0.3 (-0.2 to 0.9)	0.22	-0.3 (1.0)
TACI	6 (100%)	0 (0%)	9.2 (1.8)	7.9 (1.6)	-1.3 (--2.5 to 0.03)	0.05	-0.9 (1.3)
Body Cell Mass (kg)							
non-TACI	19 (56%)	15 (44%)	28.3 (7.9)	27.4 (6.5)	-0.9 (-3.5 to 1.7)	0.49	-1.4 (6.4)
TACI	5 (83%)	1 (17%)	31.2 (4.9)	29.6 (4.8)	-1.7 (-3.4 to 0.2)	0.07	-0.9 (1.0)

Table 3.6. Body composition changes between admission and discharge by type of stroke, continued

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day (between MF-BIA tests)
Muscle Mass (kg)							
non-TACI	19 (58%)	14 (42%)	22.4 (5.5)	24.4 (14.0)	2.0 (-2.9 to 6.9)	0.41	0.4 (4.8)
TACI	5 (83%)	1 (17%)	26.8 (5.5)	24.8 (5.0)	-2.0 (-4.1 to 0.04)	0.05	-1.3 (1.7)

Table 3.6. Body composition changes between admission and discharge for patients with Total Anterior Circulation Infarct (TACI) and those with non-TACI stroke subtype.

Body cell mass admission (non-TACI) not normally distributed (Shapiro-Wilk: $p<0.05$); Median=26.7 g, interquartile range=22.5 to 30.3 g

Body cell mass discharge (non-TACI) not normally distributed (Shapiro Wilk: $p=0.01$); Median=26.1 g, interquartile range=22.5 to 30.4 g

Muscle mass discharge (non-TACI) not normally distributed (Shapiro-Wilk $p<0.05$); Median=20.0 g; Interquartile range= 17.7 to 26.7 g

3.4.3 Extent of body composition changes by stroke severity

When analyses was stratified by stroke severity mild (NIHSS 1-9) and severe (NIHSS≥10) strokes, both patients experiencing mild and severe stroke experienced fat free mass losses with sever strokes extent of losses being higher. Half the individuals in each group experienced fat free mass losses. Similarly, both mild and sever strokes experienced fat mass gains with the extent of fat mass gain being twice as much in severe stroke compared to mild strokes. Fifty eight percentage of mild and 67% of severe strokes experienced fat mass gains. Extent of protein mass losses were higher in severe strokes with similar proportion of participant experiencing protein mass losses in both groups. Only body cell mass losses were experienced in the mild strokes as opposed to sever strokes that experienced gains (see discussion). No muscle mass losses were observed in either group. None of any of the body composition changes was statistically significant. Table 3.7 describes body composition changes between admission and discharge in patients with mild strokes (NIHSS≤9) and severe stroke (NIHSS≥10).

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day
Fat Free Mass (kg)							
NIHSS score <10	17(57%)	13 (43%)	51.2 (10.1)	50.7 (9.6)	-0.4 (-1.2 to 0.4)	0.32	-0.5 (1.4)
NIHSS score ≥10	3 (60%)	2 (40%)	55.7 (10.5)	53.8 (10.6)	-2.0 (-5.7 to 1.8)	0.22	-0.4 (1.4)
Fat mass (kg)							
NIHSS score <10	12 (40%)	18 (60%)	27.2 (10.8)	27.6 (11.0)	0.3 (-0.6 to 1.2)	0.45	0.4 (1.6)
NIHSS score ≥10	3 (60%)	2 (40%)	25.9 (3.1)	26.5 (2.8)	0.6 (-3.0 to 4.2)	0.65	0.2 (1.5)
Protein mass (kg)							
NIHSS score <10	20 (69%)	9 (31%)	7.0 (3.0)	6.8 (3.1)	-0.2 (-0.7 to 0.3)	0.40	-0.3 (1.0)
NIHSS score ≥10	4(80%)	1 (20%)	8.8 (2.1)	7.7 (1.8)	-1.1 (-2.8 to -0.5)	0.13	-0.8 (1.3)
Body Cell Mass (kg)							
NIHSS score <10	18 (60%)	12 (40%)	28.9 (8.4)	27.2 (5.6)	-1.7 (-4.4 to 1.0)	0.20	-1.8 (6.7)
NIHSS score ≥10	3 (60%)	2 (40%)	30.5 (5.9)	33.9 (9.4)	3.4 (-5.3 to 12.1)	0.34	0.6 (1.7)

Table 3.7. Body composition changes between admission and discharge by stroke severity continued

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day
Muscle Mass (kg)							
NIHSS score <10	17 (59%)	12 (41%)	22.5 (5.9)	24.6 (14.5)	2.1 (-3.5 to 7.6)	0.46	0.4 (5.1)
NIHSS score ≥10	2 (40%)	3 (60%)	26.0 (6.3)	26.6 (9.3)	0.6 (-8.4 to 7.3)	0.85	0.9 (2.3)

Table 3.7. Body composition changes between admission and discharge stratified by stroke severity by NIHSS score for patients with an NIHSS⩽9 and NIHSS⩾10.

Body cell mass admission (NIHSS<10) not normally distributed (Shapiro-Wilk: p<0.05); Median=26.8 g, interquartile range=22.5 to 31.5 g

Muscle mass admission (NIHSS<10) not normally distributed (Shapiro Wilk: p=0.03); Median=20.1 g, interquartile range=17.7 to 27.7 g

Muscle mass discharge (NIHSS<10) not normally distributed (Shapiro-Wilk: p<0.05); Median=20.0 g; Interquartile range= 17.1 to 27.6 g

3.4.4 Difference in fat free mass and body composition changes between groups

No statistically significant differences were observed between groups. Fat free mass losses were higher in NBM, TACI, and NIHSS ≥10 from non-NBM, non-TACI, and NIHSS 1-9 as shown in the positive mean difference observed in table 3.8 (NBM, TACI, and NIHSS ≥10 were subtracted from the average mean of non-NBM, non-TACI, NIHSS 1-9) respectively. Fat mass gains were higher in the modified diet, NBM, TACI, and NIHSS ≥10 compared to their corresponding normal oral diet, non-NBM, non-TACI, and NIHSS 1-9 respectively; negative mean difference. Protein mass losses were higher in the modified diet, NBM, TACI, and NIHSS ≥10 compared to their corresponding respective groups of normal oral diet, non-NBM, non-TACI, and NIHSS 1-9. There were no consistent finding in gains and losses of body cell mass and muscle mass for the modified diet, NBM, TACI, and NIHSS ≥10 compared to their respective normal oral diet, non-NBM, non-TACI, and NIHSS 1-9 as can be seen from tables 3.5 to 3.7. Table 3.8 present the mean difference and their 95% Confidence interval between fat free mass, fat mass, protein mass, muscle mass, and body cell mass for normal oral diet vs. modified diet groups, non-NBM vs. NBM groups, non-TACI vs. TACI stroke classification, and NIHSS 1-9 vs. NIHSS≥9 scores.

	Mean difference	95% CI	p-value
Fat Free Mass (kg)			
Normal Oral Diet vs. Modified diet	0.1	-1.7 to 1.6	0.91
non-nil by mouth vs. NBM	1.6	-0.54 to 4.0	0.14
non-TACI vs. TACI	0.7	-1.4 to 2.8	0.50
NIHSS 1-9 vs. NIHSS≥10	1.5	-0.72 to 3.8	0.18
Fat Mass (kg)			
Normal Oral Diet vs. Modified diet	-0.3	-2.0 to 1.3	0.70
non-nil by mouth vs. NBM	-1.3	-3.5 to 0.92	0.25
non-TACI vs. TACI	-0.8	-2.9 to 1.3	0.44
NIHSS 1-9 vs. NIHSS≥10	-0.3	-2.7 to 2.1	0.8
Protein Mass (kg)			
Normal Oral Diet vs. Modified diet	0.7	-0.39 to 1.8	0.20
non-nil by mouth vs. NBM	0.4	-1.1 to 1.9	0.56
non-TACI vs. TACI	0.9	-0.44 to 2.3	0.18
NIHSS 1-9 vs. NIHSS≥10	0.9	-0.35 to 2.2	0.15
Muscle Mass (kg)			
Normal Oral Diet vs. Modified diet	3.6	-5.7 to 12.8	0.44
non-nil by mouth vs. NBM	3.2	-9.3 to 15.8	0.61
non-TACI vs. TACI	4.1	-7.5 to 15.7	0.48
NIHSS 1-9 vs. NIHSS≥10	1.5	-12.2 to 15.2	0.82
Body Cell Mass (kg)			
Normal Oral Diet vs. Modified diet	-1.0	-5.9 to 4.0	0.69
non-nil by mouth vs. NBM	-1.2	-7.9 to 5.4	0.71
non-TACI vs. TACI	0.8	-5.4 to 7.0	0.80
NIHSS 1-9 vs. NIHSS≥10	3.4	-12.1 to 1.9	0.15

Table 3.8. The mean difference and their 95% Confidence intervals between fat free mass, fat mass, protein mass, muscle mass, and body cell mass for normal oral diet vs. modified diet groups, non-NBM vs. NBM groups, non-TACI vs. TACI stroke classification, and NIHSS 1-9 vs. NIHSS≥10 scores.

3.4.5 The effect of type of feeding regimen, type of stroke, and stroke severity on extent of body composition changes after stroke

Univariate logistic regression analysis between NBM (reference group non-NBM), TACI (reference non-TACI) or stroke severity (NIHSS≥10) (reference NIHSS <10) did not show any statistically significant increased or decreased risk on fat free mass loss, protein mass loss, muscle mass loss, body cell mass loss and fat mass gain. Table 3.9 presents the results of the Univariate logistic regression analysis for the risk of NBM, TACI, and NIHSS≥10 risk on fat free mass loss, protein mass loss, muscle mass loss, and fat mass gain in NBM, TACI, and NIHSS≥10 (stroke severity) patients.

	OR	95% CI	p-value
Nil-by Mouth			
Fat Free Mass loss	NA*	NA	≥0.1
Fat Mass gain	3.4	0.34-33.3	0.30
Protein Mass loss	1.9	0.19-19.2	0.60
Muscle Mass loss	2.8	0.28-27.8	0.38
Body Cell Mass loss	1	0.15-6.7	1
Modified diet			
Fat Free Mass loss	2.2	0.5 to 9.9	0.32
Fat Mass gain	1.4	0.3 to 5.9	0.63
Protein Mass loss	2.5	0.5 to 13.9	0.30
Muscle Mass loss	2.0	0.5 to 9.2	0.37
Body Cell Mass loss	1.2	0.3 to 5.2	0.77

Table 3.9. Unadjusted Risk of body composition changes, continued

	OR	95% CI	p-value
TACI			
Fat Free Mass loss	1.4	0.2 to 8.7	0.72
Fat Mass gain	1.6	0.2 to 9.8	0.60
Protein Mass loss	<0.923	NA	0.99
Muscle Mass loss	3.7	0.4 to 35.1	0.26
Body Cell Mass loss	4	0.4 to 37.5	0.23
NIHSS≥10			
Fat Free Mass loss	1.63	0.3 to 10.3	0.61
Fat Mass gain	0.71	0.1 to 4.1	0.70
Protein Mass loss	0.8	0.1 to 5.3	0.82
Muscle Mass loss	0.65	0.1 to 3.8	0.63
Body Cell Mass loss	0.31	0.1 to 2.0	0.21

Table 3.9. Unadjusted Risk of fat free mass, protein mass, muscle mass, and body cell mass loss and fat mass gain in patients who have a NBM feeding regimen, or modified diet, or total anterior circulation infarct or sever stroke NIHSS≥10. *OR=1.9E9

3.4.5.1 Results of sensitivity analysis

In the sensitivity analysis difference in the selected body composition changes between admission and discharge were not statistically significant within group or between groups. Tables 3.10 to 3.15 present sensitivity analysis for the difference between admission and discharge body composition changes stratified by men and women (Table 3.10) modified diet and normal oral diet (Table 3.11), non-NBM and NBM feeding regimen (Table 3.12), type of stroke being non-TACI and TACI (Table 3.13), stroke severity by NIHSS<10 and NIHSS ≥10 (Table 3.14), mean differences between body composition variables in all of the examined (groups presented in Tables 3.10 to 3.14) are presented in Table 3.15.

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day
Fat Free Mass (%)							
Men	6 (55%)	5 (45%)	72.3 (7.0)	72.3 (6.5)	0.0 (-1.9 to 1.8)	0.97	1.4 (0.9)
Women	2 (33%)	4 (67%)	57.5 (7.2)	58.3 (8.0)	0.8 (-5.4 to 6.9)	0.76	<0.01 (2.3)
Fat mass (%)							
Men	4 (36%)	7 (64%)	27.7 (7.0)	28.4 (6.9)	0.7 (-1.2 to 2.6)	0.44	0.2 (0.9)
Women	4 (67%)	2 (33%)	42.5 (7.2)	41.6 (8.3)	-0.9 (-7.4 to 5.5)	0.73	0.1 (2.4)
Protein mass (kg)							
Men	5 (45%)	6 (55%)	10.4 (3.1)	9.7 (2.4)	-0.7 (-2.7 to 1.2)	0.42	0.1 (0.7)
Women	4 (67%)	2 (33%)	6.6 (2.6)	6.7 (2.3)	0.1 (-2.1 to 2.2)	0.92	0.1 (0.8)
Body Cell Mass (%)							
Men	6 (55%)	5 (45%)	38.9 (3.5)	39.8 (7.4)	0.9 (-4.2 to 6.0)	0.88	0.1 (1.4)
Women	2 (40%)	3 (60%)	31.1 (4.0)	32.2 (6.4)	1.1 (-1.9 to 4.2)	0.36	0.5 (1..1)

Table 3.10. Sensitivity stratified analyses of selected body composition by sex, continued

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day
Muscle Mass (%)							
Men	6 (55%)	5 (45%)	33.8 (3.4)	34.1 (5.5)	0.3 (-3.9 to 4.6)	0.70	0.1 (1.3)
Women	3 (60%)	2 (40%)	19.0 (2.1)	18.2 (1.1)	-0.8 (-2.7 to 1.3)	0.36	0.5 (1.2)

Table 3.10. Stratified analyses of selected body composition mean daily changes by percentages of body weight for men and women whom test dates on admission and discharge were $\geqslant$48 hours.

Fat free mass admission (men) not normally distributed (Shapiro-Wilk: p=0.013), Median=71.9%, Interquartile range=67.4 to 72.8%

Fat free mass discharge (men) not normally distributed (Shapiro-Wilk: p=0.03), Median=70.9%, Interquartile range=68.2 to 72.7%

Fat mass admission (men) not normally distributed (Shapiro-Wilk: p=0.02), Median=28.2%, Interquartile range=27.2 to 32.5%

Fat mass admission (men) not normally distributed (Shapiro-Wilk: p=0.04), Median= 29.9%, Interquartile range=27.5 to 32.8%

Muscle mass discharge (men) not normally distributed (Shapiro-Wilk: p<0.0001), Median=32.8%, Interquartile range=31.0 to 34.8%

Body cell mass discharge (men) not normally distributed (Shapiro-Wilk: p<0.0001), Median=37.5%, Interquartile range=35.5 to 39.8%

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day
Fat Free Mass (%)							
Normal oral	5 (56%)	4 (44%)	65.4 (8.1)	65.9 (7.3)	0.5 (-2.7 to 3.8)	0.74	0.2 (1.4)
Modified diet	5 (63%)	3 (37%)	69.0 (12.0)	69.0 (12.2)	0.02 (-3.2 to 3.2)	0.99	0.4 (1.6)
Fat mass (%)							
Normal oral	5 (56%)	4 (44%)	34.7 (8.1)	33.9 (7.4)	- 0.8 (-4.2 to 2.8)	0.63	0.3 (1.5)
Modified diet	3 (37%)	5 (63%)	30.9 (12.0)	32.1 (12.2)	1.1 (-2.0 to 4.2)	0.43	0.6 (1.5)
Protein mass (%)							
Normal oral	4 (44%)	5 (56%)	9.1 (3.1)	9.3 (2.8)	0.2 (-1.35 to 1.8)	0.74	0.1 (0.8)
Modified diet	6 (75%)	2 (25%)	9.2 (4.0)	8.0 (2.5)	-1.2 (-3.7 to 1.3)	0.29	0.2 (0.5)
Body Cell Mass (%)							
Normal oral	4 (44%)	5 (56%)	36.0 (5.1)	37.6 (9.9)	1.6 (-5.6 to 8.8)	0.61	01 (1.7)
Modified diet	5 (63%)	3 (37%)	37.0 (5.4)	37.3 (5.6)	0.3 (-2.2 to 2.9)	0.77	0.4 (1.0)

Table 3.11. Sensitivity stratified analyses of selected body composition by normal oral vs. modified diet

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day
Muscle Mass (%)							
Normal oral	5 (63%)	3 (37%)	30.6 (6.3)	31.7 (8.9)	1.1 (-4.6 to 6.8)	0.66	0.1 (1.7)
Modified diet	6 (75%)	2 (25%)	30.8 (6.0)	29.5 (5.2)	-1.3 (-4.2 to 1.5)	0.31	0.3 (1.0)

Table 3.11. Sensitivity stratified analyses of selected body composition mean daily changes by percentages of body weight for normal oral diet and modified diet for patients and whom duration between admission and discharge MF-BIA test was ⩾48 hours

Muscle mass (normal oral diet) not normally distributed (Shapiro-Wilk: p=0.001), Median=32.1%, Interquartile range=26.4 to 33.6 %

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day
Fat Free Mass (%)							
non-NBM	6 (46%)	7 (54%)	65.2 (9.9)	65.9 (10.0)	0.7 (-1.5 to 3.0)	0.49	0.2 (1.2)
NBM	4 (100%)	0 (0%)	73.1 (8.6)	71.8 (8.3)	-1.3 (-9.0 to 6.5)	0.63	1.1 (1.9)
Fat mass (%)							
non-NBM	8 (62%)	5 (38%)	34.9 (9.9)	34.0 (10.0)	-0.9 (-3.2 to 1.5)	0.43	0.3 (1.3)
NBM	1 (25%)	3 (75%)	26.7 (8.5)	30.0 (9.1)	3.3 (-2.6 to 9.3)	0.17	1.5 (1.6)
Protein mass (%)							
non-NBM	7 (54%)	6 (46%)	9.3 (3.6)	8.9 (2.6)	-0.4 (-2.2 to 1.3)	0.61	<0.1 (0.7)
NBM	3 (75%)	1 (25%)	8.6 (3.3)	8.1 (3.3)	-0.5 (-2.9 to 1.9)	0.54	0.4 (1.4)
Body Cell Mass (%)							
non-NBM	7 (54%)	6 (46%)	35.4 (5.3)	37.3 (8.8)	1.9 (-3.2 to 7.1)	0.42	0.5 (2.2)
NBM	2 (50%)	2 (50%)	38.8 (3.8)	41.0 (4.4)	2.2 (-1.4 to 5.7)	0.15	0.9 (1.2)

Table 3.12. Sensitivity stratified analyses of selected body composition by non-NBM vs. NBM, continued

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day
Muscle Mass (%)							
non-NBM	7 (54%)	6 (46%)	29.7 (6.7)	38.7 (31.4)	9.0 (-10.8 to 28.9)	0.34	3.0 (10.9)
NBM	3 (75%)	1 (25%)	32.1 (2.5)	31.5 (5.0)	-0.6 (-5.4 to 4.3)	0.74	0.5 (1.4)

Table 3.12. Sensitivity stratified analyses of selected body composition mean daily changes by percentages of body weight for nil-by-mouth (NBM) and non-NBM diet for patients and whom duration between admission and discharge MF-BIA test was ⩾48 hours

Fat mass discharge (NBM) not normally distributed (Shapiro-Wilk: p=0.01), Median=30.6%, Interquartile range=22.0 to 31.4%

Body cell mass discharge (non-NBM) not normally distributed (Shapiro-Wilk: p=0.01), Median=35.9 %, Interquartile range=31.1 to 38.0 %

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change kg/day
Fat Free Mass (%)							
non-TACI	8 (57%)	6 (43%)	66.7 (11.0)	67.1 (10.7)	0.4 (-1.9 to 2.7)	0.72	0.1 (1.6)
TACI	2 (67%)	1 (33%)	68.7 (2.8)	68.3 (2.9)	-0.4 (-10.7 to 9.9)	0.87	<0.1 (0.6)
Fat mass (%)							
non-TACI	8 (57%)	6 (43%)	33.3 (11.0)	32.9 (10.7)	-0.4 (-2.8 to 2.0)	0.72	0.1 (1.7)
TACI	1 (33%)	2 (67%)	31.2 (3.0)	33.7 (1.7)	2.5 (-6.6 to 11.7)	0.35	0.1 (0.9)
Protein mass (%)							
non-TACI	7 (50%)	7 (50%)	8.6 (3.6)	8.2 (2.8)	-0.4 (-2.0 to 1.2)	0.62	<0.1 (0.8)
TACI	3 (100%)	0 (0%)	11.4 (0.6)	10.7 (1.2)	-0.7 (-4.0 to 2.6)	0.46	0.1 (0.2)
Body Cell Mass (%)							
non-TACI	7 (50%)	7 (50%)	36.0 (5.6)	37.4 (8.6)	2.8 (-1.9 to 7.3)	0.45	0.8 (2.1)
TACI	2 (67%)	1 (33%)	38.7 (1.3)	37.5 (2.8)	-1.1 (-10.7 to 8.4)	0.66	0.1 (0.5)

Table 3.13. Sensitivity stratified analyses of selected body composition by type of stroke, continued

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change kg/day
Muscle Mass (%)							
non-TACI	7 (54%)	6 (46%)	29.9 (6.3)	30.0 (7.8)	0.1 (-3.3 to 3.6)	0.93	0.1 (1.3)
TACI	2 (67%)	1 (33%)	34.1 (1.2)	32.9 (2.6)	-1.2 (-9.8 to 7.5)	0.62	0.1 (0.4)

Table 3.13. Stratified analyses of selected body composition mean daily changes by percentages of body weight for patients with TACI vs. non-TACI stroke sub classification and whom duration between admission and discharge MF-BIA test was ≥48 hours.

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day
Fat Free Mass (%)							
NIHSS score <10	5 (50%)	5 (50%)	64.7 (10.9)	65.1 (10.1)	0.4 (-2.8 to 3.6)	0.79	0.1 (1.5)
NIHSS score ≥10	3 (75%)	1 (25%)	66.8 (6.6)	66.1 (6.9)	-0.7 (-8.1 to 6.7)	0.78	0.7 (2.0)
Fat mass (%)							
NIHSS score <10	5 (50%)	5 (50%)	35.4 (10.8)	34.8 (10.2)	-0.6 (-4.0 to 2.7)	0.69	0.2 (1.6)
NIHSS score ≥10	2 (50%)	2 (50%)	33.0 (6.7)	35.7 (5.8)	2.7 (-3.5 to 8.9)	0.26	1.0 (1.9)
Protein mass (%)							
NIHSS score <10	5 (50%)	5 (50%)	7.7 (3.2)	8.2 (3.3)	0.5 (-0.7 to 1.7)	0.38	0.3 (0.6)
NIHSS score ≥10	3 (75%)	1 (25%)	10.2 (1.1)	9.8 (1.9)	-0.4 (-3.0 to 2.1)	0.61	0.3 (0.7)
Body Cell Mass (%)							
NIHSS score <10	5 (50%)	5 (50%)	34.9 (5.6)	36.3 (7.2)	1.3 (-3.3 to 5.9)	0.53	0.6 (2.1)
NIHSS score ≥10	1 (25%)	3 (75%)	36.4 (3.3)	43.9 (11.7)	7.5 (-11.4 to 18.2)	0.22	1.8 (1.7)

Table 3.14. Sensitivity stratified analyses of selected body composition by stroke severity, continued

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day
Muscle Mass (%)							
NIHSS score <10	6 (67%)	3 (33%)	29.1 (6.3)	28.7 (6.0)	0.4 (-2.0 to 1.2)	0.61	<0.1 (0.6)
NIHSS score ≥10	2 (50%)	2 (50%)	30.4 (4.0)	33.8 (11.5)	3.4 (-11.4 to 18.2)	0.52	0.2 (2.2)

Table 3.14. Stratified analyses of selected body composition mean daily changes by percentages of body weight for patients with National Institute of Health Stroke Severity Score (NIHSS) of NIHSS<10 vs. NIHSS≥10 and whom duration between admission and discharge MF-

	Mean difference	95% CI	p-value
Fat Free Mass (%)			
Men vs. Women	-0.8	-5.2 to 3.6	0.71
Normal Oral Diet vs. Modified diet	0.5	-4.0 to 4.7	0.80
non-nil by mouth vs. NBM	2.1	-2.8 to 6.9	0.38
non-TACI vs. TACI	0.8	-4.7 to 6.3	0.75
NIHSS<10 vs. NIHSS≥10	1.1	-4.7 to 6.9	0.69
Fat Mass (%)			
Men vs. Women	1.6	-3.0 to 6.2	0.46
Normal Oral Diet vs. Modified diet	-1.9	-2.5 to 6.2	0.37
non-nil by mouth vs. NBM	-4.2	-0.5 to 8.9	0.08
non-TACI vs. TACI	-3.0	-2.6 to 8.6	0.28
NIHSS<10 vs. NIHSS≥10	-3.3	-2.4 to 9.1	0.23
Protein Mass (%)			
Men vs. Women	-0.8	-3.7 to 2.0	0.55
Normal Oral Diet vs. Modified diet	1.5	-1.2 to 4.1	0.26
non-nil by mouth vs. NBM	0.1	-3.2 to 3.3	0.96
non-TACI vs. TACI	0.3	-3.3 to 3.9	0.85
N NIHSS<10 vs. NIHSS≥10	0.9	-1.1 to 3.0	0.36
Muscle Mass (%)			
Men vs. Women	1.3	-5.0 to 7.7	0.67
Normal Oral Diet vs. Modified diet	2.4	-3.3 to 8.2	0.38
non-nil by mouth vs. NBM	0.6	-6.2 to 7.4	0.86
non-TACI vs. TACI	1.3	-6.2 to 8.9	0.71
NIHSS<10 vs. NIHSS≥10	-3.8	-10.6 to 3.1	0.48

Table 3.15. Sensitivity analysis mean difference, continued

	Mean difference	95% CI	p-value
Body Cell Mass (%)			
Men vs. Women	-0.2	-7.8 to 7.3	0.95
Normal Oral Diet vs. Modified diet	3.2	-4.6 to 11.0	0.40
non-nil by mouth vs. NBM	-1.6	-9.6 to 6.1	0.67
non-TACI vs. TACI	2.6	-6.2 to 11.4	0.54
NIHSS<10 vs. NIHSS≥10	-8.2	-15.8 to 3.4	0.20

Table 3.15. Presents the mean difference and their 95% Confidence intervals between selected body composition changes (as percentages of body weight) for men vs. women, normal oral vs. modified diet, non-NBM vs. NBM, non-TACI vs. TACI stroke classification, and NIHSS <10 vs. NIHSS≥10 scores groups, and whom duration between admission and discharge MF-BIA test was ≥48 hours

3.4.5 Objective outcomes

Of the 40 participants 29 were discharged to home, seven discharged to rehabilitation, two died during acute stay, and two transferred to another hospital (at city of usual residence).

Statistically significant changes between admission and discharge were observed for patients with fat free mass loses discharged to home, but no statistically significant changes in fat free mass loss occurred among patient discharged to rehabilitation or died (n=2) during hospital stay (referred to as poor outcomes). Table 3.16 presents mean fat free mass, protein mass, muscle mass, and body cell mass losses and fat mass gains on admission and discharge and their mean change between admission and discharge per group.

As opposed to patients discharged to home, muscle mass loss was statistically significant among patients with poor outcomes. Table 3.17 shows average differences in fat free mass, protein mass, muscle mass, and body cell mass losses and fat mass gains between participants discharged to home and those discharge to rehabilitation or dead at discharge.

The result of the linear regression analysis examining the relationship between fat free mass, protein mass, muscle mass, and body cell mass losses and fat mass gain impact on length of hospital stay found no statistically significant relationships. Table 3.18 presents the results of the linear regression analysis for the impact of fat free mass, protein mass, muscle mass, and body cell mass losses and fat mass gains on length of hospital stay.

Sensitivity analysis by discharge destination after excluding all participant where the duration between admission and discharge MF-BIA test was <48 hour stratified analysis for body composition change on admission and discharge for patients discharged to home and those discharged to rehabilitation or died during acute hospital stay are presented in Table 3.19. There were no statistically significant differences.

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	average change (95% CI) kg	p-value	Change rate kg/day
Fat Free Mass (kg)							
Discharge to Home	17 (58.6%)	12 (41.4%)	53.6 (9.8)	52.9 (9.4)	-0.7 (-1.4 to 0.0)	0.05	0.3 (0.7)
Discharge to Rehabilitation or death	2 (22.2%)	7 (77.8%)	46.2 (7.0)	45.1 (6.6)	-1.1 (-2.5 to 0.2)	0.1	0.3 (0.5)
Fat mass (kg)							
Discharge to Home	12 (41.4%)	17 (58.6%)	25.9 (9.1)	26.3 (9.4)	0.4 (-0.3 to 1.1)	0.2	0.2 (0.8)
Discharge to Rehabilitation or death	3 (33.3%)	6 (66.7%)	26.9 (13.3)	27.8 (13.1)	0.8 (-0.8 to 2.4)	0.3	0.3 (0.5)
Protein mass (kg)							
Discharge to Home	19 (65.5%)	9 (35.5%)	8.1 (2.6)	7.8 (2.8)	-0.4 (-0.9 to 0.2)	0.2	0.2 (0.6)
Discharge to Rehabilitation or death	7 (77.8%)	2 (22.2%)	5.4 (2.5)	4.8 (2.2)	-0.6 (-1.1 to 0.1)	0.08	0.2 (0.2)
Body Cell Mass (kg)							
Discharge to Home	17 (58.6%)	12 (41.4%)	30.1 (8.1)	28.5 (6.5)	-1.8 (-4.5 to 1.2)	0.3	1.4 (6.4)
Discharge to Rehabilitation or death	6 (66.7%)	3 (33.3%)	25.1 (4.2)	24.8 (4.9)	-0.3 (-1.7 to 1.1)	0.7	0.1 (0.5)

Table 3.16. Selected body composition changes by discharge destination, continued

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	average change (95% CI) kg	p-value	Change rate kg/day
Muscle Mass (kg)							
Discharge to Home	16 (55.2%)	12 (44.8%)	24.2 (5.7)	23.9 (6.6)	-0.3 (-1.5 to 0.8)	0.6	0.3 (1.0)
Discharge to Rehabilitation or death	7 (77.8%)	2 (22.2%)	19.8 (4.4)	18.7 (4.4)	-1.0 (-2.1 to 0.0)	0.05	0.3 (0.4)

Table 3.16. Presents difference in body composition changes between admission and discharge for patients by the outcome categories of discharged to home and patients discharge to rehabilitation or were dead on discharge. *n=38 two patients were excluded for this outcome as they were transferred to another hospital (at area of residence) making it not possible to carry out a discharge MF-BIA measurement. n=37 for MM and PM measurements as equipment failed to record MM and PM at discharge for one patient discharged to home.

Body cell mass admission (home discharge) not normally distributed (Shapiro-Wilk: $p<0.0001$), Median= 27.9 g, Interquartile range=24.7 to 33.3 g

Body cell mass (home discharge) not normally distributed (Shapiro-Wilk: $p=0.02$), Median=27.0 g, Interquartile range=23.9 to 31.2 g

	mean difference	95% Confidence Intervals	p-value
Fat Free Mass (kg)	0.4	-1.0 to 1.8	0.6
Fat Mass (kg)	-0.4	-1.9 to 1.1	0.6
Protein Mass (kg)	0.2	-0.9 to 1.2	0.7
Muscle Mass (kg)	0.7	-1.4 to 2.8	0.5
Body Cell Mass (kg)	-1.4	-6.5 to 3.8	0.6

Table 3.17. Mean differences in fat free mass, protein mass, muscle mass, and body cell mass losses and fat mass gains between participants discharged to home and those discharge to rehabilitation or dead at discharge.

	Length of Hospital Stay		
	OR	95% Confidence Interval	p-value
Fat Free Mass loss (kg)	-0.36	-19.3 to 7.9	0.87
Fat Mass Gain (kg)	-0.27	-0.37 to 0.081	0.17
Protein mass loss (kg)	0.34	-0.26 to 0.37	0.74
Muscle mass loss (kg)	0.076	-0.11 to 0.15	0.72
Body Cell Mass loss (kg)	0.024	-0.033 to 0.0.65	0.51

Table 3.18. Linear regression analysis results for the impact of fat free mass, protein mass, muscle mass, and body cell mass losses and fat mass gains on length of hospital stay.

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day
Fat Free Mass (%)							
Discharge to Home	5 (63%)	3 (37%)	68.2 (5.4)	67.8 (4.8)	-0.4 (-2.6 to 1.9)	0.72	0.4 (2.7)
Discharge to Rehabilitation or death	3 (60%)	2 (40%)	64.3 (12.8)	63.6 (12.1)	-0.7 (-4.1 to 2.6)	0.61	0.6 (1.5)
Fat mass (%)							
Discharge to Home	3 (37%)	5 (63%)	31.9 (5.4)	32.1 (4.7)	0.3 (-2.0 to 2.4)	0.81	0.2 (2.6)
Discharge to Rehabilitation or death	3 (43%)	4 (57%)	35.6 (12.8)	37.5 (11.6)	1.9 (-1.2 to 5.0)	0.18	0.9 (1.4)
Protein mass (%)							
Discharge to Home	4 (50%)	4 (50%)	10.4 (1.4)	10.3 (1.4)	-0.1 (-1.6 to 1.4)	0.87	0.1 (1.8)
Discharge to Rehabilitation or death	4 (57%)	3 (43%)	7.0 (3.2)	6.7 (3.0)	-0.3 (-1.3 to 0.7)	0.48	0.2 (0.5)
Body Cell Mass (%)							
Discharge to Home	5 (63%)	3 (37%)	37.1 (3.4)	38.8 (8.8)	1.6 (-5.6 to 6.8)	0.62	1.6 (8.6)
Discharge to Rehabilitation or death	2 (29%)	5 (71%)	34.5 (6.6)	35.7 (7.3)	-1.2 (-0.7 to 3.1)	0.17	0.5 (1.0)

Table 3.19. Sensitivity stratified analyses of selected body composition changes by discharge destination

	Participants with body composition loss (%)	Participants with body composition gain (%)	Admission (kg)	Discharge (kg)	mean difference (95% CI) kg	p-value	Change rate kg/day
Muscle Mass (%)							
Discharge to Home	5 (63%)	3 (37%)	32.2 (3.8)	33.3 (7.1)	1.1 (-5.6 to 8.8)	0.66	1.1 (6.8)
Discharge to Rehabilitation or death	3 (43%)	4 (57%)	27.4 (6.1)	27.0 (6.6)	-0.4 (-2.4 to 1.7)	0.68	0.3 (1.0)

Table 3.19. Sensitivity stratified analyses of selected body composition mean daily changes by percentages of body weight for patients discharge to home vs. discharge to rehabilitation or dead and whom duration between admission and discharge MF-BIA test was $\geqslant$48

Muscle mass discharge (home) not normally distributed (Shapiro-Wilk: p=0.02), Median=32.16%, Interquartile range=30.2 to 33.6%

3.4.6 Subjective outcomes

Eighteen study participants responded to follow up questionnaire of which 10 were men and eight were women. Mean age was 69.1±9.7 years (range = 50-89 years). Their average length of hospital stay was 3.4 day (range 1-8 days), and average NIHSS score was 5.9 (range 1-21). Six of the participant had Lacunar Infarct (LACI), one participant Partial Anterior Circulation Infarct (PACI), seven had Posterior Circulation Infarct (POCI), and four total anterior Circulation Infarct (TACI). One participant was prescribed nil-by-mouth (NBM) during acute stay, 16 normal oral feeding, and one on pureed diet. On discharge 14 were discharged to home, three to rehabilitation, and one initially transferred to another hospital. There was statistically significant difference between discharge weight of those who responded compared to those who did not respond (non-respondents) with non-respondents weight being less than those who responded. Table 3.20 shows characteristics of those who responded to follow up questionnaire and those who did not. There were no statistically significant differences except the discharge weight; those who did not respond has significantly lower weight at the time of discharge compared to those who responded.

There were no statistically significant differences between those with fat free mass loss and gain in the SF36v2 scores. Similar observation was made with respect to fat mass gain and loss. No statistically significant difference was observed in the SF-36v2 individual component scores for patients with protein mass loss or gain. Body cell mass and muscles mass scores were similar with no statistically significant differences between each individual component scores. Table 3.21 a-e present the SF36v2 items scores for patients with fat free mass, fat mass, protein mass, muscle mass, and body cell mass loos and gain with the average differences between groups and p-values.

	Respondents	Non-respondent	*p-value*
Number	18 (45%)	22 (55%)	
Females (%)	8 (44%)		
Mean age (std) years	69.1 (9.7)	71.3 (10.1)	0.64
Age Range (years)	50-89	56-89	
Weight (kg)	82.6 (13.2)	70.6 (13.3)	**0.02**
Height (m)	1.7 (0.1)	1.7 (0.1)	0.40
Body Mass Index (kg/m^2)	27.9 (4.9)	25.4 (4.5)	0.11
Triceps Skin Fold thickness (mm)	10.7 (3.3)	11.9 (4.3)	0.34
Mid Arm Circumference (cm)	30.1 (3.5)	27.6 (4.8)	0.08
Handgrip Strength (kg)	23.8 (9.1)	18.5 (13.4)	0.18
Average length of Hospital say (range) days	3.4 (1-8)	4.6 (1-24)	0.33
Premorbid Rankin Score (n=38)			0.21
0 =No symptoms	11	9	
1 =No significant disability	6	8	
2 = Slight disability.	0	2	
3 = Moderate disability.	0	1	
4 = Moderately severe disability	0	0	
5 =Severe disability	0	0	
Total Anterior Circulation Infarct	4	2	
Left Side	2	2	
Right Side	2	0	
Partial Anterior Circulation Infarct	4	4	
Left Side	3	1	
Right Side	1	3	
Lacunar Infarct	6	11	
Left Side	5	5	
Right Side	1	6	
Posterior Circulation Infarct	7	5	
Left Side	3	2	
Right Side	4	3	
NIHSS Score (n=35) categories			0.37
1 to 9 (mild stroke)	14	16	
10 to 20 (moderate stroke)	1	2	
≥20 (severe stroke)	1	0	
Type of Feeding Regeimen			
Normal Oral	16	13	
Soft/mashed	0	4	
Pureed	1	1	
Nil-by-Mouth (NBM)	1	4	

Table 3.20 presents the characteristics of participants who responded to follow up questionnaire and those who did not respond.

	Participants with FFM Loss (n=6) mean score	Participant with FFM Gain (n=10)mean score	Mean difference (95% CI)	p-value
Fat Free Mass				
Physical Functioning	39 (23.4 to 55.4)	38.7 (23.4 to 57.0)	0.25 (-13.2 to 13.8)	0.97
Role Physical	38.5 (17.7 to 56.9)	36.9 (22.6 to 47.1)	1.63 (-12.5 to 15.7)	0.81
Bodily Pain	44.7 (29.2 to 62.1)	55.2 (37.2 to 62.1)	10.6 (-2.1 to 23.2)	0.09
General Health	45.8 (33.9 to 60.1)	45.0 (37.2 to 62.1)	0.80 (-7.5 to 9.2)	0.84
Vitality	44.6 (20.9 to 58.3)	42.2 (30.2 to 52.1)	2.40 (-9.6 to 14.4)	0.68
Social Functioning	44.3 (18.7 to 56.9)	37.7 (13.2 to 56.9)	6.5 (-9.4 to 22.5)	0.39
Role Emotional	37.3 (9.2 to 55.9)	44.2 (32.5 to 55.9)	8.3 (-11.0 to 24.9)	0.42
Mental Health	48.4 (19.0 to 58.5)	43 (21.9 to 58.5)	5.4 (-9.6 to 20.4)	0.45

Table 3.21a. Short Form Survey (SF36v2) mean scores for patients experiencing fat free mass (FFM) loss and gain respectively and the mean difference between both groups of patients who responded to follow up at 6 month post hospital discharge.

	Participants with FM Loss (n=7) mean score	Participants with FM Gain (n=9)mean score	Mean difference (95% CI)	p-value
Fat Mass				
Physical Functioning	36.5 (23.4 to 57.0)	40.7 (23.4 to 55.4)	4.2 (-8.8 to 17.1)	0.50
Role Physical	34.1 (17.7 to 47.1)	40.8 (17.7 to 56.9)	6.7 (-6.5 to 19.9)	0.29
Bodily Pain	55 (37.2 to 62.1)	43.7 (29.2 to 62.1)	5.5 (-0.52 to 23.2)	0.06
General Health	45.4 (38.6 to 55.3)	45.6 (33.9 to 60.1)	0.11 (-8.1 to 8.3)	0.98
Vitality	43.2 (30.2 to 52.1)	44.1 (20.9 to 58.3)	1.0 (-10.9 to 12.7)	0.87
Social Functioning	36.6 (13.2 to 56.9)	45.9 (18.7 to 56.9)	9.3 (-5.7 to 24.4)	0.20
Role Emotional	39.2 (9.2 to 55.9)	40.8 (9.2 to 55.9)	1.6 (-16.5 to 19.7)	0.85
Mental Health	44 (21.9 to 58.5)	48.2 (19.0 to 58.5)	4.2 (-10.5 to 19.0)	0.55

Table 3.21b. Short Form Survey (SF36v2) mean scores for patients experiencing fat mass (FM) loss and gain respectively and the mean difference between both groups of patients who responded to follow up at 6 month post hospital discharge.

	Participants with PM Loss (n=5) mean score	Participants with PM Gain (n=11)mean score	Mean difference (95% CI)	p-value
Protein Mass				
Physical Functioning	39.7 (23.4 to 57.0)	37 (23.4 to 48.1)	2.7 (-11.4 to 16.7)	0.69
Role Physical	40.8 (17.7 to 56.9)	31.4 (17.7 to 56.9)	9.4 (-4.3 to 23.1)	0.16
Bodily Pain	49.9 (29.2 to 62.1)	45.7 (37.2 to 53.7)	4.2 (-6.5 to 14.9)	0.54
General Health	44.7 (33.9 to 60.10	47.2 (38.6 to 55.3)	2.5 (-11.1 to 6.1)	0.55
Vitality	44.7 (20.9 to 58.3)	41.5 (27.1 to 49.0)	3.2 (-9.3 to 18.7)	0.54
Social Functioning	45.5 (18.7 to 56.9)	33.9 (13.2 to 45.9)	11.5 (-4.3 to 27.3)	0.14
Role Emotional	41.9 (9.2 to 55.9)	36.4 (9.2 to 55.9)	5.4 (-13.4 to 24.3)	0.54
Mental Health	45.7 (19.0 to 58.5)	47.7 (30.3 to 58.5)	2.0 (-18.0 to 13.9)	0.79

Table 3.21c. Short Form Survey (SF36v2) mean scores for patients experiencing protein mass (PM) loss and gain respectively and the mean difference between both groups of patients who responded to follow up at 6 month post hospital discharge.

	Participants with BCM Loss (n=6) mean score	Participants with BCM Gain (n=10)mean score	Mean difference (95% CI)	p-value
Body Cell Mass				
Physical Functioning	39.2 (23.4 to 57.0)	38.3 (23.4 to 52.8)	0.95 (-12.6 to 14.4)	0.88
Role Physical	38 (17.7 to 56.9)	37.7 (17.7 to 56.9)	0.33 (-13.8 to 14.4)	0.96
Bodily Pain	47.9 (29.2 to 62.1)	49.8 (29.2 to 62.1)	1.9 (-11.9 to 15.7)	0.78
General Health	43.4 (33.9 to 60.1)	50 (38.6 to 55.3)	5.5 (-2.2 to 13.3)	0.15
Vitality	43 (20.9 to 58.3)	44.8 (33.4 to 52.1)	1.8 (-10.3 to 13.8)	0.76
Social Functioning	45.4 (18.7 to 56.9)	36 (13.2 to 51.4)	9.5 (-6.0 to 24.9)	0.21
Role Emotional	39.9 (9.2 to 55.9)	40.3 (9.2 to 55.9)	0.43 (-18.0 to 18.8)	0.96
Mental Health	43.3 (19.0 to 58.5)	51.4 (35.9 to 58.5)	8.1 (-4.6 to 20.8)	0.19

Table 3.21d. Short Form Survey (SF36v2) mean scores for patients experiencing body cell mass (BCM) loss and gain respectively and the mean difference between both groups of patients who responded to follow up at 6 month post hospital discharge.

	Participants with MM Loss (n=6) mean score	Participants with MM Gain (n=10)mean score	Mean difference (95% CI)	p-value
Muscle Mass				
Physical Functioning	39.2 (23.4 to 57.0)	38.3 (23.4 to 52.8)	0.95 (-12.6 to 14.4)	0.88
Role Physical	38 (17.7 to 56.9)	37.7 (17.7 to 56.9)	0.33 (-13.8 to 14.4)	0.96
Bodily Pain	47.9 (29.2 to 62.1)	49.8 (29.2 to 62.1)	1.9 (-11.9 to 15.7)	0.78
General Health	43.4 (33.9 to 60.1)	50 (38.6 to 55.3)	5.5 (-2.2 to 13.3)	0.15
Vitality	43 (20.9 to 58.3)	44.8 (33.4 to 52.1)	1.8 (-10.3 to 13.8)	0.76
Social Functioning	45.4 (18.7 to 56.9)	36 (13.2 to 51.4)	9.5 (-6.0 to 24.9)	0.21
Role Emotional	39.9 (9.2 to 55.9)	40.3 (9.2 to 55.9)	0.43 (-18.0 to 18.8)	0.96
Mental Health	43.3 (19.0 to 58.5)	51.4 (35.9 to 58.5)	8.1 (-4.6 to 20.8)	0.19

Table 3.21e. Short Form Survey (SF36v2) mean scores for patients experiencing muscle mass (MM) loss and gain respectively and the mean difference between both groups of patients who responded to follow up at 6 month post hospital discharge.

No statistically significant mean differences in each of the stroke impact scale domain scores were observed between participants with fat free mass, fat mass, protein mass, muscle mass, and body cell mass losses and gains at 6 months post discharge. Table 3.21a-e present the stroke impact scale items scores for participants with fat free mass, fat mass, protein mass, muscle mass, and body cell mass losses and gains who provide a response at 6 months post discharge.

Average scores	Participants with FFM loss mean score	Participants with FFM gain mean score	Mean difference (95% CI)	p-value
Fat Free Mass				
Strength SIS*	83.3 (56.3 to 100)	68.8 (31.3 to 100)	14.6 (-24.2 to 53.4)	0.38
Memory SIS**	80 (42.9 to 100)	74.5 (39.3 to 100)	5.5 (-16.9 to 28.0)	0.61
Emotion SIS	66.7 (22.2 to 94.4)	64.2 (44.4 to 83.3)	2.4 (-20.7 to 25.5)	0.83
Communication SIS***	89 (57.1 to 100)	90.3 (67.9 to 100)	1.4 (-16.3 to 13.6)	0.85
Activities of Daily living[£]	80.3 (50 to 100)	85.0 (52.5 to 100)	4.7 (-15.3 to 24.7)	0.62
Mobility SIS[#]	79.3 (36.1 to 100)	85.7 (66.7 to 100)	6.4 (-14.5 to 27.2)	0.53
Hand Function SIS**	82.5 (65.6 to 100)	72.1 (0.00 to 100)	10.4 (-26.2 to 46.9)	0.53
Social Participation SIS [x]	91.1 (65.6 to 100)	90.6 (81.3 to 57.0)	0.5 (-27.0 to 28.1)	0.97

Table 3.22a. Stroke Impact Scale Score (SIS) mean scores for patients experiencing fat free mass (FFM) loss and gain respectively and the mean difference between both groups of patients who responded to follow up at 6 month post hospital discharge.

*Loss n=9, Gain n=5; ** Loss n=10, Gain=7;*** Loss n=11, Gain n=7; [£] Gain n=8, Loss n=7; [#] Loss n=11, Loss n=6; [x] Gain n=6, Loss n=2.

Average scores	Participants with FM loss mean score	Participants with FM gain mean score	Mean difference (95% CI)	p-value
Fat Mass				
Strength SIS*	68.8 (31.3 to 100)	85.2 (56.3 to 100)	16.4 (-10.1 to 42.9)	0.20
Memory SIS**	71.9 (39.3 to 100)	83 (42.9 to 100)	11.1 (-10.4 to 32.6)	0.29
Emotion SIS	66.3 (44.4 to 83.3)	65.1 (22.2 to 94.4)	1.2 (-24.0 to 21.6)	0.91
Communication SIS***	86.2 (57.1 to 100)	92.1 (60.7 to 100)	6.0 (-8.3 to 20.3)	0.39
Activities of Daily living[£]	81.3 (52.5 to 100)	83.9 (50 to 100)	2.7 (-17.4 to 22.8)	0.78
Mobility SIS[#]	78.6 (36.1 to 100)	83.6 (52.8 to 100)	5.0 (-15.3 to 25.4)	0.61
Hand Function SIS**	72.5 (0.00 to 100)	83.3 (40 to 100)	10.8 (-18.7 to 40.4)	0.45
Social Participation SIS[x]	88.5 (81.3 to 100)	92.5 (65.6 to 100)	4.0 (-20.4 to 28.3)	0.70

Table 3.22b. Stroke Impact Scale Score (SIS) mean scores for patients experiencing fat mass (FM) loss and gain respectively and the mean difference between both groups of patients who responded to follow up at 6 month post hospital discharge

*Loss n=6 Gain n=8; ** Loss n=8, Gain=9;*** Loss n=8, Gain=10; [£] Gain n=8, Loss n=7; [#] Loss n=7, Loss n=10; [x] Gain n=3, Loss n=5

Average scores	Participant with PM loss mean score	Participant with PM gain mean score	Mean difference (95% CI)	p-value
Protein Mass				
Strength SIS*	80 (31.3 to 100)	73.5 (37.5 to 100)	6.6 (-24.3 to 37.4)	0.65
Memory SIS**	83.1 (39.3 to 100)	67.9 (53.6 to 89.3)	10.2 (-6.5 to 37.0)	0.16
Emotion SIS	66.7 (27.8 to 94.4)	63.9 (22.2 to 88.9)	2.8 (-21.0 to 26.6)	0.81
Communication SIS***	93.8 (60.7 to 100)	81 (57.1 to 100)	12.8 (-1.1 to 26.7)	0.07
Activities of Daily living[£]	83.4 (50.0 to 100)	80 (55.0 to 100)	3.4 (-19.2 to 26.1)	0.75
Mobility SIS[#]	85.7 (52.8 to 100)	71.7 (36.1 to 97.2)	14.0 (-6.8 to 34.8)	0.17
Hand Function SIS[#]	79.6 (0.00 to 100)	75 (35.0 to 100)	4.6 (-28.3 to 37.5)	0.77
Social Participation SIS [x]	91.1 (65.6 to 100)	90.6 (84.4 to 96.9)	0.5 (-27.0 to 28.1)	0.97

Table 3.22c. Stroke Impact Scale Score (SIS) mean scores for patients experiencing protein mass (PM) loss and gain respectively and the mean difference between both groups of patients who responded to follow up at 6 month post hospital discharge

*Loss n=10 Gain n=4; ** Loss n=11, Gain n=6;*** Loss n=12, Gain=6; [£] Gain n=11, Loss n=4; [#] Loss n=12, Loss n=5; [x] Gain n=6, Loss n=2

Average scores	Participant with BCM loss mean score	Participant with BCM gain mean score	Mean difference (95% CI)	p-value
Body Cell Mass				
Strength SIS*	78.9 (37.5 to 100)	77.1 (31.3 to 100)	1.8 (-26.6 30.2)	0.89
Memory SIS**	77.1 (39.3 to 100)	78.6 (53.6 to 100)	1.4 (-21.2 to 24.1)	0.90
Emotion SIS	60.3 (22.2 to 94.4)	73.4 (52.8 to 88.9)	13.1 (-8.8 to 35.1)	0.22
Communication SIS***	92.5 (60.7 to 100)	84.7 (57.1 to 100)	7.8 (-6.5 to 22.2)	0.26
Activities of Daily living[£]	83.8 (50.0 to 100)	81.1 (52.5 to 100)	2.7 (-17.4 to 22.8)	0.78
Mobility SIS[#]	82.1 (52.8 to 100)	80.6 (36.1 to 100)	1.5 (-19.6 to 22.7)	0.88
Hand Function SIS[***]	78.5 (35.0 to 65.6)	77.9 (0.00 to 100)	0.64 (-29.9 to 31.2)	0.97
Social Participation SIS [x]	86.7 (65.6 to 100)	95.3 (84.4 to 100)	8.6 (-13.7 to 30.9)	0.38

Table 3.22d. Stroke Impact Scale Score (SIS) mean scores for patients experiencing body cell mass (BCM) loss and gain respectively and the mean difference between both groups of patients who responded to follow up at 6 month post hospital discharge

*Loss n=8 Gain n=6; ** Loss n=10, Gain n=7;*** Loss n=11, Gain=7; [£] Gain n=8, Loss n=7; [#] Loss n=11, Loss n=6; [x] Gain n=4, Loss n=4

Average scores	Participant with muscle mass loss mean score	Participant with muscle mass ain mean score	Mean difference (95% CI)	p-value
Muscle Mass				
Strength SIS*	78.9 (37.5 to 100)	77.1 (31.3 to 100)	1.8 (-26.6 30.2)	0.89
Memory SIS**	77.1 (39.3 to 100)	78.6 (53.6 to 100)	1.4 (-21.2 to 24.1)	0.90
Emotion SIS	60.3 (22.2 to 94.4)	73.4 (52.8 to 88.9)	13.1 (-8.8 to 35.1)	0.22
Communication SIS***	92.5 (60.7 to 100)	84.7 (57.1 to 100)	7.8 (-6.5 to 22.2)	0.26
Activities of Daily living[£]	83.8 (50.0 to 100)	81.1 (52.5 to 100)	2.7 (-17.4 to 22.8)	0.78
Mobility SIS[#]	82.1 (52.8 to 100)	80.6 (36.1 to 100)	1.5 (-19.6 to 22.7)	0.88
Hand Function SIS[***]	78.5 (35.0 to 65.6)	77.9 (0.00 to 100)	0.64 (-29.9 to 31.2)	0.97
Social Participation SIS [x]	86.7 (65.6 to 100)	95.3 (84.4 to 100)	8.6 (-13.7 to 30.9)	0.38

Table 3.22e. Stroke Impact Scale Score (SIS) mean scores for patients experiencing muscle mass (MM) loss and gain respectively and the mean difference between both groups of patients who responded to follow up at 6 month post hospital discharge.

*Loss n=8 Gain n=6; ** Loss n=10, Gain=7;*** Loss n=11, Gain=7; [£] Gain n=8, Loss n=7; [#] Loss n=11, Loss n=6; [x] Gain n=4, Loss n=4

In patients with fat free mass loss SIS overall stroke recovery scores did not show any statistical significance differences compared to those with fat free mass gain. Patients with fat free mass loss however scored higher in BI scores than patients with fat free mass gain. This was opposite to the findings for the PCS. MCS scores were higher in patients with fat free mass loss.

SIS overall stroke recovery and BI scores were lower in patients with fat mass loss compared to fat mass gain with statistical significance (p=0.05). The PCS were not coherent with SIS overall stroke recovery and BI scores. However, no statistically significant difference was observed between the two groups.

The mean difference in the overall SIS stroke recovery for participants with protein mass loss and protein mass gain was statistically significant (p=0.02). A mean difference of 0.22 (-33.7 to 33.2) was observed with those having protein mass gains mean score being higher than participants with protein mass loss. The Barthel Index scores were higher in patients with protein mass loss compared to those with protein mass gain with the PCS following the same trend. Interestingly patients with muscle mass loss and body cell mass loss scored higher in the SIS overall patients reported stroke recovery and BI compared with patients with body cell mass and muscle mass gains (difference muscle mass p=0.05 and difference body cell mass p=0.01). The PCS and MCS scores were marginally different showing no statistically significant differences between the two groups.

Table 3.22 shows the differences in the mean scores of Barthel Index Score (BI), Stroke Impact Scale (SIS) overall stroke recovery, the SF36v2 Physical Component Summary (SF36v2 PCS), and the SF36v2 Mental Component Summary (SF36v2 MCS) scores for patients who responded to the six month follow up questionnaire evaluation.

Average scores	Participant with Body Composition loss	Participant with Body Composition Gain	Mean difference (95% CI)	p-value
Fat Free Mass				
SIS overall	83.3 (30 to 100)	82.0 (4 to 100)	6.9 (-15.4 to 29.1)	0.52
BI scores	89.7 (60 to 100)	76.4 (50 to 95)	7.7 (-25.6 to 41.1)	0.61
SF36v2 PCS	41.0 (28.7 to 58.1)	43.5 (33.4 to 59.1)	2.5 (-13.2 to 8.2)	0.62
SF36v2 MCS	46.1 (14.0 to 68.3)	42.7 (31.7 to 54.9)	3.4 (-11.6 to 18.4)	0.64
Fat Mass				
SIS overall	70.6 (30 to 95)	90 (70 to 100)	19.4 (-0.11 to 38.9)	0.05
BI scores	79.3 (4 to 100)	94.67 (25 to 75)	15.4 (-15.5 to 46.4)	0.30
SF36v2 PCS	42.5 (33.4 to 59.1)	41.6 (28.7 to 58.1)	0.9 (-11.4 to 9.7)	0.86
SF36v2 MCS	41.8 (31.7 to 54.9)	47.2 (14.0 to 68.3)	6.7 (-19.8 to 9.1)	0.44
Protein Mass				
SIS overall	85.8 (4 to 100)	86.0 (60 to 100)	0.22 (-33.7 to 33.2)	0.02
BI scores	87.7 (50 to 100)	64.0 (30 to 90)	23.7 (-2.9 to 50.4)	0.90
SF36v2 PCS	43.4 (28.7 to 59.1)	38.9 (33.4 to 49.2)	4.5 (-6.5 to 15.5)	0.40
SF36v2 MCS	45.9 (14.0 to 68.3)	42.4 (36.4 to 51.1)	3.5 (-8.7 to 15.6)	0.64

Table 3.23. Follow questionnaire responses mean scores stratified by body composition changes, continued

Average scores	Participant with Body Composition loss	Participant with Body Composition Gain	Mean difference (95% CI)	p-value
Muscle Mass				
SIS overall	89.5 (70 to 100)	65 (30 to 90)	24.5 (0.7 to 48.3)	0.05
BI scores	94.8 (75 to 100)	74 (4 to 100)	20.8 (-18.9 to 60.4)	0.24
SF36v2 PCS	42 (28.7 to 59.1)	41.9 (33.8 to 54.7)	0.15 (-10.7 to 10.9)	0.98
SF36v2 MCS	44.3 (14.0 to 68.3)	45.7 (31.7 to 56.0)	1.5 (-16.5 to 13.7)	0.84
Body Cell Mass				
SIS overall	89.5 (70 to 100)	65 (30 to 90)	24.5 (0.7 to 48.3)	0.01
BI scores	94.8 (75 to 100)	74 (4 to 100)	20.8 (-18.9 to 60.4)	0.24
SF36v2 PCS	42 (28.7 to 59.1)	41.9 (33.8 to 54.7)	0.15 (-10.7 to 10.9)	0.98
SF36v2 MCS	44.3 (14.0 to 68.3)	45.7 (31.7 to 56.0)	1.5 (-16.5 to 13.7)	0.84

Table 3.23. Stroke impact scale (SIS) overall stroke recovery, barthel index, and physical component (PCS) and mental component (MCS) summary mean scores for patients experiencing fat free mass, fat mass, protein mass, muscle mass, and body cell mass loss and gains respectively and the mean difference between both groups of patients who responded to follow up at 6 month post hospital discharge.

SIS: Stroke Impact Scale; BI: Barthel Index Score; SF36v2: Short Form Survey 36 version 2

3.5 Discussion

Although there were observed differences within and between groups in fat free mass and body composition changes between normal oral diet vs. Modified diets, non-NBM vs. NBM, non-TACI vs. Non-TACI, and NIHSS 1-9 vs. NIHSS≥10, none of these difference were statistically significant except for muscle mass losses for modified diet, muscle mass and protein mass losses for TACI. Except for muscle mass loss for patients discharged to rehabilitation and fat free mass loss for patients discharged to home that were statistically significant (p=0.05), no other statistically significant differences in body composition changes were observed between participants discharged to home vs. participants discharge to rehabilitation or dead during acute stay.

Those who responded had higher weight on discharge compared to non-responders With respect to responses, the only statically significant scores were reported in the SIS overall patients reported stroke recovery scores. They were reflected by higher scores observed for participants with fat mass gain compared to those with fat mass loss (p=0.05), for participants with muscle mass and body cell mass loss compared to those with muscle mass (p=0.05) and body cell mass (p=0.01) gains, and marginally lower scores participants with protein mass loss compared to those with participant with protein mass gain (p=0.02). No other results were statically significant. Most results in the subjective outcomes were inconsistent, did not correlate with the finding that suggest loss of lean body mass tissue and gain of fat mass can jeopardize functional status and overall activity level (61, 167, 168).

3.5.1 Other studies findings

To the best of my knowledge this is the first study which attempted to understand the changes in body composition in acute stroke setting using a portable, validated method. With small sample size, I did not find significant results except marginally significant

protein mass loss (p=0.06) was observed in the whole sample and also a significant muscle mass loss was observed in those who underwent modified diet regimen. This is reflected as significantly higher proportion of people who were discharged to a rehabilitation setting/died had muscle mass loss compared to proportion of people who had muscle mass gain in this group.

Fat free mass, protein mass, muscle mass losses and fat mass gains observed in modified diet, NBM, TACI, and NIHSS ≥10 groups can be related to the severity of their condition rendering them bedridden, with a heightened stress response and making such body composition changes inevitable and this observation is in line with the existing evidence. Being inactive and bedridden can contribute to lean tissue mass losses (163), and the stress response evident by increased serum cortisol level in acute stroke patients (56) may explain the loss in lean body tissues (166). In addition, the increased fat mass gain can be related to their inactive bedridden state. Their use of active tissue such as muscles is very minimal which may result in fat tissue accumulation and active tissue loss (164, 165).

Smithard et al. (208) examined the effect of nutritional status markers in patients with swallowing difficulties and reported a deterioration in anthropometric indices and albumin levels over a month period (208). The decline in upper arm anthropometric and serum albumin levels in the Smithard's study are also seen in the body composition changes observed in our study population considering that these measures are used to assess lean body tissue (209, 210). Davalos et al also reported a similar finding showing decline in MAC, TSF, and serum albumin between admission and week one and two of hospitalization (56).

To my knowledge this is first study which assessed the changes in individual body components examined as a whole perhaps more accurately than regional anthropometric measures. The regional anthropometric measurements require some training. The lack of reproducibility of TSF due to margin of error between measurements makes the validity of this method questionable (175). MAC utility in assessing whole body

composition of fat free mass is also questionable. MAC is a localized measure to evaluate arm muscle area and thus unlikely to represent whole body lean mass tissue (178). Including serum albumin in assessing protein malnutrition is limited by the fact it is influenced by intake and loss (e.g. proteinuria) (80, 81).

3.5.2 Study Limitations

The main limitation of my study is the relatively small sample size. This in combination with requirement to analyse the data by feeding regimen or categorisation by other characteristics such as stroke severity made the sample even smaller to make any firm conclusions. Nevertheless, I have shown that patients with stroke on modified diet, NBM feeding regimen, and patients with TACI had consistent body composition changes with the majority experiencing fat free mass loss, fat mass gain, and muscle mass, and protein mass losses.

The length of hospital stay was not long enough to observe statistically significant changes across all examined body composition indices (mean 3.9, range 1-24 days) only three patients had a length of hospital stay ≥10 days (11, 12, and 24 days respectively). This is due to development of stroke services locally with extra bed capacity for acute rehabilitation in the community had impact on the patient flow and hence length of stay in acute unit situated at the main hospital site had become much shorter during the study data collection period compared to the study protocol development stage.

Objective outcomes in the form of discharge destination or death did not provide a statically significant interpretation although higher frequency of those discharged to rehabilitation services or those who died experienced fat free mass loss and fat mass gain compared to those discharge to home. Given that even in stroke patients on normal oral diet showed changes in their body composition during their acute hospital stay, MF-BIA may be used to tailor the individual nutritional needs in severe strokes which are associated with immobilization and swallowing difficulty. Whether such targeted

nutritional assessment and appropriate nutritional support would be associated with clinical and cost effectiveness need to be tested in a randomised trial setting. Whether particular type of nutritional supplementation is better than other may also require investigation.

3.5.3 Conclusion and future research:

Consistent results of fat free mass loss, fat mass gain, and protein mass, muscles mass, and body cell mass losses were only observed in patient with NBM feeding regimen and TACI stroke classification. Fat free mass loss, fat mass gain, and protein mass, muscles mass, and body cell mass loss were observed more in patients receiving NBM feeding regimen and patients with TACI suggesting that the severity of their condition may contributed to such body composition changes. Most patients with a stroke severity score NIHSS ≥10 had fat free mass loss, fat mass gain, and protein mass loss (and higher than those with NIHSS 1-9), but there were body cell mass and muscle mass gains making such results unrealistic and may be due to chance. These varied findings seen in NIHSS ≥10 strokes do not allow to draw conclusions or observe trends unlike NBM or TACI patients.

Equipment malfunction was suspected. Follow up data did not lead to any conclusion regarding the relationship between the body composition changes that occurred during the acute hospital stay and the longer term subjective outcomes. This may be due to the fact that patients on NBM, those experienced TACI, or with NIHSS≥10 patients did not respond to questionnaires examining subjective outcomes. Only the most medically fit patients with none of the former described condition mainly responded with a low response rate <50% (18 out of 40 participants). Objective outcomes in the form of discharge destination or death did not show a trend although higher frequency of those discharged to rehabilitation services or those who died experienced fat free mass loss and fat mass gain compared to those discharge to home. This could be simply due to small sample size.

In summary, my investigation shows interesting observations regarding body composition changes in patient on modified diet, NBM feeding regimen, patients who experienced TACI, and those with a moderately severe stroke assessed by NIHSS ≥10. Due to a small sample a firm conclusion on the relationship between body composition changes and type of feeding regimen, stroke classification, stroke severity, and objective outcomes such as mortality cannot be drawn. Nevertheless my work is novel and provides some normative data of body composition changes occurring during an acute hospital stay which lay the foundation for sample size calculations and deriving minimally clinically significant change for future studies. My research contribution is therefore novel and future research can be built on this foundation of new knowledge. Further research is required to observe statistically significant findings warrant of further research in the form of clinical trials to understand the impact of targeted intervention on body composition changes in acute stroke.

Chapter 4: The diagnostic accuracy of Maltron BioScan 920-2 multi-frequency bioelectrical impedance analysis in diagnosing dehydration after stroke

Abstract

Background and aims: Non-invasive methods for detecting water-loss dehydration following acute stroke would be clinically useful. I evaluated the diagnostic accuracy of multi-frequency bioelectrical impedance analysis (MF-BIA) against the reference standards, serum osmolality and osmolarity.

Methods: Patients admitted to an acute stroke unit were recruited over six months from April to October of the year 2011. Blood samples for electrolytes and osmolality were taken within 20 minutes of MF-BIA. Total body water (TBW%), intracellular water (ICW%) and extracellular water (ECW%) were calculated using MF-BIA equipment and also calculated from MF-BIA generated impedance measures using published equations for older people. These were compared to hydration status (based on measured serum osmolality and also calculated osmolarity). The most promising Receiver Operating Characteristics curves were plotted.

Results: A total of 27 stroke patients were recruited (mean age 71.3 years ± 10.7 years). Only a TBW% cut-off at 46% was consistent with current dehydration (serum osmolality >300mOsm/kg) and TBW% at 47% with impending dehydration (calculated osmolarity ≥295-300mOsm/L) with sensitivity and specificity both >60%. Even here diagnostic accuracy of MF-BIA was poor, a third of those with dehydration were wrongly classified as hydrated and a third classified as dehydrated were well hydrated.

Conclusions: MF-BIA appears ineffective at diagnosing water-loss dehydration after stroke and cannot be recommended as a test for dehydration.

4.1 Background

4.1.1 Dehydration prevalence and prognosis

Stroke complications such as dysphagia, associated medication and depression, may make the maintenance of adequate dietary fluid intake difficult after stroke. Scarce data is available on the prevalence of dehydration in stroke patients. However with the available evidence from stroke and non-stroke studies one can understand the importance of such condition on outcomes.

Studies report that dehydration is common after stroke. Bhalla (98) found that 30% of their 167 stroke patients had raised serum osmolality (>296 mOsm/kg). This was further reflected in another later study that suggested that almost a quarter of patients (n=102) were dehydrated during their hospital stay (on day nine post admission) (121). Although both studies were carried out in small samples they raised attention on the magnitude of the problem. A more recent study by Rowat and colleagues (2012) that examined stroke patients clinical data register of two hospital (n=2591) reported that dehydration was present in 62% of this population (211).

Dehydration in general and stroke specifically can increase the risk of poor outcome and mortality. In care homes, it was reported that very high serum osmolality (>308 mOsml/kg) in elderly residents, living in a continuing care, predicted marginally significant increased mortality (75% of 20 residents with high serum osmolality, compared to 53% of 38 residents with lower osmolality, p=0.053), and median survival time was significantly reduced (p=0.025) (212). In stroke the risk is similar. Bhalla et al 2000 suggested that the risk of mortality increased by more than two fold in dehydrated patients (n=50) compared to those not dehydrated (n=117); (OR 2.4, 95%CI 1.0 t 5.9) (98).

The prognosis of dehydration post-stroke is not limited to mortality only but also to morbidity. In the 102 acute ischaemic stroke patients included, raised serum osmolality (>297mOsm/kg, in 24% of their patients) on day 9 following admission was associated with increased odds of venous thromboembolism (OR 4.7, 95% CI 1.4 to 16.3) (121). The largest study examining the prevalence and prognosis of dehydration in stroke patients (n=2591) suggested that dehydrated patient have higher probability of being dead or dependent at hospital discharge compared to those not dehydrated ($p<0.0001$) (211).

Dehydration may not only affect objective outcome of mortality but may also have poor prognosis on full recovery and quality of life. The risk of mortality and poor outcomes of dehydration diagnosed in hospital settings can have negative prognosis on short and long term outcomes. Patients discharged from hospital and diagnosed with dehydration on admission were more likely to die at 30 day ($p=0.037$) and six months ($p=0.002$), with a suggested increase in dehydration incidence rate of 3.5% during hospital stay (n=1416) reaching to a 533 dehydrated patient in the four year study periods; 67% of the dehydrated patients had available data and were entered in the final outcome analysis(213). Dehydration can also affect the quality of life. It could be that dehydration decreases muscular strength through initiating active tissue, fat free mass and mainly muscles mass, loss resulting in general weakness. Finn et al 1996, suggested that fat free mass loss is initiated by cellular dehydration in sepsis and critically ill patients, and such changes in fat free mass are associated with reduced functional capacity (214).

Given that dehydration is prevalent after stroke and its prognostic significance, diagnosing dehydration becomes a priority in its management. There are several methods to assess water-loss (or hypertonic) dehydration including clinical and biochemical assessments some of which I will discuss briefly in the following section.

4.1.2 Dehydration in clinical Setting: physical and biochemical assessment

Despite the dehydration council creating the DEHYDRATION mnemonic listing 12 indicators to be used in dehydration screening (64), the diagnosis of dehydration remains a dilemma. Physicians misdiagnosed dehydration in a third of patients admitted to a hospital (215). This can be attributed to the variety of available methods in diagnosing dehydration. Methods used to assess water-loss dehydration in clinical settings in older people include urinary, haematological, and physical assessments (216). Using serum osmolarity (>295 mOsm/l) and sodium (>145 mmol/L) as a reference for dehydration Thomas found that of those patients diagnosed as dehydrated using physical assessment, only 17% had a serum osmolarity >295 mOsm/l (217). Physical assessment differs from physician to physician and may include some or all of the following: capillary refill time, skin turgor, longitudinal tongue furrows, tongue dryness, orthostatic hypotension, urine colour and volume and many more. This may be exacerbated by poor inter-observer agreement, as with capillary refill time. Anderson found only 70% agreement in classifying patients as normal vs. abnormal (Kappa=0.38) in their study on clinically stable emergency department patients (n=209) (218, 219).

Capillary refill time have proven to be unreliable as a recent review suggested (220) and is also affected by environmental factors, with a decrease in capillary refill time as the temperature rises, showing the importance of training and standardising the use of such tests (218). Skin turgor is another method used in assessing dehydration in adults, but again skin elasticity changes with ageing reduces the validity of the test, as results can rely on physiological skin changes rather than state of dehydration (221). There are indications that tongue dryness and longitudinal tongue furrows may be more reliable. Gross et al evaluated 38 signs of dehydration among 60+ year old patients at two teaching hospitals. They evaluated medical records to judge whether patients were dehydrated and used this as the dehydration reference (but did not report serum osmolality) (191). Tongue dryness and longitudinal tongue furrows strongly correlated with dehydration severity as two strong indicators of dehydration ($p<0.001$ for both) (191).

Elevated serum osmolality, sodium, creatinine, and urea are used in evaluating dehydration. In general Individual components of serum osmolality, such as urea, creatinine and sodium have also been used to assess dehydration, but have been found to be inaccurate (215, 222). Serum sodium, creatinine, urea may not reflect actual dehydration. The presence of a high serum creatinine can be related to high muscle mass and muscle tissue turnover, a state of muscle metabolism and not necessarily dehydration. Creatinine serum levels are also associated with different pathologies. For example, a rise in creatinine levels can be associated with gastrointestinal bleeding, septic shock, and renal function (222) giving misleading diagnosis of dehydration. Serum sodium is another indicator used in evaluating hydration status. Thomas et al warned the use of sodium in evaluating dehydration that serum sodium may not reflect true intracellular dehydration but rather volume depletion (217). Bowker et al 1992 (219) examined urea level in patient with pre-renal condition including dehydration. They found that only in 50% of those patients urea was higher than normal 13.2 mmol/L. In addition, urea level increased in 80% of the patients with post renal obstruction or pathology (222). These findings suggest that urea does not always reflect the presence or absence of dehydration.

Of all Biochemical indicators of dehydration, serum osmolality is most frequently used as a reference standard (64, 223). Serum osmolality is the osmolar concentration or osmotic pressure of serum, so reflects the number of dissolved particles (whether they are able to permeate cell membranes or not) per kilogram of serum. Serum osmolality reflects the osmolality of intracellular fluid as cell walls are permeable to water, and as osmolality is carefully controlled by the body any change in osmolality suggests important alterations in body biochemistry. Serum osmolality is sensitive to hydration status changes. It is sensitive to change after the first day of hydration status changes (224). Where body fluids are lost along with electrolytes (through loss of blood or sweat for example) then fluid may be lost without alteration of osmolality, this state is termed “water and salt-loss” dehydration. Following stroke it is possible that there will be a reduction in fluid intake, with or without increased fluid losses associated with use of diuretics, fever, uncontrolled diabetes mellitus, etc. In such situations where body fluids are lost overall, the result is likely to be that of increased osmolality, or “water-loss” dehydration. Serum osmolality can be used alone, and without prior measurement,

as a hydration status marker (223), unlike weight change as a reference standard which depends on other body components (216). Thus serum osmolality is probably the best reference standard method to measure water-loss dehydration and the diagnostic standard against which the accuracy of other measures should be judged (64) .

While studies have used slightly varying cut-off points for serum osmolality to define dehydration (98, 121) the Dehydration Council's definition is specific to older people, with a serum osmolality 295-300 mOsm/kg equates to impending dehydration and > 300 mOsm/kg with current dehydration and this definition is used in this study. In clinical practice serum osmolality is often not assessed, but estimated from the combined concentrations of serum sodium, potassium, glucose and urea, referred to as serum osmolarity (2Na+2K+Urea+Glucose, all in mmol/L). There is a difference between measured serum osmolality and calculated osmolarity, known as the osmolar gap (as some components of osmolality are not included in the formula to calculate osmolarity) (225). In addition, and given that urea and sodium measurements may not be accurate, serum osmolarity may not reflect the true state of dehydration compared to measured serum osmolality.

Given that serum osmolality is not routinely performed in clinical practice, an alternative swift dehydration monitoring test is essential. Bioelectrical impedance analysis (MF-BIA) is one method that maybe able to help in monitoring and diagnosing dehydration. MF-BIA measurement is fairly simple, non-invasive, and can be performed in clinical settings while the patient is lying down. The MF-BIA can measure total body water (TBW), intracellular water (ICW), and extracellular water (ECW) volumes. ICW reflects water volume within body cells, and so may reflect how well the body is hydrated.

4.1.3 Evaluating dehydration using bioelectrical impedance analysis

Total body water is another component that can be assessed by bioelectrical impedance analysis. Total body water can provide information on the degree of dehydration. Physiological changes occurring in the ageing process increases the risk of dehydration. These physiological changes are related to reduced capacity in retaining water; such changes include but are not limited to reduced renal filtration rate, increased proximal tubular filtration absorption, and decreased free water clearance (64). Total body water consists of intracellular and extracellular water. Loss of intracellular water is usually defined as dehydration (226, 227). Assessing dehydration using MF-BIA can predict not only total body water, but also specific intracellular and extracellular components. Evaluating intracellular and extracellular water can provide information on the extent of tissue catabolism. As indicated earlier acute/chronic inflammation instigated during illness leads to catabolism of lean body mass resulting in fat free mass loss (174). Fat free mass loss leads to loss of cellular fluids as tissue catabolism results in intracellular fluid loss and expansion of extracellular fluid; cellular dehydration (60). Based on intracellular and extracellular water changes related to lean tissue catabolism, caloric and nutritional needs can be modified to allow tissue anabolism and prevent further catabolism. Assessing dehydration through measuring body composition values may provide information on the nutritional status and management needs of patients.

4.2 Study Objective

This study aimed to assess the levels of dehydration after stroke using the reference standard of serum osmolality and to assess whether MF-BIA can be substituted for serum osmolality in diagnosing dehydration after stroke.

Methods to assess hydration status which do not require obtaining blood samples would be helpful in situations where there is no quick and easy access to laboratory facilities such as care homes and rehabilitation services. Multi frequency bioelectrical impedance analysis (MF-BIA) can provide estimates of total body water (TBW), intracellular water (ICW), and extracellular water (ECW) volumes and as percentages of body weight, which theoretically should correspond to hydration status. If so the composition of these compartments would suggest MF-BIA as a useful non-invasive method of diagnosing dehydration that does not require medical training in operating in daily clinical practice. This chapter presents the study which assessed the diagnostic accuracy of Maltron BioScan 920-2 MF-BIA to monitor hydration status in patients with stroke.

4.3 Methodology

This cross sectional study was carried out in an acute stroke unit in the East of England (as in Chapter 3). A total of 45 stroke patients admitted within 48 hours of symptom onset were recruited between 1st April and 15th October 2011. Patients were included if older than 17 years, with newly diagnosed stroke (first ever or recurrent). Exclusions included those with severe stroke by National Institute of Health Stroke Scale (NIHSS) score >30, co-existing terminal illness, or expected survival <48 hours as judged by a stroke physician, and those who were unable to give informed consent. Routine medical, nursing and therapist care was unaffected by entry into the study. All eligible patients who provided informed consent during the study period were enrolled in the study.

Upon consent a venous blood sample was taken for serum osmolality, sodium, potassium, random glucose, creatinine and urea and the sample was analysed immediately. Co-morbidities including diabetes and renal impairment were noted. Serum osmolality was analysed by the hospital pathology laboratory using freezing point depression on an Advanced Instruments model 2020 osmometer (Advanced Instruments Inc., Massachusetts 02062 USA), and all other measures were standardised and automated. Two consecutive MF-BIA measurements (BioScan 920-2, Maltron International Ltd, Essex; using brand new equipment) were taken within 20 minutes of the blood sampling with the subject supine, before serum osmolality results were available (the assessor was blinded to hydration status). MF-BIA measurements were undertaken using the manufacturers recommended method with two electrodes attached to the skin between the talus and the 3rd and 5th digits of the foot and two more attached to the same side between the 3rd and 5th knuckles of the hand and the wrist. Participant information including anthropometrics (measured by investigator or nurse as described in details in the previous chapter), age, gender, and race were entered into the device and the measurements made over a couple of seconds. The recording was repeated a few minutes later. All measurements including blood samples, MF-BIA, and anthropometric measures were carried out in the acute stroke unit at the patients' bed

location. In the stroke unit meals are provided at specific times and patients maybe consuming snacks provided by their visitors at anytime. After measurements were recorded and saved, data were downloaded onto a laptop with Maltron MF-BIA software installed. Impedances at 5, 50 and 100 kHz, and MF-BIA calculations of total body water as a percentage of body weight (TBW%), intracellular water as a percentage of TBW (ICW%) and extracellular water as a percentage of TBW (ECW%) were noted for each recording. Modified Rankin scores (mRs, a measure of disability) were recorded by an occupational therapist.

Ethical Approval for this study was gained from Cambridgeshire I Research Ethics Committee; REC reference number 10/H0304/18 in April 2011. This part of my research was funded by the European Hydration Institute. The funder had no role in designing or conducting the study.

4.3.1 Statistical analysis

All statistical analyses were carried out using PASW 18 for Windows (Polar Engineering and Consulting, formerly known as SPSS). Mean, standard deviation (SD) and range were presented for continuous and number (percentages) were presented for categorical data (hydration status; hydrated, impending, and current dehydration). Percentages of patients diagnosed with impending (serum osmolality 295-300mOsm/kg or serum osmolarity 295-300 mOsm/L) and current dehydration (serum osmolality >300mOsm/kg or serum osmolarity >300 mOsm/L) were calculated. An average was calculated for each two consecutive measurements taken by MF-BIA of same variable for use in subsequent calculations. For the one participant where the two consecutive estimates of TBW% varied by>3% the first data set was used.

The internal consistency of MF-BIA was assessed by carrying out a reliability analysis of the 2 separate measurements of impedance at 5 kHz for each individual; this was

repeated for impedance measures at 50 and 100 kHz, and the MF-BIA equipment calculation of TBW (L).

Impedance outputs (mean from the two readings) were used to calculate TBW (L) and ECW (L) using equations developed for use in older people by Vaché (228) and Visser (229) (as quoted in Ritz(230)), and TBW%, ECW% and ICW% were calculated as percentages of body weight.

TBW%, ECW%, ICW% and ECW: ICW ratio from the internal calculations of the MF-BIA equipment, and those calculated from equations derived specifically for older people were each plotted in 2x2 tables against impending and current serum osmolality and calculated serum osmolarity. These tables were used to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), pre- and post-test probability of each for impending and current dehydration(231). Where any of these values were not calculable due to the presence of zeros in the 2x2 table, 0.1 was added to each cell of the table. As published cut-off points of TBW, ECW and ICW for dehydration are not readily available, three arbitrary cut-off points were selected for each measure (TBW%, ECW%, ICW% and the ratio).

Receiver operating characteristic (ROC) curves were created for both impending and current dehydration, then additional promising cut-off points (where cut-offs may possibly have both sensitivity and specificity >60%) were added to fill in the ROC curves. At the ends of the ROC curve, once either sensitivity or specificity was below 50%, no further outlying points were added. An acceptable cut-off point was considered to be one with both sensitivity and specificity greater than 60% and represented by the point closest to the top left corner of the ROC plot. There is no definition of “good enough” sensitivity and specificity but we chose a minimum of 60% for both as suggesting that the measure was at least promising (232). For all cut-off points I also calculated positive predictive value (PPV), negative predictive value (NPV), and positive and negative post-test probabilities. The results have been reported in line with the STARD reporting guidelines (233) .

4.3.2 Sample Size

This is an observational cross sectional study. No power calculation was performed as there was no data available previously reporting diagnostic accuracy of MF-BIA against serum osmolality. Forty five participants were a realistic sample given the time frame we were able to use for this study. I performed thorough literature search and to my knowledge, there are no previous studies of similar nature performed in this field to allow us to do formal sample size calculations. There are no data on body water values which have been shown to be related to serum osmolality.

Therefore, the objectives of this cross sectional study was to assess the diagnostic accuracy of MF-BIA, to help understand whether MF-BIA can be used to monitor hydration status in place of serum osmolality after stroke.

4.4 Results

4.4.1 Characteristics of the participants

The data from the last 18 of the 45 participants had to be discarded as their TBW% was recorded as 75% or greater (extremely high and unrealistic readings) suggesting an error in MF-BIA impedance readings occurred. This group did not differ in their clinical characteristics (such as type of stroke, age, biochemistry, or presence of peripheral oedema) from other participants. Incorrect data for these last 18 participants were removed leaving 27 participants for analysis (59% males); average age 71.3 (10.7) years. There was a technical malfunctioning in the equipment. No adverse events occurred as a result of any of the tests used.

Of the 27 remaining subjects 12 (44%) were well hydrated (serum osmolality 275 to <295mOsm/kg), 9 (33%) had impending dehydration (serum osmolality 295-300mOsm/kg) and 6 (22%) were dehydrated (serum osmolality >300mOsm/kg), see Table 4.1. Stratified by calculated serum osmolarity 8 (30%) were well hydrated (275 to <295 mOsm/L), 7 (26%) had impending dehydration, and 12 (44%) had current dehydration (>300mOsm/L) (Table 4.1). 11% (n=3) were receiving a nil-by-mouth feeding regimen because of dysphagia. One patient was on pureed diet and 19% (n=5) on soft-mashed diets due to mild dysphagia. Sixty seven percentage (n=18) were on normal oral diets without needing alteration of food texture.

4.4.2 Internal consistency and reliability of MF-BIA measurements

Cronbach's alpha was 0.960 for the reproducibility of the two impedance measures at 5 kHz (n=27), suggesting excellent internal consistency. Cronbach's alpha was similarly excellent for impedance at 50 kHz, and 100 kHz, and TBW (L) (0.974, 0.978 and 0.995 respectively).

	Serum osmolality (mOsm/kg)			Serum osmolarity (mOsm/L)		
	Hydrated	Impending dehydration	Current dehydration	Hydrated	Impending dehydration	Current dehydration
Number of participants	12 (44.4%)	9 (33.3%)	6 (22.2%)	8 (29.6%)	7 (25.9%)	12 (44.4%)
Mean Age (SD), yrs.	72.3 (12.5)	68.7 (8.0)	73.5 (11.4)	71.0 (14.5)	71.1 (9.9)	71.7 (9.1)
Age Range, yrs.	46-92	59-81	59-88	46-92	59-82	59-88
Weight (SD), kg	80.5 (17.1)	74.3 (9.0)	90.0 (13.6)	78.2 (19.6)	81.2 (9.9)	81.7 (14.5)
Height (SD), m	1.7 (0.1)	1.6 (0.1)	1.7 (0.1)	1.7 (0.1)	1.7 (0.1)	1.8 (0.1)
Body Mass Index (SD), kg/m^2	29.1 (5.4)	27.7 (2.4)	31.1 (4.2)	28.6 (5.4)	29.0 (4.2)	29.5 (4.1)
Pre-morbid Rankin Score[a]						
0 (No symptoms).	6	3	1	3	3	4
1-2 (No significant to slight) disability	5	2	2	5	2	2
3-4 (Moderate to moderately severe disability)	0	1	2	0	0	3
5 (Severe disability)	1	0	0	0	1	0
Normal Food	9	6	3	6	5	7
Pureed or soft mashed	1	3	2	1	1	4

Table 4.1. Baseline characteristics

	Serum osmolality (mOsm/kg)			Serum osmolarity (mOsm/L)		
	Hydrated	Impending dehydration	Current dehydration	Hydrated	Impending dehydration	Current dehydration
NIHSS score (stroke severity)[b]						
1-9	9	7	3	6	5	8
10-20	1	1	2	1	0	3
>21	1	0	0	0	1	0

Table 4.1. Baseline characteristics of the 27 included participants stratified by serum osmolality (directly measured) and serum osmolarity (calculated) as being hydrated or having impending or current dehydration.

Table 4.1. [a]n=23 as not all participants were assessed.

[b]n=24 as not all participants were assessed.

4.4.3 Dehydration status, osmolality and osmolarity

Current dehydration (>300 mOsm/L) diagnosed on serum osmolarity criteria was twice as common as when based on serum osmolality (>300 mOsm/kg), the reference standard (Table 4.2). As calculated osmolarity (in mOsm/L) is considered to be equivalent in clinical practice to measured osmolality (in mOsm/kg) we directly compared the two for individuals. Mean calculated serum osmolarity was 298.2±6.9 mOsm/L while mean measured serum osmolality was 295.5±7.5 mOsm/kg. When they were directly compared there was a significant difference of 2.72 (95% CI 0.6 to 4.8; p=0.014). When stratified by hydration status serum osmolarity was greater than osmolality for hydrated participants (mean difference 4.7, 95% CI 1.1 to 8.2, p=0.02) and those with impending dehydration (mean difference 2.9, 95% CI 0.2 to 5.6, p=0.04) but not for those with current dehydration (mean difference -1.4, 95% CI -7.4 to 4.5, p=0.57).

Mean serum sodium, potassium, Creatinine, urea and glucose values were always higher in those with current dehydration than those who were well hydrated, but the mean values for impending dehydration were not always between those of hydrated and currently dehydrated groups. There were few clear patterns in TBW%, ECW%, ICW% or ECW: ICW ratio by serum osmolality or calculated serum osmolarity (Table 4.2).

	Serum Osmolality (mOsm/kg)			Serum Osmolarity (mOsm/L)		
Average (SD)	Hydrated	Impending dehydration	Current dehydration	Hydrated	Impending Dehydration	Current Dehydration
Total Population (%)	12 (44.4%)	9 (33.3%)	6 (22.2%)	8 (29.6%)	7 (25.9%)	12 (44.4%)
Total Body Water% [a]	51.9 (4.0)	52.5 (5.8)	50.7 (4.2)	52.3 (3.7)	51.5 (3.6)	51.7 (5.9)
Extracellular Water % [a]	45.4 (2.8)	46.1 (2.3)	45.3 (1.0)	45.9 (3.1)	44.8 (2.5)	45.9 (1.5)
Intracellular Water% [a]	54.6 (2.8)	53.9 (2.3)	54.7 (1.0)	54.1 (3.1)	55.2 (2.5)	54.1 (1.5)
ECW:ICW	0.83 (0.1)	0.86 (0.1)	0.83 (0.03)	0.85 (0.1)	0.82 (0.08)	0.85 (0.05)
Serum Osmolality mOsm/kg	288.6 (4.3)	298.4 (1.7)	305.0 (2.6)	287.6 (4.8)	296.7 (6.5)	300.1 (5.1)
Serum Osmolarity mOsm/L	293.2 (5.8)	301.3 (4.3)	303.6 (5.2)	290.2 (3.6)	297.1 (1.1)	304.3 (3.9)
Serum Sodium mmol/l	135.8 (2.0)	140.4 (2.0)	138.7 (3.4)	134.9 (1.7)	137.9 (1.1)	140.1 (2.9)
Serum Potassium mmol/l	4.1 (0.3)	4.4 (0.3)	4.6 (0.5)	4.2 (0.43)	4.3 (0.6)	4.4 (0.3)
Serum Creatinine µmol/L	74.3 (15.1)	72.7 (6.6)	90.3 (20.6)	75.4 (10.8)	75.3 (16.9)	79.8 (18.2)
Serum Urea mmol/L	5.1 (1.1)	5.5 (1.4)	8.4 (6.6)	5.1 (1.2)	5.7 (1.0)	6.7 (4.9)
Serum Glucose mmol/L [b]	8.4 (4.3)	6.3 (1.8)	8.8 (3.9)	7.0 (2.6)	7.3 (1.7)	8.7 (4.8)

Table 4.2. Body fluid compartments and serum components stratified by hydration status (serum osmolality (measured) and osmolarity (calculated)) for the 27 participants with valid MF-BIA data.[a] expressed as a percentage of body weight, [b] n=26

4.4.4 Diagnostic accuracy of MF-BIA vs. dehydration by Serum Osmolality

No cut-off point for TBW%, ICW%, ECW% or ECW: ICW ratio (calculated by the MF-BIA equipment) had both a sensitivity and specificity above 60% for impending (Table 4.3) or current (Table 4.4) dehydration assessed by (measured) serum osmolality. None of the impending dehydration ROC curves neared the upper left hand corner. Figure 4.1 shows the ROC plot for ICW% for impending dehydration by serum osmolality and Figure 4.2 shows the ROC plot for ECW% for current dehydration by serum osmolality).

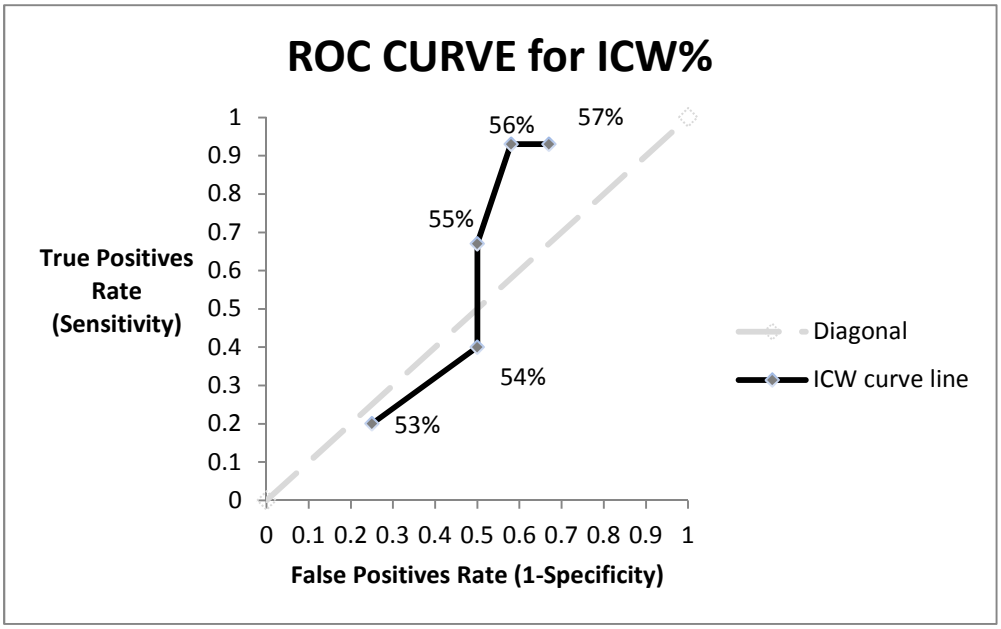

Figure 4.1. ROC curve assessing the diagnostic accuracy of MF-BIA assessment of intracellular water as a percentage of total body water (ICW% by the Maltron equations) in estimating impending dehydration (≥295 mOsm/kg).

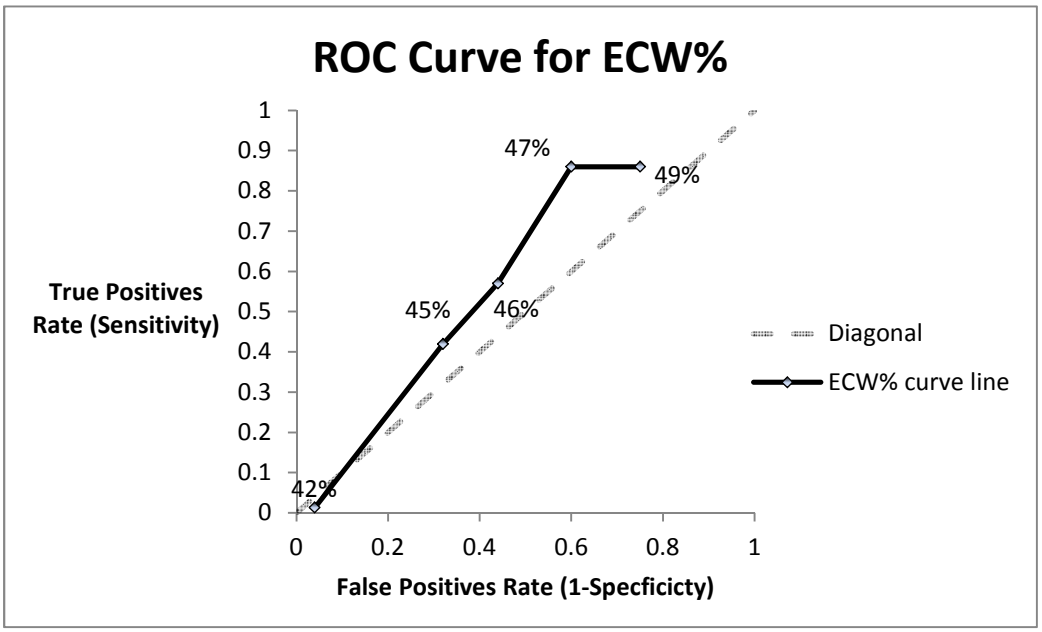

Figure 4.2. ROC curve assessing diagnostic accuracy of MF-BIA assessment of extracellular water as a percentage of total body water (ECW%) in estimating current dehydration (>300 mOsm/kg).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.13	0.92	0.67	0.46	0.56	0.54	0.67
50%	0.33	0.75	0.63	0.47	0.56	0.53	0.63
52%	0.40	0.67	0.6	0.47	0.56	0.53	0.60
54%	0.80	0.25	0.57	0.50	0.56	0.50	0.57
55%	0.87	0.08	0.54	0.33	0.56	0.67	0.54
57%	0.93	0.08	0.56	0.50	0.56	0.50	0.56
ICW%							
53%	0.20	0.75	0.50	0.43	0.56	0.57	0.50
54%	0.40	0.50	0.50	0.40	0.56	0.60	0.50
55%	0.67	0.50	0.63	0.55	0.56	0.46	0.63
56%	0.93	0.42	0.67	0.83	0.56	0.17	0.67
57%	0.93	0.33	0.64	0.80	0.56	0.20	0.64

Table 4.3. Diagnostic accuracy of MF-BIA in diagnosing impending dehydration (295-300 mOsm/kg), continued

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
ECW%							
42%	0.00	0.92	0.00	0.42	0.56	0.58	0.00
45%	0.33	0.50	0.46	0.38	0.56	0.63	0.46
46%	0.60	0.50	0.60	0.50	0.56	0.50	0.60
47%	0.80	0.33	0.60	0.57	0.56	0.43	0.60
50%	1.00	0.08	0.58	1.00	0.56	0.00	0.58
ECW:ICW							
0.60 [a]	0.01	0.99	0.50	0.45	0.56	0.56	0.50
0.80	0.13	0.58	0.29	0.35	0.56	0.65	0.29
0.85	0.60	0.50	0.60	0.50	0.56	0.50	0.60
0.90	0.80	0.25	0.57	0.50	0.56	0.50	0.57
1.10 [a]	0.99	0.01	0.56	0.50	0.56	0.50	0.56

Table 4.3. Diagnostic accuracy of MF-BIA measures (at several cut-off points) in diagnosing impending dehydration (295-300 mOsm/kg). Based on internal Maltron equations for TBW, ICW and ECW, and on the 27 participants with reliable MF-BIA data. [a] 0.1 fraction added to all 4 cells of the 2x2 table due to the presence of a zero in one of the cells that prevents at least one of the properties being calculated. PPV: positive predictive value. NPV: negative predictive value. TBW was expressed as a percentage of body weight (TBW %), and ICW and ECW were expressed as a percentage of total body water (ICW%, ECW%).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.17	0.91	0.33	0.79	0.22	0.21	0.33
50%	0.33	0.71	0.25	0.79	0.22	0.21	0.25
52%	0.33	0.62	0.20	0.77	0.22	0.24	0.20
53%	0.67	0.48	0.27	0.83	0.22	0.17	0.27
54%	1.00	0.29	0.29	1.00	0.22	0.00	0.29
55%	1.00	0.14	0.25	1.00	0.22	0.00	0.25
ICW%							
53%	0.00	0.71	0.00	0.71	0.22	0.29	0.00
55%	0.50	0.38	0.19	0.73	0.22	0.27	0.19
56%	1.00	0.29	0.28	1.00	0.22	0.00	0.29
57%	1.00	0.23	0.27	1.00	0.22	0.00	0.27

Table 4.4. Diagnostic accuracy of MF-BIA measures in diagnosing current dehydration (>300mOsm/kg), continued

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test probability (-ve)	Post-test probability (+ve)
ECW%							
42%	0.00	0.95	0.00	0.77	0.22	0.23	0.00
45%	0.50	0.62	0.27	0.81	0.22	0.19	0.27
46%	0.67	0.48	0.27	0.83	0.22	0.17	0.27
47%	1.00	0.33	0.30	1.00	0.22	0.00	0.30
49%	1.00	0.10	0.24	1.00	0.22	0.00	0.24
ECW:ICW							
0.60 [a]	0.02	1.00	0.50	0.78	0.23	0.22	0.50
0.75	0.00	0.71	0.00	0.71	0.22	0.29	0.00
0.85	0.67	0.48	0.27	0.83	0.22	0.17	0.27
0.90	1.00	0.29	0.29	1.00	0.22	0.00	0.29
0.95	1.00	0.14	0.25	1.00	0.22	0.00	0.25

Table 4.4. Diagnostic accuracy of MF-BIA measures (at several cut-off points) in diagnosing current dehydration (>300mOsm/kg). Based on internal Maltron equations for TBW, ICW and ECW, and on the 27 participants with reliable MF-BIA data.

[a] 0.1 fraction added to all 4 cells of the 2x2 table due to the presence of a zero in one of the cells.

PPV: positive predictive value. NPV: negative predictive value. TBW was expressed as a percentage of body weight (TBW %), and ICW and ECW were expressed as a percentage of total body water (ICW%, ECW%).

Diagnostic accuracy for TBW%, ICW%, ECW% and ECW: ICW calculated using the equations specifically developed for older people (228-230)(rather than those programmed into the MF-BIA equipment) compared to serum osmolality resulted in one cut-off point with both sensitivity and specificity >60% for current dehydration (Table 4.5), and none for impending dehydration (Table 4.6). TBW% with a cut-off at 46% of body weight, was diagnostic of current dehydration by osmolality with sensitivity of 67% (95% CI 49% to 85%), specificity 62% (95% CI 44% to 80%) (Table 5, Figure 4.3). The positive likelihood ratio (LR^+) for this cut-off was 1.75 and negative likelihood ratio (LR^-) was 0.54.

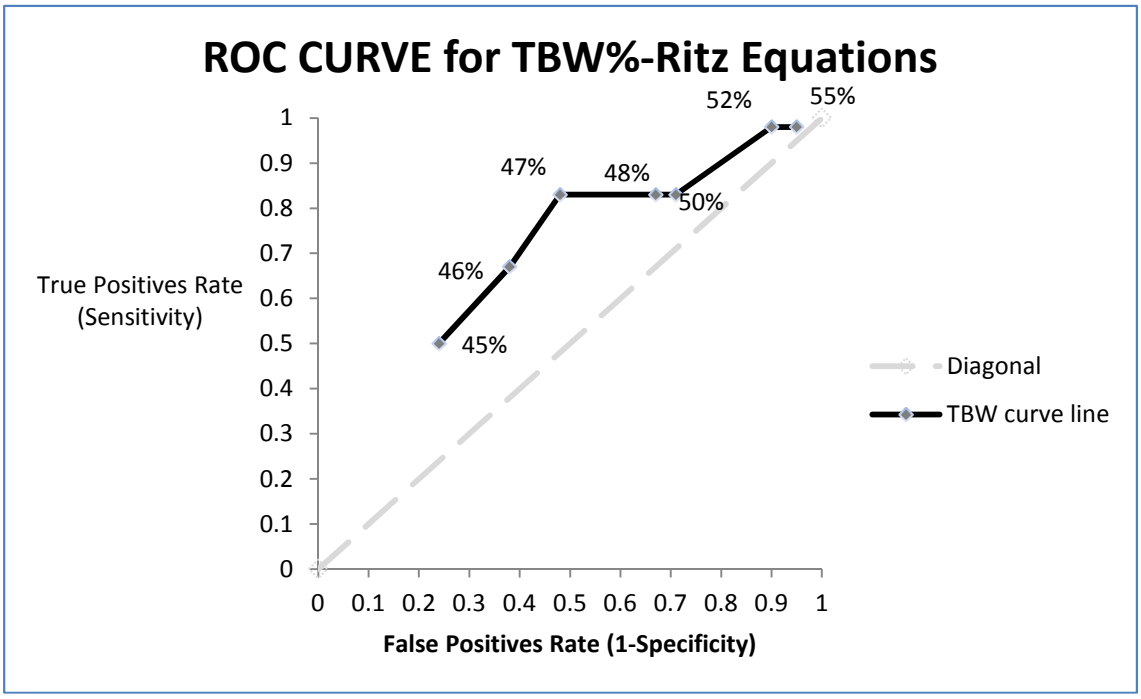

Figure 4.3. ROC curve assessing diagnostic accuracy of TBW% calculated from equations for older people15 against current dehydration by serum osmolality (>300 mOsm/kg). The 46% cut-off point had a sensitivity of 67% (95% CI 49%-85%), and specificity of 62% (95% CI 44%-80%).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.50	0.76	0.38	0.84	0.22	0.16	0.38
46%	0.67	0.62	0.33	0.87	0.22	0.13	0.33
47%	0.83	0.52	0.33	0.92	0.22	0.08	0.33
48%	0.83	0.33	0.26	0.88	0.22	0.13	0.26
50%	0.83	0.29	0.25	0.86	0.22	0.14	0.25
52%	1.00	0.10	0.24	1.00	0.22	0.00	0.24
55%	1.00	0.05	0.23	1.00	0.22	0.00	0.23
ICW%							
25%	0.33	0.76	0.29	0.8	0.22	0.20	0.29
26%	0.50	0.71	0.33	0.83	0.22	0.17	0.33
27%	0.67	0.52	0.29	0.85	0.22	0.15	0.29
28%	0.67	0.33	0.22	0.78	0.22	0.22	0.22
29%	0.83	0.29	0.25	0.86	0.22	0.14	0.25
30%	1.00	0.14	0.25	1.00	0.22	0.00	0.25

Table 4.5. Diagnostic accuracy of Ritz measures (at several cut-off points) in diagnosing current dehydration (>300mOsm/kg), continued

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
ECW%							
18%	0.33	0.76	0.29	0.80	0.22	0.20	0.29
19%	0.50	0.67	0.30	0.82	0.22	0.18	0.30
20%	0.67	0.48	0.27	0.83	0.22	0.17	0.27
21%	0.83	0.19	0.23	0.80	0.22	0.20	0.23
22%	1.00	0.14	0.25	1.00	0.22	0.00	0.25
ECW:ICW							
0.60	0.02	1.00	0.50	0.78	0.22	0.22	0.50
0.70	0.33	0.67	0.22	0.78	0.22	0.22	0.22
0.75	0.67	0.52	0.29	0.85	0.22	0.15	0.29
0.80	0.67	0.38	0.24	0.80	0.22	0.20	0.24
0.85	1.00	0.19	0.26	1.00	0.22	0.00	0.26

Table 4.5. Diagnostic accuracy of Ritz measures (at several cut-off points) in diagnosing current dehydration (>300mOsm/kg) based on alternate equations for TBW, ICW and ECW in older people (Ritz 2001), and on the 27 participants with reliable MF-BIA data.

[a] 0.1 fraction added to all 4 cells of the 2x2 table due to the presence of a zero in one of the cells that prevents at least one of the properties being calculated. PPV: positive predictive value. NPV: negative predictive value. TBW, ICW and ECW were all expressed as percentages of body weight (TBW%, ICW%, ECW%).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.40	0.83	0.75	0.53	0.56	0.47	0.75
46%	0.53	0.67	0.67	0.53	0.56	0.47	0.67
47%	0.67	0.58	0.67	0.58	0.56	0.42	0.67
48%	0.80	0.42	0.63	0.63	0.56	0.38	0.63
50%	0.87	0.42	0.65	0.71	0.56	0.29	0.65
51%	0.93	0.17	0.58	0.67	0.56	0.33	0.58
52%	0.93	0.08	0.56	0.50	0.56	0.50	0.56
ICW%							
25%	0.33	0.83	0.71	0.5	0.56	0.50	0.71
27%	0.60	0.58	0.64	0.54	0.56	0.46	0.64
28%	0.73	0.42	0.61	0.56	0.56	0.44	0.61
29%	0.80	0.33	0.60	0.57	0.56	0.43	0.60
30%	0.93	0.17	0.58	0.67	0.56	0.33	0.58
32%*	0.99	0.01	0.56	0.50	0.56	0.50	0.56

Table 4.6. Diagnostic accuracy of Ritz measures (at several cut-off points) in diagnosing impending dehydration (295-300mOsm/kg), continued

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
ECW%							
20%	0.60	0.50	0.60	0.50	0.56	0.50	0.60
21%	0.87	0.25	0.59	0.60	0.56	0.40	0.59
22%	0.93	0.17	0.58	0.67	0.56	0.33	0.58
23%	0.93	0.08	0.56	0.50	0.56	0.50	0.56
25% [a]	0.93	0.01	0.54	0.08	0.56	0.92	0.54
ECW:ICW							
0.60	0.01	0.99	0.50	0.45	0.56	0.56	0.50
0.75	0.53	0.50	0.57	0.46	0.56	0.54	0.57
0.80	0.60	0.33	0.53	0.40	0.56	0.60	0.53
0.85	0.80	0.08	0.52	0.25	0.56	0.75	0.52
0.90 [a]	0.99	0.01	0.56	0.50	0.56	0.50	0.56

Table 4.6. Diagnostic accuracy of Ritz measures (at several cut-off points) in diagnosing impending dehydration (295-300mOsm/kg) based on alternate equations for TBW, ICW and ECW in older people (Ritz 2001), and on the 27 participants with reliable MF-BIA data.

[a] 0.1 fraction added to all 4 cells of the 2x2 table due to the presence of a zero in one of the cells that prevents at least one of the properties being calculated. PPV: positive predictive value. NPV: negative predictive value. TBW, ICW and ECW were all expressed as percentages of body weight (TBW%, ICW%, ECW%).

4.4.5 Diagnostic Accuracy of MF-BIA vs. dehydration assessed by calculated Serum Osmolarity

Diagnostic accuracy for water fractions calculated using the equations for older people used in Ritz 2001 against calculated serum osmolarity resulted in one cut-off point with both sensitivity and specificity of at least 60%. TBW% at 47% of body weight was diagnostic of impending dehydration by calculated osmolarity with sensitivity and specificity of 63% (95% CI 45% to 81%) (Table 4.7; Figure 4.4). The LR+ and LR – were 1.7 and 0.6 respectively for this cut-off. No cut-offs were accurate for current dehydration (Table 4.8).

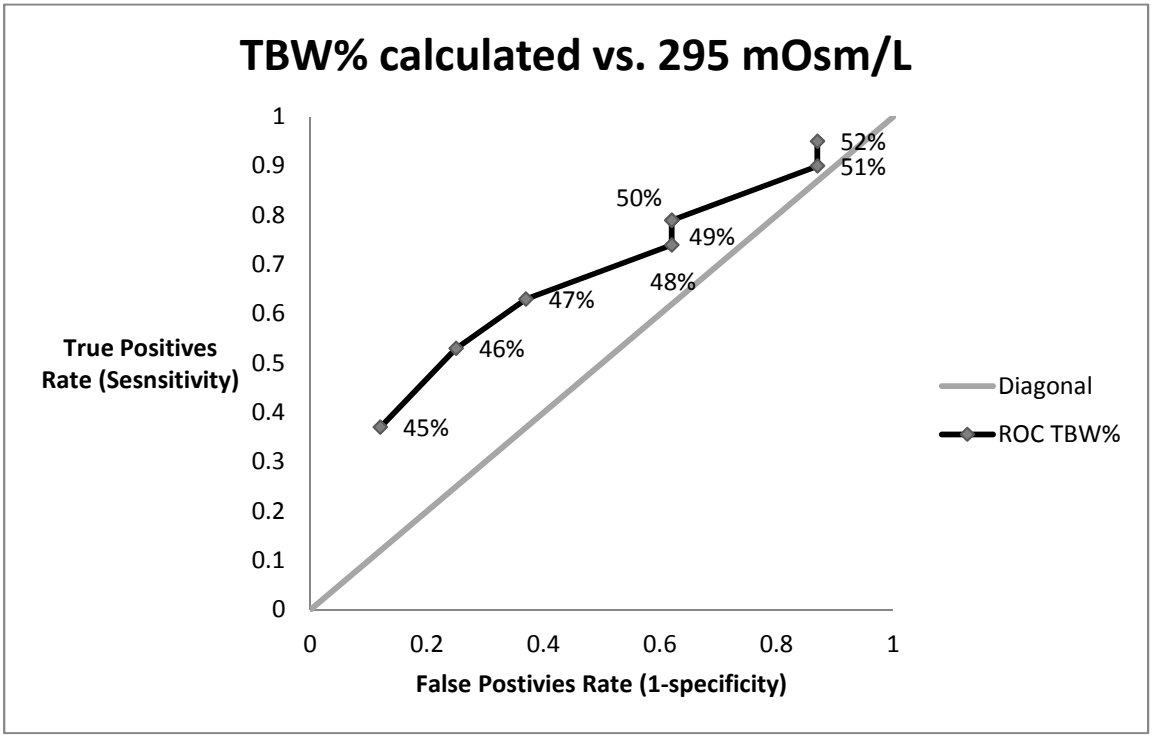

Figure 4.4. ROC curve assessing diagnostic accuracy of TBW% calculated from Ritz 2001 equations for older people against impending dehydration as calculated by serum osmolarity (≥295 mOsm/L). The 47% cut off point had a sensitivity and specificity of 63% (95%CI 45% to 81%) each.

No cut-off points for TBW%, ICW%, ECW% or ECW: ICW as calculated by the MF-BIA equipment against calculated serum osmolarity had a sensitivity and specificity above 60% for impending (≥295 mOsm/L serum osmolarity, (Table 4.9) or current dehydration (>300 mOsm/L, Table 4.10).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.37	0.88	0.88	0.37	0.70	0.63	0.88
46%	0.53	0.75	0.83	0.40	0.70	0.60	0.83
47%	0.63	0.63	0.80	0.42	0.70	0.58	0.80
48%	0.74	0.38	0.74	0.38	0.70	0.63	0.74
49%	0.79	0.38	0.75	0.43	0.70	0.57	0.75
50%	0.79	0.38	0.75	0.43	0.70	0.57	0.75
51%	0.90	0.13	0.71	0.33	0.70	0.67	0.71
52%	0.95	0.13	0.72	0.50	0.70	0.50	0.72

Table 4.7. Diagnostic accuracy of Ritz measures (at several cut-off points) in diagnosing impending dehydration (295-300mOsm/l), continued

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
ICW%							
25%	0.32	0.88	0.86	0.35	0.70	0.65	0.86
27%	0.53	0.50	0.71	0.31	0.70	0.69	0.71
28%	0.68	0.38	0.72	0.33	0.70	0.67	0.72
29%	0.74	0.25	0.70	0.29	0.70	0.71	0.70
30%	0.95	0.25	0.75	0.67	0.70	0.33	0.75
ECW%							
20%	0.58	0.50	0.73	0.33	0.70	0.67	0.73
21%	0.84	0.25	0.73	0.40	0.70	0.60	0.73
22%	0.90	0.13	0.71	0.33	0.70	0.67	0.71
23%	0.95	0.13	0.72	0.50	0.70	0.50	0.72

Table 4.7. Diagnostic accuracy of Ritz measures (at several cut-off points) in diagnosing impending dehydration (295-300mOsm/l), continued

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
ECW:ICW							
0.6	0.01	0.99	0.50	0.30	0.70	0.70	0.50
0.75	0.53	0.50	0.71	0.31	0.70	0.70	0.71
0.8	0.63	0.38	0.71	0.30	0.70	0.70	0.71
0.85	0.84	0.13	0.70	0.25	0.70	0.75	0.70
0.9 [a]	0.99	0.01	0.70	0.50	0.70	0.50	0.70

Table 4.7. Diagnostic accuracy of Ritz measures (at several cut-off points) in diagnosing impending dehydration (295-300mOsm/l) based on alternate equations for TBW, ICW and ECW in older people (Ritz 2001), and on the 27 participants with reliable MF-BIA data.

[a] 0.1 fraction added to all 4 cells of the 2x2 table due to the presence of a zero in one of the cells that prevents at least one of the properties being calculated. PPV: positive predictive value. NPV: negative predictive value. TBW, ICW and ECW were all expressed as percentages of body weight (TBW%, ICW%, ECW%).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.42	0.80	0.63	0.63	0.44	0.37	0.63
46%	0.50	0.60	0.50	0.60	0.44	0.40	0.50
47%	0.67	0.53	0.53	0.67	0.44	0.33	0.53
48%	0.83	0.40	0.53	0.75	0.44	0.25	0.53
50%	0.91	0.01	0.42	0.08	0.44	0.37	0.63
ICW%							
25%	0.33	0.80	0.57	0.60	0.44	0.40	0.57
26%	0.42	0.73	0.56	0.61	0.44	0.39	0.56
27%	0.58	0.53	0.50	0.62	0.44	0.38	0.50
28%	0.75	0.40	0.50	0.67	0.44	0.33	0.50
29%	0.83	0.33	0.50	0.71	0.44	0.29	0.50
30% [a]	0.92	0.13	0.46	0.67	0.44	0.33	0.46

Table 4.8. Diagnostic accuracy of MF-BIA (Ritz 2001) against measured serum osmolality (current dehydration), continued

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
ECW%							
19%	0.42	0.67	0.50	0.59	0.44	0.41	0.50
20%	0.58	0.47	0.47	0.58	0.44	0.42	0.47
21%	0.83	0.20	0.46	0.60	0.44	0.40	0.46
22% [a]	0.92	0.13	0.46	0.67	0.44	0.33	0.46
ECW:ICW							
0.75	0.50	0.47	0.43	0.54	0.44	0.46	0.43
0.8	0.58	0.33	0.41	0.50	0.44	0.50	0.41
0.85	0.83	0.13	0.44	0.50	0.44	0.50	0.44
0.9 [a]	0.99	0.01	0.45	0.50	0.44	0.50	0.45

Table 4.8. Diagnostic accuracy of MF-BIA (Ritz 2001) against measured serum osmolality (current dehydration) at several cut-off points in diagnosing current dehydration based on alternate equations for TBW, ICW and ECW in older people (Ritz 2001) against serum Osmolarity (>300 mOsm/L). [a] 0.1 fraction added to all 4 cells of the 2x2 table due to the presence of a zero in one of the cells that prevents at least one of the properties being calculated. PPV: positive predictive value. NPV: negative predictive value. TBW, ICW and ECW were all expressed as percentages of body weight (TBW%, ICW%, ECW%).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.16	0.99	0.97	0.34	0.70	0.67	0.97
50%	0.32	0.75	0.75	0.32	0.70	0.68	0.75
52%	0.37	0.63	0.70	0.29	0.70	0.71	0.70
53%	0.53	0.38	0.67	0.25	0.70	0.75	0.67
54%	0.79	0.25	0.71	0.33	0.70	0.67	0.71
55%	0.90	0.13	0.71	0.33	0.70	0.67	0.71
ICW%							
53%	0.16	0.63	0.50	0.24	0.70	0.76	0.50
55%	0.58	0.38	0.69	0.27	0.70	0.73	0.69
57%	0.84	0.25	0.73	0.40	0.70	0.60	0.73
59%	0.99	0.01	0.70	0.50	0.70	0.50	0.70

Table 4.9. Diagnostic Accuracy of MF-BIA against calculated serum osmolarity (impending dehydration), continued

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test probability (-ve)	Post-test probability (+ve)
ECW%							
50%	1.00	0.13	0.73	1.00	0.70	0.00	0.73
47%	0.84	0.38	0.76	0.50	0.70	0.50	0.76
46%	0.21	0.99	0.98	0.35	0.70	0.65	0.98
45%	0.42	0.63	0.73	0.31	0.70	0.69	0.73
42%	0.00	0.88	0.00	0.27	0.70	0.73	0.00
ECW:ICW							
0.6	0.01	0.99	0.50	0.30	0.70	0.70	0.50
0.75	0.16	0.63	0.50	0.24	0.70	0.76	0.50
0.80	0.21	0.63	0.57	0.25	0.70	0.75	0.57
0.9	0.84	0.38	0.76	0.50	0.70	0.50	0.76
1.1	0.99	0.01	0.70	0.50	0.70	0.50	0.70

Table 4.9. Diagnostic Accuracy of MF-BIA against calculated serum osmolarity (impending dehydration) diagnostic accuracy of MF-BIA measures (at several cut-off points) by Maltron BioScan 920-2 in diagnosing impending dehydration against calculated serum osmolarity (>295mOsm/L).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.25	0.99	0.97	0.62	0.45*	0..38	0.97
50%	0.33	0.73	0.50	0.58	0.44	0.42	0.5
52%	0.42	0.67	0.50	0.59	0.44	0.42	0.5
53%	0.42	0.67	0.47	0.58	0.44	0.42	0.47
54%	0.75	0.20	0.43	0.50	0.44	0.5	0.43
55%	0.83	0.07	0.42	0.33	0.44	0.67	0.42
ICW%							
53%	0.17	0.73	0.33	0.52	0.44	0.48	0.33
55%	0.67	0.47	0.50	0.64	0.44	0.36	0.5
57%	1.00	0.33	0.55	1.00	0.44	0.00	0.55

Table 4.10. Diagnostic accuracy of MF-BIA measures in diagnosing current dehydration against calculated serum osmolarity (>300mOsm/L), continued

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test probability (-ve)	Post-test probability (+ve)
ECW%							
49%	0.92	0.13	0.46	0.67	0.44	0.33	0.46
47%	0.83	0.26	0.48	0.67	0.44	0.33	0.48
46%	0.58	0.47	0.47	0.58	0.44	0.42	0.47
45%	0.33	0.53	0.36	0.50	0.44	0.50	0.36
42%	0.00	0.93	0.0	0.54	0.44	0.46	0.00
ECW:ICW							
0.6	0.01*	0.99	0.50	0.56	0.45*	0.44	0.50
0.75	0.00	0.60	0.00	0.43	0.44	0.57	0.00
0.85	0.58	0.47	0.47	0.58	0.44	0.42	0.47
0.9	0.83	0.27	0.48	0.67	0.44	0.33	0.48
0.95	0.92	0.13	0.46	0.67	0.44	0.33	0.46

Table 4.10. Diagnostic accuracy of MF-BIA measures by Maltron BioScan 920-2 (at several cut-off points) in diagnosing current dehydration against calculated serum osmolarity (>300mOsm/L).

4.4.6 Diagnostic accuracy of MF-BIA by men and women:

Tables 4.11 a and 4.11 b present the diagnostic accuracy of MF-BIA against measured serum Osmolality (impending dehydration) in men and women, TBW% (as a percentage of body weight) are based on internal equations in Maltron Bio-Scan 92-2 for TBW. None of the cut off points for either men or women had a sensitivity and specificity >60% respectively. Tables 4.12 a and 4.12 b presents the diagnostic accuracy of MF-BIA for men and women at several TBW% cut off points (as a percentage of body weight) against measured serum osmolality in diagnosing current dehydration (>300mOsm/kg). None of the cut off points for either men or women had a sensitivity and specificity >60% respectively.

Tables 4.13 a and 4.13 b presents the diagnostic accuracy of MF-BIA for men and women at several TBW% cut off points (as a percentage of body weight) based on alternate equations for TBW in older people (Ritz 2001) against measured serum osmolality in diagnosing impending dehydration (295-300mOsm/kg). In men only at TBW of 47% cut off sensitivity and specify was >60%, but no TBW% cut off for women was >60%. Tables 4.14 a and 4.14 b presents the diagnostic accuracy of MF-BIA for men and women at several TBW% cut off points (as a percentage of body weight) based on alternate equations for TBW in older people (Ritz 2001) against measured serum osmolality in diagnosing current dehydration (>300mOsm/kg). In men only sensitivity and specificity was >60% at 46 and 47% TBW% cut off points.

Tables 4.15 a and 4.15 b presents the diagnostic accuracy of MF-BIA for men and women at several TBW% cut off points (as a percentage of body weight) based on alternate equations for TBW in older people (Ritz 2001) against calculated serum osmolarity in diagnosing impending dehydration (295-300mOsm/L). In women only sensitivity and specificity was >60% at 45% TBW% cut off points. Tables 4.16 a and 4.16 b presents the diagnostic accuracy of MF-BIA for men and women at several TBW% cut off points (as a percentage of body weight) based on alternate equations for TBW in older people (Ritz 2001) against calculated serum osmolarity in diagnosing

current dehydration (>300mOsm/L). In men only sensitivity and specificity was >60% at 47% TBW% cut off points.

Tables 4.17 a and 4.17 b presents the diagnostic accuracy of MF-BIA for men and women at several TBW% cut off points (as a percentage of body weight) based on MF-BIA internal equations for TBW against calculated serum osmolarity in diagnosing impending dehydration (295-300mOsm/L). In women only sensitivity and specificity was >60% at 49%, 50%, and 52% TBW% cut off points showing very similar sensitivity and specificity. Tables 4.18 a and 4.18 b presents the diagnostic accuracy of MF-BIA for men and women at several TBW% cut off points (as a percentage of body weight) based on MF-BIA internal equations for TBW against calculated serum osmolarity in diagnosing current dehydration (>300mOsm/L). None of the TBW% cut off points showed a sensitivity and specificity >60%.

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.13	0.99	0.92	0.53	0.50	0.47	0.92
50%	0.13	0.88	0.50	0.50	0.50	0.50	0.50
52%	0.25	0.75	0.50	0.50	0.50	0.50	0.50
53%	0.38	0.50	0.43	0.44	0.50	0.56	0.43
54%	0.75	0.38	0.55	0.6	0.50	0.40	0.55
55%	0.88	0.13	0.50	0.50	0.50	0.50	0.50

Table 4.11a. The diagnostic accuracy of MF-BIA against measured serum Osmolality (impending dehydration) in men at several TBW% cut off points (as a percentage of body weight) are based on internal equations in Maltron Bio-Scan 92-2 for TBW.

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.14	0.75	0.50	0.33	0.64	0.67	0.50
50%	0.57	0.50	0.67	0.40	0.64	0.60	0.67
52%	0.57	0.50	0.67	0.40	0.64	0.60	0.67
54%	0.57	0.50	0.67	0.0.40	0.64	0.60	0.67
55%	0.86	0.02	0.60	0.09	0.63*	0.92	0.60

Table 4.11b. The diagnostic accuracy of MF-BIA against measured serum Osmolality (impending dehydration) in women at several TBW% cut off points (as a percentage of body weight) based on internal equations in Maltron Bio-Scan 92-2 for TBW.

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.34	0.99	0.92	0.86	0.20*	0.14	0.92
50%	0.33	0.92	0.50	0.86	0.19	0.14	0.5
52%	0.33	0.77	0.25	0.83	0.19	0.17	0.25
53%	0.67	0.62	0.29	0.89	0.19	0.11	0.29
54%	1.00	0.38	0.27	1.00	0.19	0.00	0.27
55%	1.00	0.15	0.21	1.00	0.19	0.00	0.21

Table 4.12a. The diagnostic accuracy of MF-BIA in men at several TBW% cut off points (as a percentage of body weight) against measured serum osmolality in diagnosing current dehydration (>300mOsm/kg).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.00	0.75	0.00	0.67	0.27	0.33	0.00
50%	0.33	0.38	0.17	0.60	0.27	0.40	0.17
52%	0.33	0.38	0.17	0.60	0.27	0.40	0.17
53%	0.67	0.25	0.25	0.67	0.27	0.33	0.25
54%	1.00	0.13	0.30	1.00	0.27	0.00	0.30
55%	1.00	0.13	0.30	1.00	0.27	0.00	0.30

Table 4.12b. The diagnostic accuracy of MF-BIA in women at several TBW% cut off points (as a percentage of body weight) against measured serum osmolality in diagnosing current dehydration (>300mOsm/kg).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.13	0.99	0.92	0.53	0.50	0.47	0.92
46%	0.38	0.88	0.75	0.58	0.50	0.42	0.75
47%	0.63	0.75	0.71	0.67	0.50	0.33	0.71
48%	0.75	0.50	0.60	0.67	0.50	0.33	0.60
49%	0.88	0.50	0.64	0.80	0.50	0.20	0.64
50%	0.88	0.50	0.64	0.80	0.50	0.20	0.64
51%	0.88	0.25	0.54	0.67	0.50	0.33	0.54
52%	0.88	0.13	0 .50	0.50	0.50	0.50	0.50

Table 4.13a. The diagnostic accuracy of MF-BIA in men at several TBW% cut off points (as a percentage of body weight) based on alternate equations for TBW in older people (Ritz 2001) against measured serum osmolality in diagnosing impending dehydration (295-300mOsm/kg).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
TBW%							
40%	0.29	0.50	0.50	0.29	0.64	0.71	0.50
43%	0.29	0.50	0.50	0.29	0.64	0.71	0.50
44%	0.43	0.50	0.60	0.33	0.64	0.67	0.60
45%	0.71	0.50	0.71	0.50	0.64	0.50	0.71
47%	0.71	0.25	0.63	0.33	0.64	0.67	0.63
48%	0.86	0.25	0.67	0.50	0.64	0.50	0.67
50%	0.86	0.25	0.67	0.50	0.64	0.50	0.67
52%	0.99	0.02	0.63	0.50	0.63*	0.50	0.63

Table 4.13b. The diagnostic accuracy of MF-BIA in women at several TBW% cut off points (as a percentage of body weight) based on alternate equations for TBW in older people (Ritz 2001) against measured serum osmolality in diagnosing impending dehydration (295-300mOsm/kg).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.34	0.99	0.92	0.86	0.20*	0.14	0.92
46%	0.67	0.85	0.50	0.92	0.19	0.08	0.50
47%	1.00	0.69	0.43	1.00	0.19	0.00	0.43
48%	1.00	0.46	0.30	1.00	0.19	0.00	0.30
50%	1.00	0.39	0.27	1.00	0.19	0.00	0.27
52%	1.00	0.15	0.21	1.00	0.19	0.00	0.21
55%	1.00	0.08	0.20	1.00	0.19	0.00	0.20

Table 4.14a. The diagnostic accuracy of MF-BIA in men at several TBW% cut off points (as a percentage of body weight) based on alternate equations for TBW in older people (Ritz 2001) against measured serum osmolality in diagnosing current dehydration (>300mOsm/kg).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
TBW%							
40%	0.33	0.63	0.25	0.71	0.27	0.29	0.25
43%	0.33	0.63	0.25	0.71	0.27	0.29	0.25
44%	0.33	0.50	0.20	0.67	0.27	0.23	0.20
45%	0.67	0.38	0.29	0.75	0.27	0.25	0.29
47%	0.67	0.25	0.25	0.67	0.27	0.33	0.25
48%	0.67	0.13	0.22	0.50	0.27	0.50	0.22
50%	0.67	0.13	0.22	0.50	0.27	0.50	0.22
52%	0.97	0.01	0.28	0.50	0.28*	0.50	0.28
55%	0.97	0.01	0.28	0.50	0.28*	0.50	0.28

Table 4.14b. The diagnostic accuracy of MF-BIA in women at several TBW% cut off points (as a percentage of body weight) based on alternate equations for TBW in older people (Ritz 2001) against measured serum osmolality in diagnosing current dehydration (>300mOsm/kg)

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.09	0.98	0.92	0.34	0.68*	0.66	0.92
46%	0.37	0.98	0.98	0.42	0.68*	0.58	0.98
47%	0.55	0.80	0.86	0.44	0.69	0.56	0.86
48%	0.64	0.40	0.70	0.33	0.69	0.67	0.70
49%	0.72	0.40	0.73	0.40	0.69	0.60	0.73
50%	0.72	0.40	0.72	0.40	0.69	0.60	0.73
51%	0.82	0.20	0.69	0.33	0.69	0.67	0.69
52%	0.91	0.20	0.71	0.50	0.69	0.50	0.71

Table 4.15a. The diagnostic accuracy of MF-BIA in men at several TBW% cut off points (as a percentage of body weight) based on alternate equations for TBW in older people (Ritz 2001) against calculated serum osmolarity in diagnosing impending dehydration (295-300mOsm/L).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
TBW%							
40%	0.38	0.67	0.75	0.29	0.73	0.71	0.75
43%	0.38	0.67	0.75	0.29	0.73	0.71	0.75
44%	0.50	0.67	0.80	0.33	0.73	0.67	0.80
45%	0.75	0.67	0.86	0.50	0.73	0.50	0.86
46%	0.75	0.33	0.75	0.33	0.73	0.67	0.75
47%	0.75	0.33	0.75	0.33	0.73	0.67	0.75
48%	0.88	0.33	0.78	0.50	0.73	0.50	0.78
50%	0.88	0.33	0.78	0.50	0.73	0.50	0.78
52%	0.99	0.03	0.72	0.50	0.72*	0.50	0.72

Table 4.15b. The diagnostic accuracy of MF-BIA in women at several TBW% cut off points (as a percentage of body weight) based on alternate equations for TBW in older people (Ritz 2001) against calculated serum osmolarity in diagnosing impending dehydration (295-300mOsm/L).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.18	0.99	0.92	0.66	0.38	0.34	0.92
46%	0.33	0.80	0.50	0.67	0.38	0.33	0.50
47%	0.67	0.70	0.57	0.78	0.38	0.22	0.57
48%	0.83	0.50	0.50	0.83	0.38	0.17	0.50
49%	0.83	0.40	0.46	0.8	0.38	0.20	0.46
50%	0.83	0.40	0.46	0.80	0.38	0.20	0.46

Table 4.16a. The diagnostic accuracy of MF-BIA for men at several TBW% cut off points (as a percentage of body weight) based on alternate equations for TBW in older people (Ritz 2001) against calculated serum osmolarity in diagnosing current dehydration (>300mOsm/L).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test Probability (-ve)	Post-test probability (+ve)
TBW%							
40%	0.33	0.60	0.50	0.43	0.55	0.57	0.50
43%	0.33	0.60	0.50	0.43	0.55	0.57	0.50
44%	0.33	0.40	0.40	0.33	0.55	0.67	0.40
45%	0.67	0.40	0.57	0.50	0.55	0.50	0.57
46%	0.67	0.20	0.50	0.33	0.55	0.67	0.50
47%	0.67	0.20	0.50	0.33	0.55	0.67	0.50
48%	0.83	0.20	0.56	0.50	0.55	0.50	0.56
49%	0.83	0.20	0.56	0.50	0.55	0.50	0.56
50%	0.83	0.20	0.56	0.50	0.55	0.50	0.56

Table 4.16b. The diagnostic accuracy of MF-BIA in women at several TBW% cut off points (as a percentage of body weight) based on alternate equations for TBW in older people (Ritz 2001) against calculated serum osmolarity in diagnosing current dehydrati

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.09	0.98	0.92	0.34	0.68*	0.66	0.92
50%	0.09	0.80	0.50	0.29	0.69	0.71	0.50
52%	0.18	0.60	0.50	0.25	0.69	0.75	0.50
53%	0.36	0.40	0.57	0.22	0.69	0.78	0.57
54%	0.73	0.40	0.73	0.40	0.69	0.60	0.73
55%	0.90	0.20	0.71	0.50	0.69	0.50	0.71

Table 4.17a. The diagnostic accuracy of MF-BIA for men at several TBW% cut off points (as a percentage of body weight) based on MF-BIA internal equations for TBW against calculated serum osmolarity in diagnosing impending dehydration (295-300mOsm/L).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.26	0.97	0.95	0.34	0.72*	0.66	0.95
48%	0.50	0.67	0.80	0.33	0.73	0.67	0.80
49%	0.63	0.67	0.83	0.40	0.73	0.60	0.83
50%	0.63	0.67	0.83	0.40	0.73	0.60	0.83
52%	0.63	0.67	0.83	0.40	0.73	0.60	0.83
53%	0.75	0.33	0.75	0.33	0.73	0.67	0.75
54%	0.87	0.03	0.70	0.08	0.72*	0.92	0.70

Table 4.17b. The diagnostic accuracy of MF-BIA for women at several TBW% cut off points (as a percentage of body weight) based on MF-BIA internal equations for TBW against calculated serum osmolarity in diagnosing impending dehydration (295-300mOsm/L).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.18	0.99	0.92	0.66	0.38*	0.34	0.92
50%	0.17	0.90	0.50	0.64	0.38	0.36	0.50
52%	0.33	0.80	0.50	0.67	0.38	0.33	0.50
53%	0.50	0.60	0.43	0.67	0.38	0.33	0.43
54%	0.67	0.30	0.36	0.60	0.38	0.40	0.36
55%	0.83	0.10	0.36	0.50	0.38	0.50	0.38

Table 4.18a. The diagnostic accuracy of MF-BIA for men at several TBW% cut off points (as a percentage of body weight) based on MF-BIA internal equations for TBW against calculated serum osmolarity in diagnosing current dehydration (>300mOsm/L).

Cut-off point	Sensitivity	Specificity	PPV	NPV	Pre-test Probability	Post-test probability (-ve)	Post-test probability (+ve)
TBW%							
45%	0.34	0.98	0.96	0.55	0.54*	0.45	0.96
50%	0.50	0.40	0.50	0.40	0.55	0.60	0.50
52%	0.50	0.40	0.50	0.40	0.55	0.60	0.50
53%	0.67	0.20	0.50	0.33	0.55	0.67	0.50
55%	0.82	0.02	0.50	0.08	0.54*	0.92	0.50

Table 4.18b. The diagnostic accuracy of MF-BIA for women at several TBW% cut off points (as a percentage of body weight) based on MF-BIA internal equations for TBW against calculated serum osmolarity in diagnosing current dehydration (>300mOsm/L).

4.6 Discussion

Only 60% (n=27) participant data was included in the analysis and 40% (n=18). Although I tried different ways of calculating TBW, ICW and ECW, and defined dehydration using both serum osmolality and serum osmolarity (16 sets of calculations assessing at least 5 cut-off points each, for both impending and current dehydration, i. e over 160 2x2 tables), only 2 cut-off points had both sensitivity and specificity of at least 60%. Limited diagnostic accuracy was observed for TBW% at 46% when calculated using equations developed for older people (sensitivity 67%, specificity 62%) for current dehydration by measured osmolality (>300 mOsmol/kg), but positive and negative likelihood ratios were poor (1.75 and 0.54 respectively). Similarly TBW at 47%, only with equations developed for older people, showed limited diagnostic accuracy (sensitivity 63% and specificity 63%, LR^+ 1.7 and LR^- 0.6) for impending dehydration as assessed by calculated serum osmolarity (≥295 mOsmol/L). When internal equipment equations for estimating TBW were used no cut off were even minimally diagnostic. In this population of 27 people with recent strokes, MF-BIA did not fulfil its promise as a diagnostic tool for water-loss dehydration.

Calculated serum osmolarity was not good at predicting those with current dehydration by the reference standard, measured serum osmolality, and using calculated osmolarity resulted in 44% of our population being labelled as having current dehydration, compared to 22% by serum osmolality.

4.6.1 Diagnostic Accuracy

The limited diagnostic accuracy for current dehydration by osmolality at TBW% of 46% (sensitivity 67%, specificity 62%) using the impedance output from MF-BIA to calculate TBW% suggests that only 67 of every 100 people with current dehydration by serum osmolality will be “positive” using TBW% as the test, meaning that 33 of every

100 with current dehydration will be missed. Similarly the specificity of 62% suggests that for every 100 people without current dehydration 62 will have a negative test but 38 will have a positive test[1]. This is a very high level of false positives and negatives, suggesting that MF-BIA is not useful in diagnosing water-loss dehydration. The test's positive (PPV) and negative (NPV) predictive values[2] as well as pre and post test probabilities provide more information on the utility of TBW% at the 46% cut off point. The PPV of 33% (equivalent to the positive post-test probability of 33%) suggests that only 33% of those who are diagnosed as having current dehydration by MF-BIA truly have current dehydration by serum osmolality. The NPV of 87% is clearly better, meaning that 87% of those diagnosed as not having current dehydration are truly without current dehydration (and this is another way of stating the negative post-test probability of 13%). The positive likelihood ratio (LR+) was 1.75 and negative likelihood ratio (LR-) 0.54[3] suggesting that for a person "positive" for dehydration by this test the odds are 1.75 that dehydration is present compared to 1.00 for a person "negative" for dehydration.

Studies evaluating the utility of MF-BIA in diagnosing dehydration in clinical settings are scarce. The findings of my study suggest that MF-BIA is not a useful diagnostic tool and are in broad agreement with those of Olde Rikkert et al. They found that in dehydrated geriatric patients (n=53) the sensitivity of diagnosing dehydration using 100 kHz MF-BIA measurements was only 14% - very poor sensitivity, and sensitivity was not improved when other frequencies were tested (234).

[1] Sensitivity is the proportion of people who have the disorder who test positive. Specificity is the proportion of people who do not have the disorder who test negative.

[2] The positive predictive value is the ratio of true positives to all positives, and represents the proportion of those with a positive result that are correctly diagnosed (according to the reference standard). The negative predictive value is the proportion of those with a negative result that are correctly diagnosed (so test negative on the reference standard).

[3] The likelihood ratio for a positive result (LR+) tells you how much the odds of dehydration increase when a test is positive. The likelihood ratio for a negative result (LR-) tells you how much the odds of dehydration decrease when a test is negative.

4.6.2 The importance of MF-BIA results

Leaving the mathematics of diagnostic accuracy of MF-BIA aside and observing data generated by MF-BIA also suggested that MF-BIA generated outcomes are not coherent with the diagnosis of dehydration. Table 2 suggested no significant difference in MF-BIA measures between hydrated, impending, and current dehydration groups. The intracellular water content reflects information on the state of hydration at the cellular level. Cellular hydration status can change within minutes under the effects of stress, nutrients, hormones, and other factors (235).

Therefore MF-BIA measures do not appear to usefully reflect changes observed in serum osmolality or osmolarity or to sensitively identify the dehydrated state at the cellular level.

The state of hydration at a cellular level is important. If MF-BIA fails to identify dehydration as a sole method in diagnosing the hydration status this can result in loss of body tissue. Haussinger et al (235) suggested that a well hydrated cell increases anabolic processes, but a dehydrated cell shifts metabolism to catabolic processes especially at the muscle tissue. If recovery is to occur in a highly stressed patient after stroke, we want to be able to make sure that they are in an anabolic state rather than in a catabolic state that can affect liver function and may influence general weakness (muscle catabolism) that can influence functional recovery if experienced, or delayed rehabilitation recovery. Dehydration has been documented to correlate with poor outcomes after stroke. Bhalla (98) found that the 30% of their 167 stroke patients who had raised serum osmolality (>296 mOsm/kg) had increased risk of mortality at 3 months (OR 2.4, 95%CI 1.0 t 5.9). Kelly (121) found that in their 102 acute ischaemic stroke patients raised serum osmolality (>297mOsm/kg, in 24% of their patients) on day 9 following admission was associated with increased odds of venous thromboembolism (OR 4.7, 95% CI 1.4 to 16.3).

4.6.3 The Convenience of the Maltron BioScan 920-2

The Maltron website states that *"The BioScan 920-2 Multi-frequency Analyser with its unique features is a rapid, non-invasive, inexpensive method for evaluating hydration and nutrition status"* (236) . Among other things it suggests applications in "fluid retention", "effects of hydration and dehydration" and "estimation of Total Body Water, Extra/Intracellular Water". I was unable to verify this.

Despite the Maltron website reporting that it is "quick, safe and easy" and "no assistance or technical knowledge is required" (236) the machine is not user friendly. Without a keyboard, data entry and saving of data are slow and may result in errors and data loss. Re-running a second measurement for the same participant requires re-entering all the same information again or the new test overwrites existing data. Analysed data are not easily accessible to visual check without downloading the full data set, and there is no warning when unrealistic readings are registered. On- site readout of each variable for each participant was time consuming and unrealistic in an acute stroke unit. All data had to be downloaded first for a swift read out making it disadvantageous if discrepancies are present causing data loss.

Approaching the same participant again would ethically require further consent if patient is still eligible (48 hours time frame) and would require another serum osmolality test; a considerably invasive procedure as it requires venepuncture. MF-BIA equipment was used before in a previous research and no discrepancies were encountered. First 20 patients' data was checked for discrepancies. None was present giving confidence to the investigator.

4.6.4 Strengths and weaknesses

Study strengths include the use of both serum osmolality and calculated osmolarity as reference standards, conducted a population with high levels of dehydration, and recording serum osmolality and other serum measures (sodium, potassium, glucose, urea) within 20 minutes of MF-BIA measurements (enabling me to capture cellular hydration status as evaluated by MF-BIA and its coherence with reference serum values).

Weaknesses included small sample size and loss of MF-BIA data from several participants due to equipment malfunction. MF-BIA machine malfunction occurred unexpectedly. I checked data of first 20 patients for any discrepancies and none was present giving me the confidence in the equipment. The data of the last 18 patients only included in this dehydration study was omitted as discrepancies occurred. The possible explanation is that towards the end of my PhD study, other researchers were interested to examine the utility of MF-BIA for their own future studies. Therefore a training session was provided and they also tested the machine. This might have re-set the machine somehow causing error in measurements for the last 18 patients included in the hydration study.

In summary MF-BIA is not appropriate for the diagnosis of water-loss dehydration after stroke. Diagnostic accuracy is far too low to usefully diagnose dehydration current or impending dehydration at any selected cut-off point.

Chapter 5: Validation studies of the BioScan 920-2 multi-frequency bio electrical impedance machine in patients with recent ischaemic stroke or transient ischaemic attack against the Dual X-ray Absorptiometry scan

Abstract

Introduction: In the clinical study, the assessments of body composition changes were conducted using the multi-frequency bioelectrical impedance analysis (MF-BIA) (Maltron BioScan 920-2). However, dual-x ray absorptiometry (DEXA) is considered as the Gold Standard measurement. Therefore an external validation study of MF-BIA measurement using BioScan against DEXA was conducted. Two internal validation studies were also conducted to assess the reproducibility of the MF-BIA machine.

Methods: Ten participants were recruited for the external validation of whom seven participated in the longitudinal study (Chapter 3). Fat free mass and fat mass measurements recorded by MF-BIA machine immediately after the Dual X-ray Absorptiometry (DEXA) scan were used to validate MF-BIA against DEXA as primary measures along with protein mass, muscle mass and body cell mass. Additionally, two internal validation studies were conducted; (1) 10 consecutive measurements of MF-BIA recorded for each participant after the DEXA scan examination in 10 participants attending DEXA examination, and (2) two consecutive measurements recorded on both admission and discharge for each participants of the longitudinal study. Bland and Altman analysis was carried out to examine the extent of agreement between MF-BIA and DEXA for the external validation. Cronbach's-α was calculated for the reliability analysis to assess internal validity of MF-BIA.

Results: Of the ten participants included in external validation study, five were of normal weight (20.0-25.0 kg/m^2), four were overweight (25.0-29.9 kg/m^2), and one was obese (≥30.0 kg/m^2). There was strong correlation between MF-BIA and DEXA with r^2 values of 0.884 and 0.778 for fat free mass and fat mass, respectively. According to Bland and Altman analysis both MF-BIA and DEXA did not differ in their measurements. Internal consistency of MF-BIA measurement was excellent with fat free mass and fat mass assessed on admission and discharge (Cronbach's Alpha > 0.9 for both; n=40). Internal consistency was also excellent for 10 MF-BIA measurements measured at the same time of the external validation with (Cronbachs-α value > 0.9).

Conclusion: The findings suggested good internal consistency of MF-BIA and also showed good agreement and correlation of MF-BIA with DEXA with regards to fat mass and fat free mass measurements in stroke and TIA patient population.

5.1 Background

The assessment of individual components of the body composition is not carried out routinely in daily clinical practice. Kotler et al highlighted that "the assessment of body composition in clinical arena is lagging behind scientific and technological development" (237). It has been recognized that assessing nutritional status in clinical setting is useful (238). Body composition data can provide an understanding of the nutritional status and needs of an individual patient in clinical practice. Body composition measurement can be a complex and time consuming procedure depending on the method used. Multi-frequency bioelectrical Impedance Analysis (MF-MF-BIA) can be one simple and swift method to measure body composition (please see rationale in the Chapter 3), but its validity against reference standard methods in stroke and transient ischaemic attack (TIA) patient population is not known. This chapter presents the validation studies of Maltron BioScan 920-2, Multi-frequency BIA machine used in the clinical longitudinal study.

5.1.1 Assessment of body composition

Assessment of body composition can be done using simple, low technology methods as well as advanced methods. Established methods that are used to assess body composition include skin fold thickness (56), underwater weighing and dilution method (174), neutron activation analysis (239), determination of total body potassium (240), magnetic resonance imaging (MRI) (241), and dual x ray absorptiometry (DEXA) (63).

5.1.1.1 Upper Arm Anthropometrics:

Upper arm anthropometrics such as triceps skin fold (TSF) and mid arm circumference (MAC) are nutritional assessment methods that can provide estimates of fat free mass and fat mass of an individual. Skin fold thickness is used as a nutritional assessment method in clinical settings for bedridden or very ill patients who cannot undergo other

methods that require a certain level of mobility (242). Despite the utility of upper arm anthropometrics as a nutritional assessment technique in bedridden patients, their accuracy and usefulness have been questioned. In one study, the diagnostic accuracy of TSF and MAC in assessing severe malnutrition (<5th percentile for age) was examined against that of Body Mass Index (BMI) < 18 kg/m^2 and the Subjective Global Assessment Tool (SGA) scores of 158 patients admitted to a hospital. The authors found that the sensitivity of TSF in diagnosing severe malnutrition (as defined above) compared to similar diagnosis using BMI and SGA was poor with sensitivities of 62% and 38% for BMI and SGA, respectively (243). Sensitivity of the MAC was better compared to TSF especially against SGA, but still relatively poor with sensitivity values of 66% and 61% for BMI and SGA, respectively (243). This lack of sensitivity is may be related to the fact that both TSF and MAC provide measure of specific fat and muscle mass distribution in certain body area (upper arm) unlike BMI which provide a measurement of body mass of whole body without providing any estimation in fat or muscle mass or the pattern of distribution of fat. Therefore, using BMI or TSF or MAC as a criterion or gold standard measure for body composition is clearly not appropriate.

5.1.1.2 Underwater weighing method

One of the more complex methods is underwater weighting or hydrodensitometry method. Underwater weighting relies on the estimation of body fat from calculated body density using a validated mathematical equation. The subject's body mass is calculated by dividing the measured weight by gravitational force in air and while in a water tank. First, subject's mass is calculated in air (M_{air}) by dividing weight (kg) by gravitational force (N; neutons). To measure weight in water, the subject sits in a stainless steel chair placed on a Toledo platform scale in an aluminium water tank with a controlled water temperature between 35-36 ^{o}C, and is submerged into water up to the neck. Mass in water is determined (M_{water}) by multiplying volume of water times it density at 35-36 ^{o}C (which equates to 0.994). The difference between body mass in air and water (M_{air} - M_{water}) divided by the density of water at a temperature of 35-36 ^{o}C (which equates to 0.994) is used to calculate the volume of displaced water which is equal to body volume (244).

To calculate body volume accurately using this method, correction must be made by subtracting residual gas volume (described below) from body volume. First residual volume need to be measured. This can be done using a nitrogen analyser available in the water tank. The nitrogen analyser consists of a stopcock and spirometer (244). The nitrogen washout method is used to calculate the residual gas lung volume in lungs. In this method the subject breathes air through the stopcock. After a full expiration the subject is then connected to the spirometer filled with 100% oxygen. The subject is then asked to inhale and exhale once every three seconds. At the third exhalation residual air volume is calculated using the formulae used in Rahn 1949 from nitrogen concentration percentage in the total volume of exhaled air in the spirometer (245). Once residual gas lung volume is subtracted from body volume, body density is calculated using the difference between M_{air} and M_{water} (246) (as described above). Brozek equation is then used to calculate body fat from body density as below (247);

$$\text{Body Fat} = 4.57/\text{body density} - 4.142) \times 100$$

Validation of the Brozek equation for estimating body fat against body fat estimated by dual-X ray absorptiometry (DEXA) suggest that it is very accurate in estimating body fat (248). Despite its accuracy (248)underwater weighing is only used for research purposes and not for clinical purposes as it is not an easy method to use (174). Furthermore because the subject's body is required to be submerged in the water except the head, it is difficult to use in pregnant women, obese people, elderly, and people with disability (246), hence not pragmatic to use across patient populations. The approximation of residual lung volume can be inaccurate sometimes resulting in imprecise body volume estimation and making it one of the main limitations of this method (249).

5.1.1.3 Dilution method

The dilution method is used to measure total body water, extracellular, and intracellular water. Fat free mass (FFM) can be calculated from total body water volume by multiplying total body water by 0.732 which is the FFM constant (250); the constant value is derived based on the fact that water content of the lean or fat free tissue in human is 73%. In this method, measuring of the total body water volume is done by administration of a dose of tracer labelled water into the subject either orally or intravenously. The water is usually labelled with tritium, deuterium, or oxygen-18. Before the dose is administered a sample of urine, or blood is collected from the subject. Two to three hours after the labelled water administration the same pre-dose sample type and quantity is collected (251). The principle behind the dilution method is that the tracer will reach equilibrium in the compartments intended to measure by distributing equally in these compartments given that this tracer is not metabolized (252). Total body water (TBW) can be calculated as in formulae below. The formulae assumes that the volume of a compartment (total body water (V)) can be calculated from ratio of the difference in the administered (D and excreted (E) dose concentrations to the difference of the concentration of the collected fluid (d_t) after tracer dose administration and its concentration before dose administration (d_0).

$$V=k1 \times k2 \; xk3 \; xk4 \times \{(D-E)/ (d_t-d_0)\}$$

Correction factors are k_1, k_2, k_3, and k_4 (251). Fat free mass can then be calculated given that total body water is a constant and present in 73% of fat free mass (253). Fat free mass can then be subtracted from body weight to estimate fat mass. The dilution method can also be used to calculate extracellular water (ECW) as the same way as calculating total body water by using deuterated bromide or chloride which diffuses in ECW space. ECW can be subtracted from TBW to calculate Intracellular water volume (ICW) (174).

The dilution method is considered to be one of the reference methods to other body composition methods such as Dual X-ray absorptiometry (251). However, the requirements for a sophisticated equipment and setting make it difficult to perform in daily clinical practice. In addition, it is not as a swift body composition assessment in clinical setting as the samples must be relocated to larger facilities for analysis (254).

5.1.1.4 Total Body Potassium

Total body potassium method is used to estimate fat free mass (255, 256). Potassium isotopes known as potassium-40 [^{40}K] is fractionally present in the body and emits gamma rays radiation (257). The emission of gamma rays allows for ^{40}K counting and body composition assessment given that the potassium isotope content in fat free mass is constant (255). For total body potassium counting, the subject lays in a supine position between two sodium iodide detectors (which trap gamma rays emission) for 15 minutes in an enclosed room to allow the trapping of emitted gamma rays from the subject only and not radiation from the naturally occurring ^{40}K. The gamma rays are trapped by the sodium iodide (NaI) detectors and converted to total body potassium value (186).

Other detectors are also available such as potassium chloride crystal bottles used by Kehayias and colleagues (258). Total body potassium method is a precise method with only small variance between the ^{40}K body pool reflecting actual fat free mass content (259). The precision of total body potassium method was further examined in older people by Kehayias and colleagues and they documented that total body potassium was precise in showing a decreasing trend with reduced fat free mass and an increasing trend with increased fat mass in ageing subjects experiencing sarcopenia (258). The main drawback of total body potassium counting method is that it requires sophisticated and expensive set up (detectors, special chamber, etc.) that are not easily available for clinical usage.

5.1.1.5 Magnetic Resonance Imaging (MRI)

Body composition can be measured using more advanced techniques such as Magnetic Resonance Imaging (MRI). MRI method involves exposing the human body components to a magnetic field. The body consists of atoms as the case of all naturally occurring subjects. When the nucleus of an atom, consisting of neutron and protons, is exposed to the magnetic field, the protons position themselves perpendicular to the magnetic field. The time taken for the protons to align with the magnetic field is called longitudinal relaxation time (T1). This alignment or orientation is lost once the disappearing magnetic causes protons to rotate back to their initial positions. This process releases energy as they realign to their pre-exposure position. The energy released can be captured as radiofrequency. The time for the protons to return to their original orientation, before the application of the magnetic field, is expressed as transverse relaxation time (T2).

Both T1 and T2 differ between different tissues. The detection of radiofrequency at different interval allows the determination of the volume of each tissue (260, 261). The main advantage of MRI is that it allows for the imaging of each different body tissue compartment including subcutaneous and visceral adipose tissue unlike other methods discussed so far which allow quantification of fat mass and fat free mass only. MRI also shows good accuracy. The mean variance between MRI estimated visceral and subcutaneous fat and actual weights measured of the three human cadavers was <10% (262). Other validation studies include work by Engstorm and colleagues demonstrating that MRI provided accurate measurement of the cross sectional area of human cadaver thighs compared to anatomical standard (AN) measurement(263). The high resolution images of MRI allowed for good estimation of muscle volume as MRI values were within 7.5% of the AN standard (263). MRI also showed good accuracy in estimating body composition volumes in animals (264). The main disadvantage of MRI that it is relatively expensive, not quick to perform, requires a certain extent of subject mobility, and it is not advisable to carry out measurement if the person has any medical devices such as a pacemaker.

5.1.1.6 Dual Energy X-ray Absorptiometry (DEXA)

The Dual Energy X-ray Absorptiometry (DEXA) is used as a reference method in evaluating body composition (251). Therefore it has been increasingly used in both research and clinical settings. DEXA was first used to measure bone and soft tissue composition (265). The DEXA was developed based on the same principles as Dual Photon Absorptiometry (DPA) which generates gamma rays through a radionuclide source. The principle behind DPA used in measuring body composition is that when a photon is directed at a subject, the intensity of the photons is reduced as they travel through the subject body. The photons exiting the subject can be quantified by the detector on the opposite side of the subject allowing for body composition calculation using different formulae (251).

The DPA have been shown to have excellent agreement with body fat measured by underwater weighing (UWW), total body potassium (TBK), and the dilution methods with a fat mass of 16.7±4.9 kg for DPA and a combined average fat mass of the three methods of 17.6±5.9 kg leading to a correlation coefficients between 0.79 and 0.99; p values= between 0.01 and 0.001(266). With further technological advancement, the photon source of DPA was replaced with X-ray generating tubes resulting in currently used DEXA technology (265).

The DEXA is considered to be the best body composition measurement technique with a precision error of less than 1.0 kg for fat mass and relative error of less than 0.8 kg for fat free mass percentage (63). It also has low radiation exposure; the radiation exposure in each measurement is less than 0.1 microGy (63) which is less than a whole day exposure to radiation emitted from the sun in a sunny summer day in the Western Europe such as UK. While DEXA method is considered to be gold standard measurement of body composition, it is still relatively expensive, time consuming to perform ranging from 15-20 minutes for one measurement, and inconvenient for patients with disability or limited mobility – the person needs to be able to lay flat during the examination. All these factors make DEXA not pragmatic to be used routinely in clinical practice for purposes such as screening for all patients.

The methods discussed above are costly, challenging, complex and they cannot be performed to everyone in daily clinical practice (237). These methods are not quick as they require a patient/person to travel to the location of the facility. They also require the presence of an expert technician and cannot be calibrated by a researcher or a clinician without previous appropriate training. This has led to further development in new methods which can evaluate body composition accurately that are cheap, convenient, easy to perform and easily accessible. Multi-frequency Bioelectrical Impedance Analysis (MF-BIA) is one of the newer methods which were used in my thesis work. I discuss briefly below (please refer to Chapter 3 for more details) on the MF-BIA method in measuring body composition.

5.1.1.7 Multi-frequency Bioelectrical Impedance Analysis

Multi-frequency bioelectrical impedance analysis (MF-BIA) is one of the newer methods that can assess body composition. Body composition data which can be collected by MF-BIA include fat free mass (Kg), fat free mass percentage, fat mass (Kg), fat mass percentage, total body water (L), total body water percentage, extra and intracellular water (L), extra to intracellular water ratio, body cell mass (Kg) and percentage, extracellular mass (Kg) and percentage, Creatinine clearance rate (ml/min), glomerular filtration rate (ml/min), protein mass (Kg), mineral mass (Kg), mineral mass percentage, total body calcium and potassium (g), muscles mass (Kg), glycogen mass (g), dry weight (Kg), extracellular fluid (L), plasma fluid-intravascular (L), interstitial fluid-extravascular, body volume (L), and body density (Kg/L).

In brief, specific equations programmed in the MF-BIA machine is used to calculate the body composition components simultaneously based on the quantitative value of the resistance imposed on the flowing electrical current by different components (tissues) of the body. The underlying principle of this measurement method is that while some components in the extracellular space impedes the electrical current from flowing through the body, the intracellular components allow it to flow freely (267). For

example, body components such as adipose tissue are non-conductive to electrical current while lean tissues such as muscle, and other elements such as electrolytes and water, are conductive. Therefore, when an electrical current passes through the human body it faces resistance from the adipose tissue, but passes through the non-adipose tissue to complete its circuit without any resistance or impedance.

The difference in conductivity between different tissues is used to calculate fat mass and fat free mass using a validated formula already programmed in the MF-BIA equipment taking into account of factors such as gender, height, weight, and age (62). The MF-MF-BIA technique can measure body composition using a single frequency current (SF-MF-MF-BIA) or a multi-frequency current (MF-BIA). In SF- BIA a single current of a known quantity, usually 50 kHz, is used (183), while MF-BIA uses electrical currents of several frequencies of incremental values (5, 50, 100, 200, etc., ...up to 500 kHz); Maltron BioScan 920-2, MF-BIA machine, I used in my study measure the body components using electrical current frequencies of (5, 50, 100, and 200 kHz).

In MF-BIA currents of various frequencies are passed through the body tissues separately and impedance is generated for each frequency. Electrical currents' input and output difference for each frequency is measured and the difference is used in validated equations already integrated in the equipment to calculate body compositions. Both SF-BIA and MF-BIA use empirical linear regression equations to generate results and the results are available to the investigator instantly (183). MF-BIA has been previously used in clinical settings in several conditions. These include but are not limited to older patients (234), patients after coronary artery bypass graft (CABG) (268), patients with HIV (269), and those on dialysis (270). The advantages of MF-BIA include being easy to use, non-invasive, and requires minimal training to operate the equipment (271). The main disadvantage of MF-BIA is that there are several manufacturers and not all are validated therefore a validation against a reference standard body composition assessment method is required to ascertain the reliability and for future clinical use in specific patient/participant populations.

5.1.2 Validation of MF-BIA against DEXA

The validation studies of MF-BIA were usually conducted against DEXA measurement as the gold standard method and therefore, I validated MF-BIA [BioScan 920-2, Maltron International Essex, United Kingdom) machine used in my project against DEXA. Previous validation studies of MF-BIA against DEXA are somewhat limited, conducted in specific populations' e.g. healthy volunteers but not in stroke/TIA patient population. However, it has been shown that the accuracy of MF-BIA measurement is dependent on the participant's body mass index. One recent study by Schafer et al (43) examined the validity of MF-BIA compared to DEXA in healthy subjects across a range of BMI categories. The MF-BIA overestimated fat mass in obese (30.0-30.9 kg/m^2) subjects compared to DEXA ($p<0.0001$); difference 4.11 ± 0.34, and in overweight (25.0-29.9 kg/m^2) subjects ($p\leq0.006$); difference of 0.95 ± 0.33. Despite MF-BIA's overestimation of fat mass, the authors highlighted that MF-BIA measurements did show body fat percentage agreement with DEXA in the normal (18.5-24.9 kg/m^2) and overweight BMI categories with a mean difference of -1.56% (limits of agreement -6.7% to +3.6%) and +0.58% (limits of agreement -3.8% to +5.0%), respectively.

The agreement with DEXA appears to be weaker in people whose BMI values were in obese range (i.e. BMI >30 kg/m^2); mean difference was 3.50% (-2.2 to +8.8%). In their study, MF-BIA overestimated fat free mass in subjects with normal and overweight BMI categories compared to DEXA with a difference of 2.08 ± 0.32 ($p<0.0001$) and 0.71 ± 0.33 ($p\leq0.04$) respectively. Overall conclusion was that MF-BIA is in agreement with DEXA when measuring normal and overweight subjects although overestimation occurs in obese subjects, and therefore caution should be taken in interpreting MF-MF-BIA results in obese subjects (272).

There is a dearth of data on the use of MF-BIA method in evaluating body composition changes after stroke/TIA. One study compared body composition changes after stroke between the paretic and non-paretic leg of patients (n = 35) (273). It used the DEXA method in evaluating body composition, indicating that significant losses in lean body mass and bone density loss occurred in the paretic leg compared to the non-paretic leg after stroke; $p<0.05$ (273).

Efforts to have reference FFM and FM values were made mainly on healthy subjects (274). Norm FFM and FM reference values in specific populations are unknown and still less well studied. Further validation of available machines should be carried out against reference method DEXA in larger studies and across wide range of specific populations in clinical setting.

5.2 Study Objective

The objective of this study is to externally validate MF-BIA against gold standard DEXA in patients with recent stroke/TIA. The validation of MF-BIA against DEXA can provide information on the level of agreement between major components of interest, fat mass and fat free mass, measured using MF-BIA and their corresponding values estimated by DEXA for the same study participant. This study not only sought to carry out an external validation for MF-BIA against DEXA, but also examined the internal consistency of MF-BIA measurements for the same participants recorded several times as well as using measurement data from the longitudinal study described in Chapter 3.

5.3 Methodology

The MF-BIA used in the study (Maltron BioScan 920-2, Maltron International Co. Essex, United Kingdom), was validated against DEXA machine (Hologic Discovery, Hologic Inc. Massachusetts, USA) located at the Clinical Research Trials Unit in the Norwich Medical School of the University of East Anglia (UEA). The Clinical Research Trial Unit at the UEA is a National Health Service affiliated facility that has provisional Clinical Trial Unit registration with the National Institute for Health Research (NIHR) in England.

5.3.1 External validation study

For external validation of MF-BIA against DEXA (referred in this chapter as MF-BIA validation study), 10 participants with recent stroke/TIA who met the inclusion criteria were studied. The majority of participants for external validation (n = 7) were drawn from the longitudinal study participants as described in the Chapter 3. The remaining three participants were enrolled into the MF-BIA validation study only because their expected acute hospital stay was very short to provide meaningful results for the longitudinal study or they were not interested in participating in the longitudinal clinical study but agreed to participate in this sub-study. Study participants were mainly stroke patients (n = 8) and the remaining two patients experienced transient ischemic attack (TIA). TIA patients were also included in the validation study as the purpose of the sub-study is to evaluate the agreement of the measurements between two different techniques in people with recent cerebrovascular event (stroke or TIA) as opposed to assessment of changes in body composition after a stroke.

At the time of study enrolment, I described the objectives of the validation study to potentially eligible patients. I explained that the MF-BIA equipment used in the study can be very useful in evaluating body composition but it has not been validated in stroke/TIA patient population and this MF-BIA validation study will allow us to understand if the values provided by the MF-BIA equipment are reproducible by a gold

standard method, DEXA. I also explained the potential benefit of research that if MF-BIA could be reliably used to measure fat mass and fat free mass in stroke/TIA population, it may allow further research in the future that can lead to recommendation of the MF-BIA use in clinical practice considering that it is quick making it a useful tool for health care professionals in assessing the nutritional status and needs of patients.

5.3.2 Sample size

For correlation, a sample size of 8 would have 90% power to detect a correlation of 0.9 at the 5% level of significance. I therefore recruited ten participants, six with a recent stroke and 4 with a transient ischaemic attack (TIA), from the acute stroke unit at the Norfolk and Norwich University 140 Hospital, UK.

5.3.3 Procedure

Inclusion criteria: The inclusion criteria are the same as the inclusion criteria for the longitudinal study described in the Chapter 3, except that TIA patients were also eligible for this validation study.

5.3.4 Exclusion Criteria

Exclusion criteria for the MF-BIA validation is the same as the exclusion criteria detailed in the longitudinal study in the Chapter 3.

5.3.5 Invitations

After hospital discharge, each patient who consented to take part in the MF-BIA validation study was contacted by phone to set a convenient date and time for the participant and the research team to perform the DEXA scan, and MF-MF-BIA measurements for both external validation against DEXA and one of the internal validation studies using 10 repeated measures of MF-BIA. An invitation letter to attend the Clinical Research Trials Unit with the information such as direction to CRTU (standard UEA campus map with CRTU location clearly marked), the appointment date and time was then sent to the participants by post. The letter also included other information such as the duration of the procedure etc. A car parking pass was also included in the postal package. Attendance was confirmed by contacting participants by telephone three days prior to their CRTU visit.

A consultant physician caring for the participant during their stay at the acute stroke unit in Norfolk and Norwich University Hospital wrote a request (Appendix XVI) for a DEXA scan for each participant as per the requirement of the CRTU Standard Operating Procedures (SOP). Whole body scan was requested from the four options available including hip, spine, or forearm, because the indication for the study was to measure body composition as opposed to the other purpose, e.g. assessment of osteoporosis. Radiation exposure confirmation of directed dose and appropriate approvals checklist was filled by the radiation expert to carry the scans.

Upon participant's arrival to the Clinical Research Trial Unit (CRTU) on the examination date, pre-scan assessment interview was performed by the researcher as described in detail below.

5.3.5 Pre-DEXA scan interview

The interview was aimed at ensuring participant's safety (Appendix VII). I used the standard SOP documents of CRTU for DEXA examination (Appendix XVIII). First the participant was asked if they had any medical procedure within the last seven days that involved the use of contrast media, arterial, iodine, barium, and nuclear medicine isotope study. All participants answered NO.

Participants were also asked if they are wearing any metal device or object such as button, zips, belts, mobile phone, etc. The participant was requested to remove them if they were wearing or carrying with them any of such items.

Finally the participant was asked if they had any surgery that resulted in having metal device fixed on them such as pacemaker leads, radioactive seeds, metal implants, hip replacement, surgical staples, or any metal foreign bodies such as shrapnel, radio-opaque catheters or tubes, and bullets. If any of the answer was YES it was not an issue but the practitioner carrying out the DEXA scan would assess if they interfere with the scans (Appendix XIX).

The second informed consent specifically for DEXA procedure was obtained immediately prior to DEXA scan examination. This was required for all participants intended to take part in any DEXA scan for research purposes as per CRTU SOP (Appendix X).

5.3.6 Dual X-Ray Absorptiometry (DEXA) Scan

The DEXA examination was carried out using Hologic Discovery (Hologic Discovery, QDR series, Hologic Inc. Massachusetts, USA); image 1. Patients were asked to lie down flat on their back for the scan within the marked area. It was checked to ensure

that the patient lied between the marked lines, one above the head and one below the feet. This marked area guarantees that the all parts of the body are exposed to the X-ray to obtain a full body scan. The patient's feet were tied with a tape to ensure that they are kept close together. Once the scan was ready to take place the machine was run while the machine operator (the technician who has appropriate qualification to operate the scanner) and I stood in the designated area behind a barrier that protect the radiation exposure to the examiners. The duration of the scan was exactly seven minutes. Once the scanning finished the participant was helped to sit upright slowly.

Image 5.1. (Hologic Discovery, QDR series, available in the Clinical Research Trial Unit (CRTU) at the Norwich Medical School, University of East Anglia

5.3.7 Bioelectrical Impedance Analysis

Immediately after performing the DEXA examination, the participant's body composition measurement was carried out using multi-frequency bioelectrical impedance analysis (MF-BIA) as described details in the Chapter 3. Briefly, the participant's weight was recorded by asking to take off their shoes and stand on the weight meter while wearing light clothing. Weight was recorded to the nearest 0.1

kilogram (Kg). While on the weight meter, the participant was asked to stand upright and straight to measure their height using the stadiometer. The stadiometer was slid from the above until the headpiece of stadiometer touched the top of the skull of the participant comfortably. Height was then recorded to the nearest decimal point in centimetre (cm).

The participant was then asked to lie down in a supine position on the bed in the examination room at the CRTU and made comfortable. Participant information, a given ID number, age, gender, height, weight, and ethnicity were all entered into MF-BIA machine prior to body composition measurement. This information is used by MF-BIA machine to calculate body composition components using pre-programmed formulas as described in the Chapter 3 and the introduction section of this chapter. Once all the relevant necessary information was entered the preparation for the measurement was carried out. Electrodes from the equipment were attached to the patients using sticky patches similar to ECG patches as described in the Chapter 3.

The reasoning behind placing the patches on the participant after entering the information not before is to ensure that they are not contaminated with skin secretions if they stay for a longer period of time which may interfere with electrical current flow and the accuracy of the body composition measurements. The cables of the MF-MF-BIA machine were then attached to the patches with the red coloured cable (positive) being closer to the heart and the black coloured cable (negative) farthest. A total of ten MF-BIA measurements were carried out for each participant consecutively.

5.3.8 Internal validation studies of MF-BIA

First Internal Validation Study: The first for internal validation of MF-BIA came from the source of data from the ten MF-BIA measurements recorded in 10 participants who attended DEXA examination as described above which were measured for the external validation purpose. The comparison of MF-BIA values among these 10 measurements

within an individual were also used to evaluate the internal consistency of MF-BIA. The data is presented as First Internal Validation study in the Results section.

Second Internal Validation Study: As described in the longitudinal study of this thesis (Chapter 3), two consecutive measurements were made using MF-BIA for each participant both at the time of admission and on discharge (n=40). The purpose of these two measurements on each occasion was also to evaluate the internal consistency of MF-MF-BIA on both at the time of admission and at hospital discharge separately in a larger number of participants. Therefore, this second internal validation study of MF-BIA was based on a total of 80 pairs of MF-BIA measurements in 40 participants.

5.3.8 Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences SPSS of the product line Predictive Analytics Software (PASW version 18.0).

5.3.8.1 External Validation of MF-BIA against DEXA

For external validation against DEXA, mean values for fat free mass, fat free mass percentage, fat mass and fat mass percentage were calculated from MF-BIA measurements and compared to their corresponding values measured by DEXA. First, the means of first two MF-BIA measures (out of 10) was calculated to examine the agreement with DEXA. Then comparisons were made with average of first three MF-BIA measurements, first four MF-BIA measurements, and so on until the average of the all 10 measurements was used. Therefore, for each participant a total of nine comparisons were made between MF-BIA and DEXA measurements. The rationale being to explore the number of MF-BIA measurements that provide the optimum level of agreement between MF-BIA and DEXA after which strength of correlation did not improve further. This will aid in understanding how many MF-BIA measurement should be recorded for an average that results in most precise measurement similar to

measurement recorded by the gold standard DEXA for fat mass and fat free mass. All analyses were repeated stratified by BMI category as the existing literature suggests some overestimation in obese subjects.

Bland Altman method for assessing agreement between two methods of clinical measurements was used for the external validation purpose (275). The Bland Altman method includes a test of linearity step and limit of agreement step. For the test of linearity (Figures 5.11-5.44), each measurement from DEXA for fat free mass, fat mass and their percentages were plotted respectively against MF-BIA corresponding measurements optimum mean. A line was fitted and r^2 calculated to understand the strength of relation (not agreement) between the two variables.

For limits of agreements, upper and lower limits were first calculated (Mean difference ± (2 x standard deviation of difference)). The overall mean of each measurement of fat free mass, fat mass, and their percentages from both MF-BIA and DEXA (Optimum MF-BIA mean and DEXA measurement) was plotted against the mean difference of their corresponding values (difference between optimum mean for each measurement by MF-BIA and DEXA). The plotted points were examined for falling within the limits of agreement (upper and lower limits) or beyond the limits of agreement as in Bland Altman method (275).

5.3.8.2 Internal validation

For the first internal validation study, ten consecutive measurements of MF-BIA recorded were examined for MF-BIA reliability. The first two MF-BIA fat free mass, fat mass, and their percentages measurements Cronbachs Alpha values were calculated respectively. The same step was repeated for the first three, first four and so on until all ten measurements Cronbachs Alpha Values were calculated. The purpose is to find the optimum number of measurements of MF-BIA to obtain the highest Cronbachs Alpha value (to be most reliable).

For the second internal validation study, two MF-BIA measurements for admission were examined to validate the internal consistency of MF-MF-BIA using reliability

analysis. Cronbachs Alpha values, confidence intervals, and p-values were calculated. The same reliability analysis was carried out for the discharge measurements.

5.4 Results

Ten participants were recruited for the external validation study (mean age 66 years, age range 50-82 years, 70% men). Of the ten participants six were diagnosed with ischaemic stroke type and four were diagnosed with Transient Ischaemic attack at the time of admission to the acute stroke unit. Five participants had a normal weight BMI 20-24.9 kg/m^2), four were overweight (BMI 25-29.9 kg/m^2), and one participant was obese (≥ 30 kg/m^2). The sample characteristics of patients included in the second internal validation study are presented in the Table 3.1 of Chapter 3.

5.4.1 External validation

The mean age of the 10 participants for the external validation and the first internal validation study was 66 years (SD 11.1 years, range 50-82 years, 70% men), of whom six had an ischemic stroke (mean NIHSS = 3.2; range 1-8) and four a TIA. Five participants had a normal weight (BMI 20-24.9 kg/m^2), four were overweight (BMI 25-29.9 kg/m^2), and one participant was obese (≥ 30 kg/m^2). The mean age of the 40 patients included in the second internal validation study was 70.3 years (SD 9.9 years, range 50-89 years, 55% men), all had an ischemic stroke (mean NIHSS = 5.1; range 1-22). Three were underweight (<20 kg/m2), eight were normal weight, 21 were overweight and 8 were obese.

Table 5.1 shows the comparison between fat fee mass, fat free mass percentage, fat mass, and fat mass percentages mean of the ten participants measured MF-BIA BioScan 920-2 (after calculating optimum mean of ten measurements) compared to the reference standard Hologic Discovery DEXA mean for the same ten participants No statistically significant differences were observed for all of the body composition indices between two measurement methods.

	Measurement	Mean difference (95% CI)	p-value
Fat Free Mass (kg)			
Mean MF-BIA BioScan 920-2 (std) kg	55.5 (14.1)	0.6 (-2.9 to 4.1)	0.71
Mean Hologic Discovery DEXA (std) kg	54.9 (13.7)		
Fat Free Mass %			
Mean MF-BIA BioScan 920-2 (std) kg	72 .0(11.6)	1.1 (-3.9 to 6.0)	0.64
Mean Hologic Discovery DEXA (std) kg	70.9 (8.5)		
Fat Mass (kg)			
Mean MF-BIA BioScan 920-2 (std) kg	22.0 (10.7)	0.8 (-2.7 to 4.4)	0.61
Mean Hologic Discovery DEXA (std) kg	21.2 (8.8)		
Fat Mass %			
Mean MF-BIA BioScan 920-2 (std) kg	28.0 (11.6)	0.4 (-4.7 to 5.4)	0.9
Mean Hologic Discovery DEXA (std) kg	27.7 (9.1)		

Table 5.1. Fat Free Mass, Fat Free Mass percentage, Fat Mass, and Fat mass percentages mean of the ten participants measured MF-BIA BioScan 920-2 (after calculating optimum mean of ten measurements) compared to the reference standard Hologic Discovery DEXA mean for the same ten participants; included are mean differences and 95% Confidence intervals (95% CI).

Table 5.2 shows R-squared and mean differences averages values of fat free mass, fat mass percentages, fat mass and fat mass percentages measurements for the external validation of by MF-BIA BioScan 920-2 against Hologic Discovery Dual X-ray absorptiometry for all the study sample population and stratified by Body Mass Index kg/m^2 categories. There was a statistically significant correlation between fat free mass, fat mass and their percentages with no statically significant mean differences between both methods of measurements for all body composition indices measured. When stratified by BMI category, only fat free mass (kg) values measured by both DEXA and MF-BIA BioScan 920-2 showed statistically significant strong correlation (r-squared >0.7) in overweight subjects. No statistically significant mean differences between both methods of measurements for all other body composition indices measured were observed when stratified analyses were conducted by BMI category.

.

	R-squared correlation	p-value for correlation	Mean Difference	p-value
Fat Free Mass (kg)				
All BMI categories	0.94	<0.0001	0.6 (-2.9 to 4.1)	0.71
Normal BMI (20-25 kg/$m^{2)}$	0.435	0.23	2.7 (-4.9 to 10.3)	0.38
Overweight BMI (25-30 kg/m^2)	0.943	0.03	1.9 (-1.8 to 5.7)	0.2
Fat Free Mass %				
All BMI categories	0.805	0.005	1.1 (-3.9 to 6.0)	0.64
Normal BMI (20-25 kg/$m^{2)}$	0.128	0.55	4.1 (-6.8 to 14.9)	0.36
Overweight BMI (25-30 kg/m^2)	0.882	0.09	2.5 (-2.9 to 7.8)	0.24
Fat Mass (kg)				
All BMI categories	0.882	0.001	0.8 (-2.8 to 4.4)	0.61
Normal BMI (20-25 kg/$m^{2)}$	0.182	0.47	1.0 (-6.9 to 9.0)	0.74
Overweight BMI (25-30 kg/m^2	0.742	0.14	2.7 (-2.1 to 7.5)	0.173
Fat Mass %				
All BMI categories	0.794	0.006	0.4 (-4.7 to 5.4)	0.87
Normal BMI (20-25 kg/$m^{2)}$	0.225	0.42	2.1 (-9.2 to 13.5)	0.63
Overweight BMI (25-30 kg/m^2	0.757	0.13	3.2 (-3.1 to 9.5)	0.21

Table 5.2. Fat free mass, fat mass, and their percentages measured by two different methods, Dual X-ray absorptiometry (DEXA) and Multi-frequency Bioelectrical Impedance Analysis (MF-BIA) BioScan 920-2 for the entire study sample, and stratified by body mass index .

There were excellent correlations between Hologic Discovery DEXA and MF-BIA BioScan 920-2 measurements using any of MF-BIA BioScan 920-2 averages of first two, first three, first four, and so on until all 10 measurements for all indices measured, fat free mass and fat mass and their percentages for each participant in the study sample. R^2 was > 0.8 and >0.6 for fat free mass and fat free mass percentage respectively. R^2 was > 0.7 and >0.6 for fat mass and fat mass percentage respectively. Table 5.3 shows r-squared values for fat free mass, fat free mass percentage, fat mass, and fat mass percentages of the external validation for each of the averages of the first two, three, four, five, six, seven, eight, ninth and all ten measurements recorded by MF-BIA BioScan 920-2 against Hologic Discovery Dual X-ray (DEXA) absorptiometry for the 10 participants who participated in the external validation study.

	First two	First three	First four	First five	First six	First seven	First eight	First nine	All ten
Fat Free Mass (kg)	0.881	0.882	0.882	0.882	0.881	0.882	0.882	0.882	0.884
Fat Free Mass %	0.648	0.649	0.649	0.654	0.648	0.649	0.648	0.654	0.648
Fat Mass (kg)	0.782	0.781	0.782	0.787	0.782	0.783	0.786	0.787	0.778
Fat Mass %	0.633	0.632	0.633	0.64	0.633	0.634	0.639	0.641	0.63

Table 5.3. R-squared values per measurement repetitions for fat free mass, fat free mass percentage, fat mass, and fat mass percentages of the external validation for each of the averages of the first two, three, four, five, six, seven, eight, ninth and all ten measurements recorded by MF-BIA BioScan 920-2 against Hologic Discovery Dual X-ray absorptiometry for each of the 10 participants who participated in the external validation study.

Test of linearity

Figure 5.1 shows the test of linearity diagram/plot for fat free mass of DEXA values in kg plotted against their corresponding values measured by MF-BIA BioScan 920-2 for the 10 study participants using optimum average of 10 measurements for each participant. All points lied along the linearity line. The correlation coefficient was excellent ($r=0.940$; $p<0.0001$). In two participants, the values lied almost on the linearity line (i.e. almost exactly the same results between DEXA and MF-MF-BIA) indicating a substantial agreement.

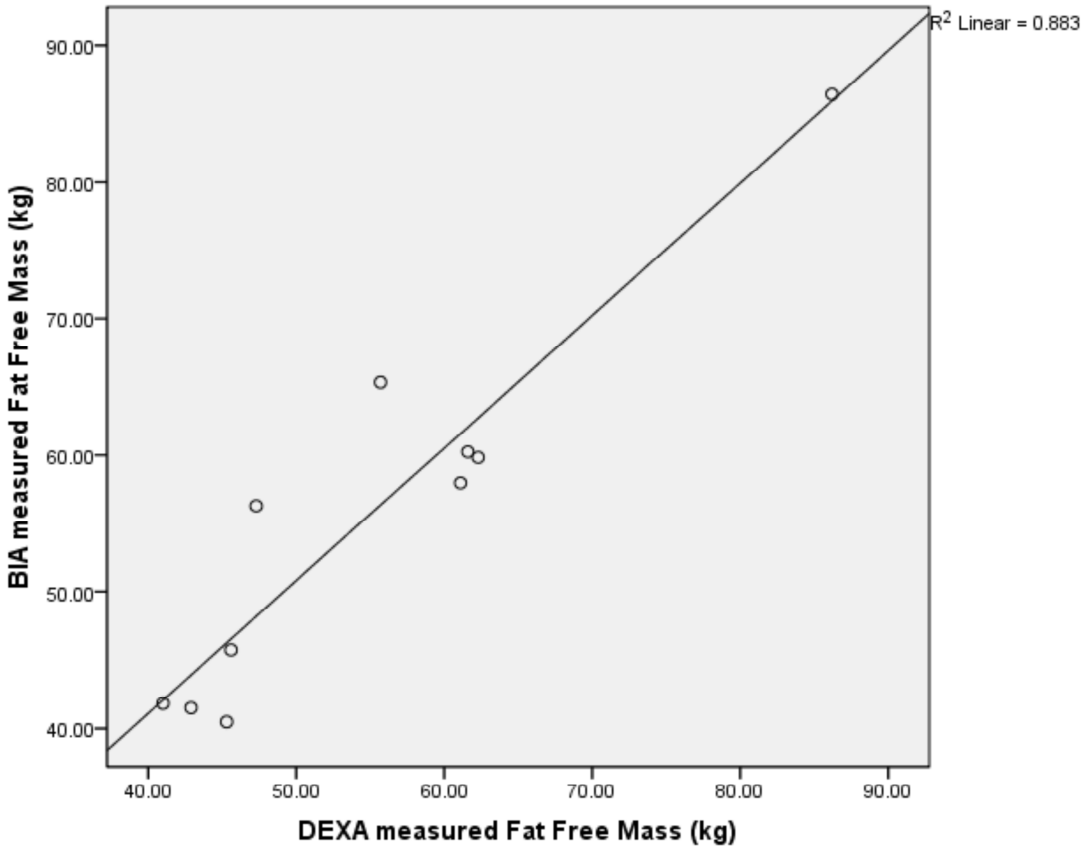

Figure 5.1. Test of linearity diagram for fat free mass of DEXA values in kg plotted against their corresponding values measured by MF-BIA BioScan 920-2 for the 10 study participants

Figure 5.2 shows the test of linearity diagram for fat fee mass percentages of DEXA values plotted against their corresponding values measured by MF-BIA BioScan 920-2 for the 10 study participants using the optimum average of 10 measurements for each participant. Fat free mass percentages by DEXA and MF-MF-BIA for all 10 subjects were close the linearity line with three participants being very close to the linearity line. The correlation coefficient was r=0.805; p=0.005 (table 2). One point was on the linearity line suggesting a 100% agreement between MF-MF-BIA and DEXA measurements in that individual.

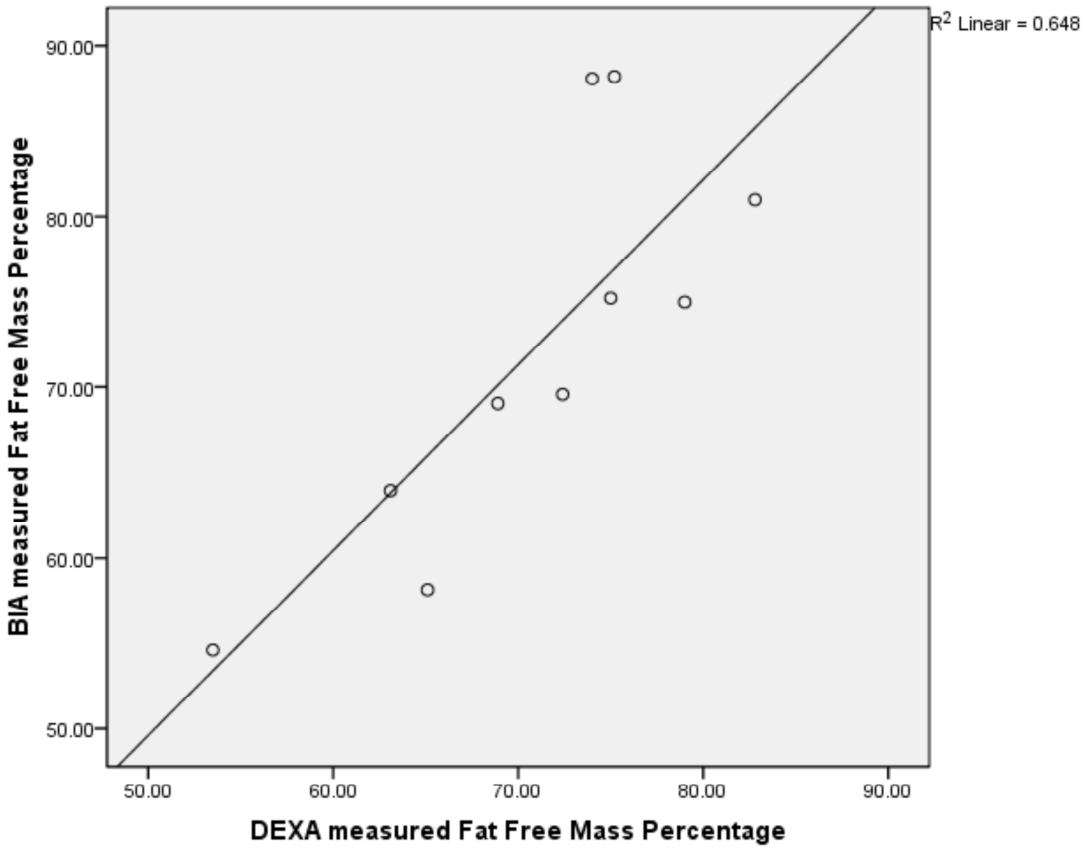

Figure 5.2. Test of linearity diagram for fat free mass percentage of DEXA values plotted against their corresponding values measured by MF-BIA BioScan 920-2 for the 10 study participants.

Figure 5.3 shows the test of linearity diagram for fat free mass of DEXA values in kg plotted against their corresponding values measured by MF-BIA BioScan 920-2 for the 10 study participants using the optimum average of 10 measurements for each participant. The correlation coefficient for average fat mass of 10 MF-MF-BIA measurements and DEXA was 0.882 (p=0.001). All values lied either just above or below the linearity line, and in no participant the measurements by two methods lied exactly at the linearity line to suggest perfect agreement.

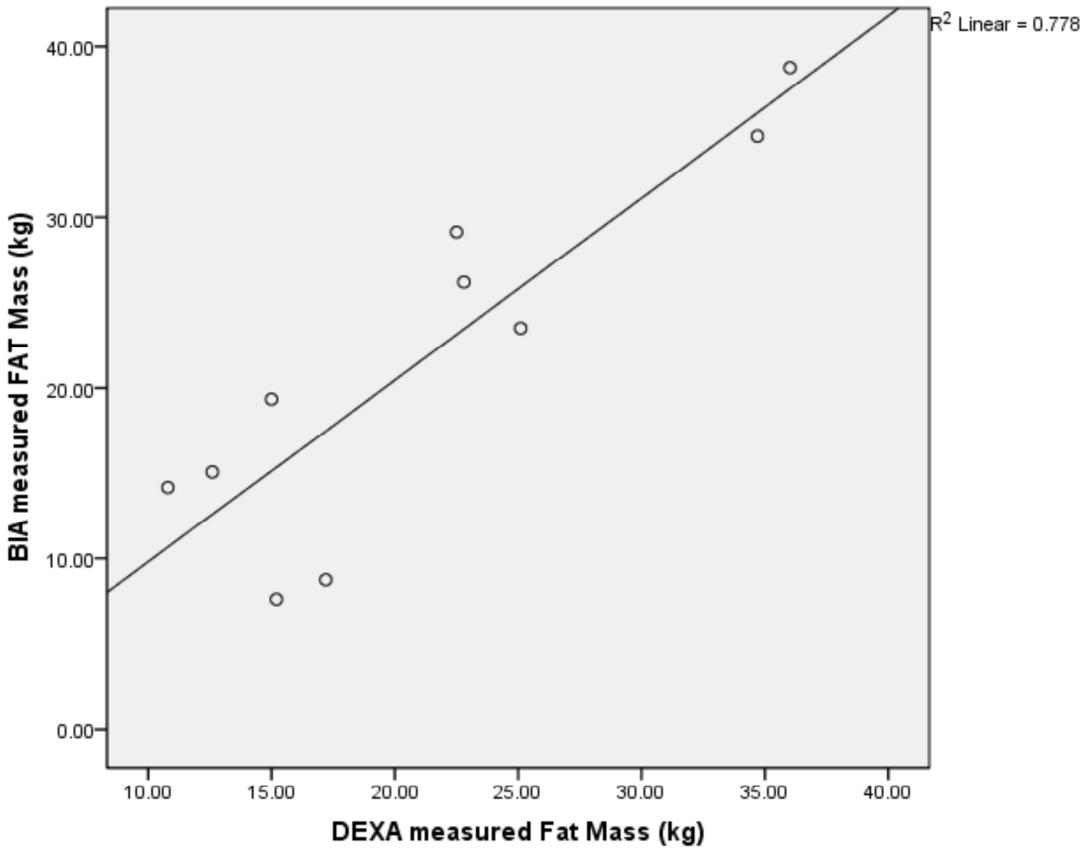

Figure 5.3. Test of linearity diagram/plot for fat mass of DEXA values in kg plotted against their corresponding values measured by MF-BIA BioScan 920-2 for the 10 study participants

Figure 5.4 shows the test of linearity diagram for fat mass percentages of DEXA values kg plotted against their corresponding values measured by MF-BIA BioScan 920-2 for the 10 study participants using optimum average of 10 measurements for each participant. When plotting fat mass percentage measured by MF-MF-BIA against its corresponding values measured by DEXA, I found that no point was on the linearity line they were all laying across the linearity line suggesting not an exact agreement. The correlation coefficient however was 0.794 indicating a significant correlation (p=0.006).

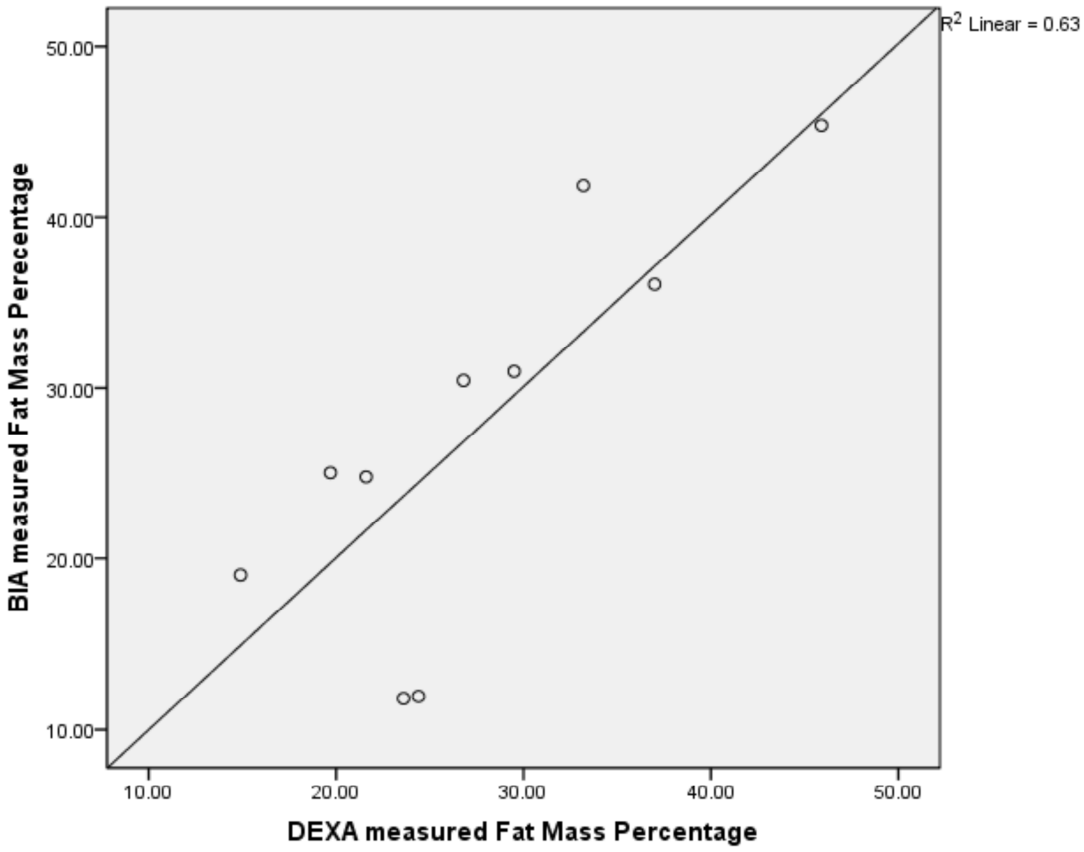

Figure 5.4. Test of linearity diagram for fat mass percentages of DEXA values kg plotted against their corresponding values measured by MF-BIA BioScan 920-2 for the 10 study participants

Figure 5.5 shows the difference of fat free mass (kg) mean of 10 MF-MF-BIA measurement using the optimum average of 10 MF-BIA measurements and DEXA measurement plotted against the mean difference between MF-BIA optimum average and DEXA. Plotting the difference against the mean for fat free mass resulted in all points lying within the limits of agreement. DEXA and MF-MF-BIA results both signify the same clinical interpretation according to Bland an Altman with a lower and upper limit of –-9.17 to 10.37.

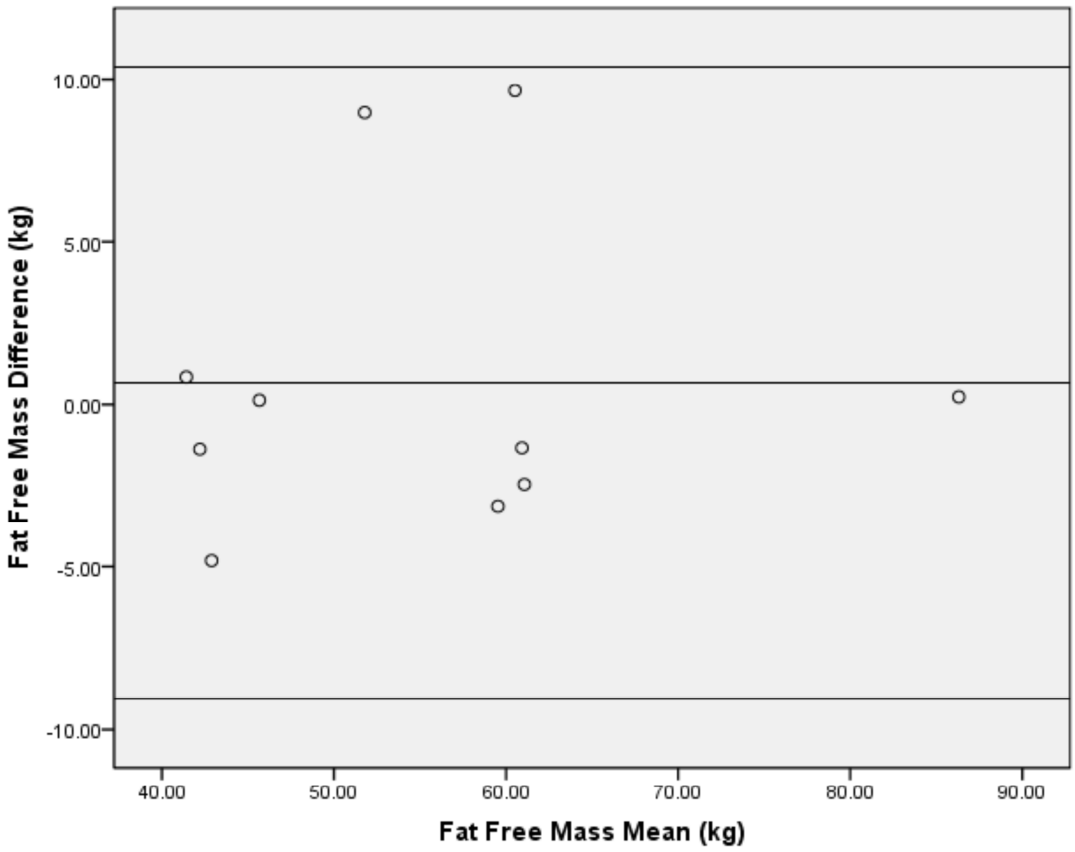

Figure 5.5. Showing the difference of fat free mass (kg) mean of 10 MF-MF-BIA measurements and DEXA measurement plotted against the mean difference between MF-BIA optimum average and DEXA.

Figure 5.6 shows the difference of fat free mass percentage mean of 10 MF-BIA measurements using optimum average of 10 MF-BIA and DEXA measurement plotted against the mean difference between MF-BIA optimum average and DEXA. Plotting the difference against the mean for fat free mass percentage resulted in all points lying within the limits of agreement DEXA and MF-BIA results both signify the same clinical interpretation according to Bland an Altman with a lower and upper limit of -12.86 to 14.98.

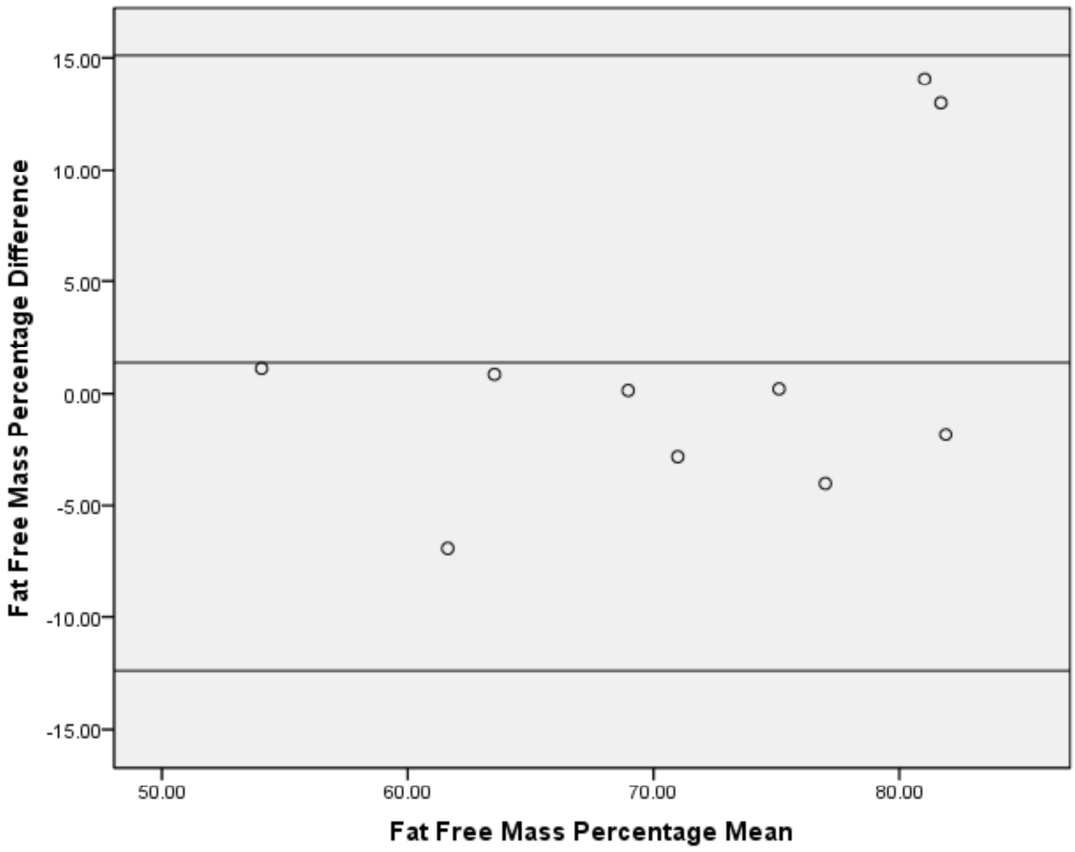

Figure 5.6. Limits of agreement plot showing the difference of fat free mass percentage mean of 10 MF-MF-BIA measurements and DEXA measurement plotted against the mean difference between MF-BIA optimum average and DEXA.

Figure 5.7 shows the difference of fat free mass percentage mean of 10 MF-MF-BIA measurement using optimum average of 10 MF-BIA and DEXA measurement plotted against the mean difference between MF-BIA optimum average and DEXA. All point lied in between the upper and lower limit. Fat mass as in fat free mass all points lied within the limits of agreement with the lower -9.19 and upper at 10.87. This outcome suggests that both MF-MF-BIA and DEXA fat mass results provide the same clinical interpretation.

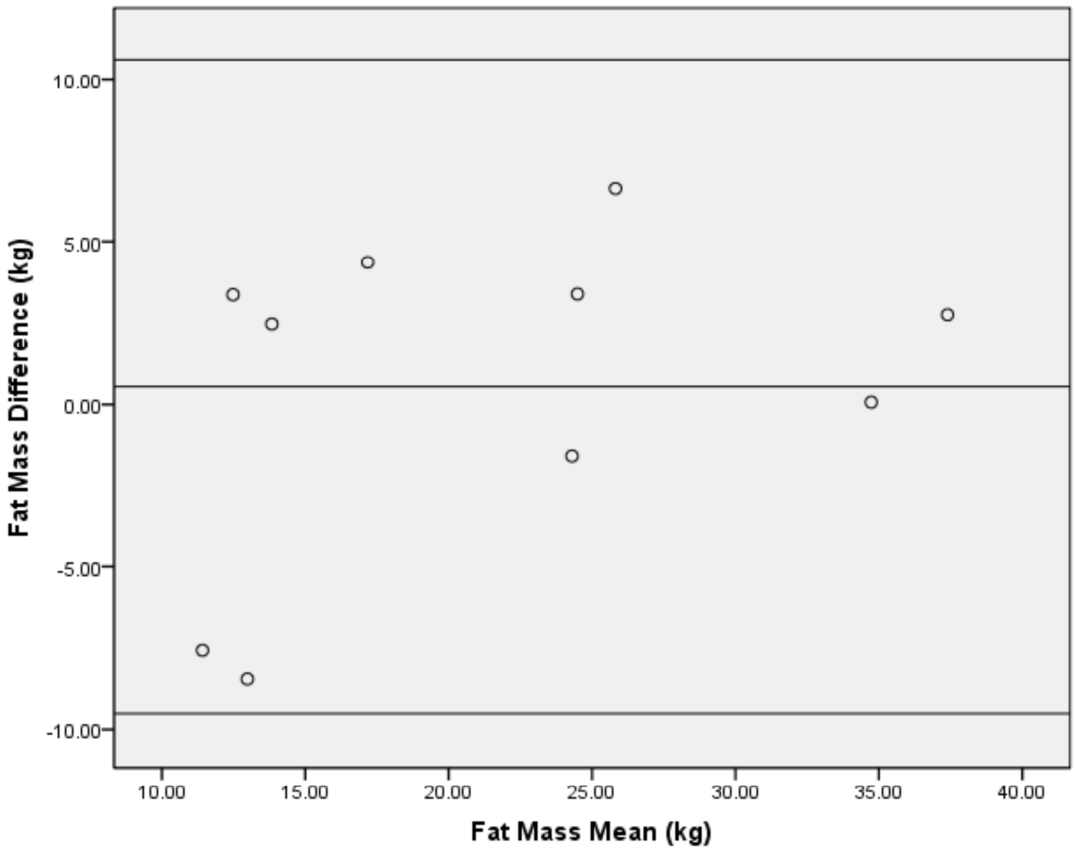

Figure 5.7. Limits of agreement FM plot showing the difference of fat mass (kg) mean of 10 MF-MF-BIA measurements and DEXA measurement plotted against the mean difference between MF-BIA optimum average and DEXA.

Figure 5.8 shows the difference of fat mass percentage mean of 10 MF-MF-BIA measurement using optimum average of 10 MF-BIA and DEXA measurement plotted against the mean difference between MF-BIA optimum average and DEXA. All point lied in between the upper and lower limit. Fat mass percentages points were within the limits of agreement with a lower -13.73 and upper at 14.49. This outcome suggests that both MF-MF-BIA and DEXA fat mass percentage do not vary or provide different result interpretation.

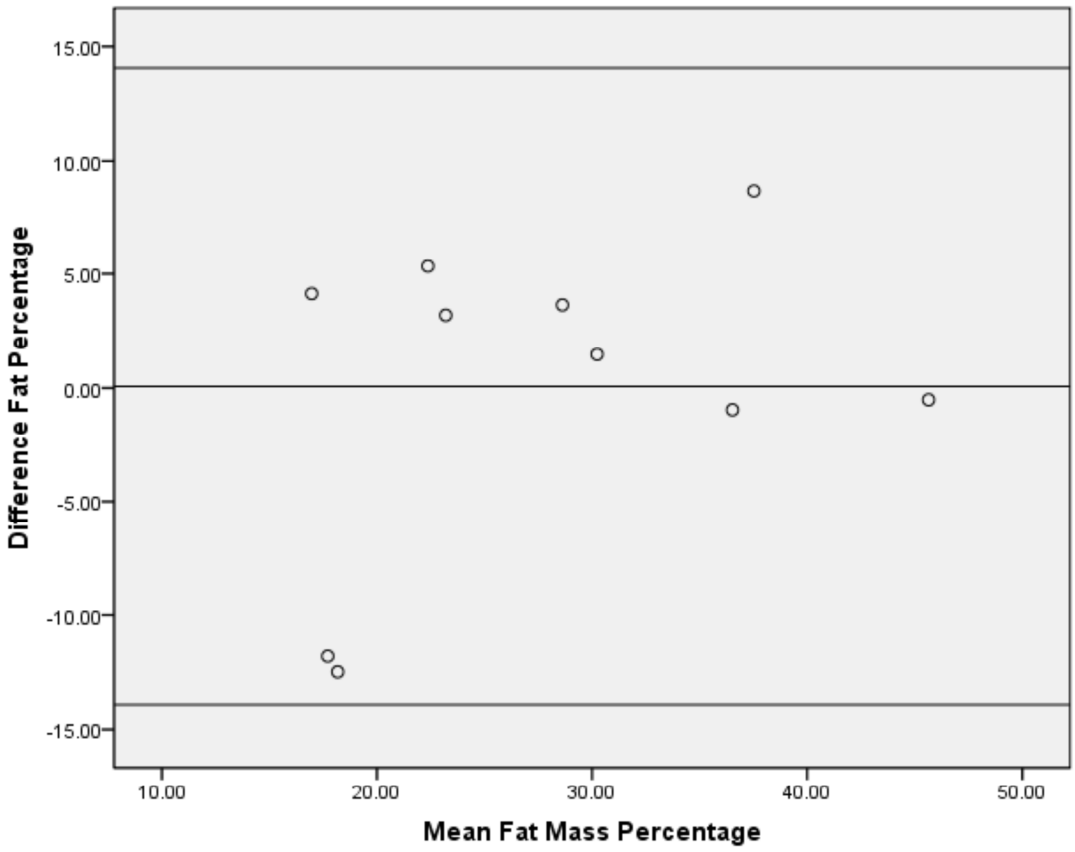

Figure 5.8. Limits of agreement FM% plot showing the difference of fat mass percentage mean of 10 MF-MF-BIA measurements and DEXA measurement plotted against the mean difference between MF-BIA optimum average and DEXA.

5.4.2 First Internal Validation of BioScan 920-2 MF-BIA

In ten participants who were included in the external validation study, the reliability analysis to evaluate the internal consistency of MF-BIA BioScan 920-2 to measure fat free mass and fat mass and their percentages suggested almost perfect agreement between each of the 10 measurements for each component within the same individual. The Cronbachs alpha values were excellent as Table 5.4 below demonstrates (In Table 5.4 individual participants are designated as 1^{st}, 2^{nd}, 3^{rd}, and so on). No statistically significant difference was observed between each single measure for each participant.

	Individual Cronbachs alpha values for each of the 10 participants									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Fat Free Mass (kg)	1	1	1	1	1	1	1	1	1	1
Fat Free Mass %	1	1	0.99	1	1	1	1	1	1	1
Fat Mass (kg)	1	1	0.99	1	1	1	1	1	1	1
Fat Mass %	1	1	0.99	1	1	1	1	1	1	1

Table 5.4. The Cronbachs alpha values for each participant's fat free mass, fat free mass percentage, fat mass and fat mass percentage recorded 10 times by MF-BIA BioScan 920-2 for the 10 participants who participated in the external validation with DEXA part of the study.

5.4.3 Second Internal validation studies of BioScan 920-2 MF-BIA

The internal consistency of BioScan 920-2 MF-BIA for the measurements of fat free mass, fat free mass percentage, fat mass, and fat mass percentage, protein mass, muscle mass, and body cell mass recorded twice consecutively both on admission and discharge were excellent. Table 5.5 shows the Cronbachs alpha values for each of the two Fat free Mass, Fat free mass percentage, fat mass, and fat mass percentage, protein mass, muscle mass, and body cell mass recorded on admission and discharge in the longitudinal study (Chapter 3) for the 40 participants.

	Admission	95% Confidence Intervals	Discharge	95% Confidence Intervals
Fat Free Mass (kg)	0.998	0.997-0.998	0.999	0.997-0.999
Fat Free Mass %	0.997	0.994-0.998	0.994	0.902-0.973
Fat Mass (kg)	0.999	0.997-0.999	0.997	0.994-0.998
Fat Mass%	0.997	0.994-0.998	0.959	0.922-0.978
Protein Mass (kg)	0.989	0.979-0.994	0.957	0.917-0.978
Muscle Mass (kg)	0.997	0.994-0.998	0.969	0.940-0.984
Body Cell Mass (kg)	0.998	0.996-0.999	0.995	0.990-0.997

Table 5.5. Internal consistency assessed using Cronbachs alpha values for each of the two fat free Mass, fat free mass percentage, fat mass, and fat mass percentage, protein mass, muscle mass, and body cell mass recorded on admission and discharge in 40 patients who participated the Longitudinal study (Chapter 3).

The internal consistency of BioScan 920 MF-BIA remained excellent with high Cronbachs alpha values when analysis were stratified by quartiles of BMI for both admission and discharge measurements (Table 5.62). BMI quartiles for the study sample were first quartile (16.08 to 23.36 kg/m^2), second quartile (24.8 to 26.10 kg/m^2), third quartile (26.12 to 28.86 kg/m^2), and fourth quartile (28.92 to 39.35 kg/m^2). There was also no significant difference between first and second measures of BioScan 920-MF-BIA for all measurements conducted. Table 5.6a and 5.6b present the internal consistency Cronbachs-α values for the first and second measurements for fat free mass, fat mass, protein mass, body cell mass, and muscle mass estimated by MF-BIA on admission and discharge stratified by BMI quartile.

	Admission		Discharge	
Fat Free Mass (kg)	Cronbachs Alpha	95% Confidence Intervals	Cronbachs Alpha	95% Confidence Intervals
1st quartile	0.995	0.981 to 0.999	0.999	0.996 to 1.0
2nd quartile	1	0.998 to 1.0	0.996	0.95 to 0.999
3rd quartile	0.998	(0.991 to 0.999	1	
4th quartile	1		1	
Fat Free Mass %				
1st quartile	0.988	0.953 to 0.997	0.997	0.987 to 0.999
2nd quartile	0.998	0.9963 to 1.0)	0.986	0.947 to 0.996
3rd quartile	0.99	0.959 to 0.997	1	
4th quartile	0.999	0.998 to 1.0	0.683	-0.274 to 0.921
Fat Mass (kg)				
1st quartile	0.988	0.951 to 0.997	0.983	0.931 to 0.996
2nd quartile	0.998	0.991 to .999	0.985	0.944 to 0.996
3rd quartile	0.979	0.917 to 0.995	0.99	0.956 to 0.998

Table 5.6a. MF-BIA Internal consistency by BMI quartile, continued

	Admission		Discharge	
Fat Mass %				
1st quartile	0.988	0.953 to 0.997	0.997	0.988 to 0.999)
2nd quartile	0.999	0.994 to 1.0	0.986	0.947 to 0.996
3rd quartile	0.989	0.958 to 0.997	0.499	-1.2 to 0.887
4th quartile	0.999	0.998 to 1.0	0.986	0.945 to 0.997

Table 5.6a. MF-BIA Internal consistency by BMI quartile: assessed using Cronbachs alpha value for each of the two Fat free Mass, Fat free mass percentage, fat mass, and fat mass percentage recorded on admission and discharge by quartiles of body mass index in the in 40 patients participated in the longitudinal study (Chapter 3).

	Admission		Discharge	
Protein Mass (kg)	Cronbachs Alpha	95% Confidence Intervals	Cronbachs Alpha	95% Confidence Intervals
1st quartile	0.985	0.94 to 0.996	0.994	0.976 to 0.999
2nd quartile	0.984	0.928 to 0.996	0.728	-0.097 to 0.932
3rd quartile	0.985	0.983 to 0.996	1 0	0.999 to 1.0
4th quartile	1	0.998 to 1.0	1.0	0.999 to 1.0
Muscle Mass (kg)				
1st quartile	0.987	0.948 to 0.997	0.652	-0.401 to 0.914
2nd quartile	1	0.998 to 1.0	1	
3rd quartile	0.999	0.996 to 1.0)	1	
4th quartile	1	.999 to 1.0	1	
Body Cell Mass (kg)				
1st quartile	0.979	0.916 to 0.995	0.997	0.989 to 0.999
2nd quartile	0.998	0.991 to 0.999	0.992	0.971 to 0.998
3rd quartile	1	0.99 to 1.0	0.984	0.928 to 0.996
4th quartile	0.999	0.996 to 1.0	1	0.99 to 1.0

Table 5.6b. Internal consistency assessed using Cronbachs alpha value for each of the two protein mass, muscle mass, and body cell mass recorded on admission and discharge by quartiles of body mass index in the in 40 patients participated in the longitudinal study (Chapter 3).

5.5 Discussion

This validation study confirms the usefulness of MF-BIA measurement using BioScan 920-2 in measuring fat and fat free mass other components such as protein mass, muscle mass and cell mass in people with recent stroke and TIA. All Cronbachs alpha values observed were > 0.9 with no statistical significant differences between any two consecutive measurements in all 40 participants of longitudinal study. The internal consistency was also excellent for the first two, three and so on until 10 measurements for each of the 10 participants included in the external validation. Cronbachs alpha values suggested excellent MF-BIA BioScan 920-2 reliability.

There was also a high level of agreement between MF-BIA BioScan 920-2 and DEXA Hologic Discovery. When plotted using Bland & Altman methods for a comparing two different methods, all of the values of fat free mass, fat mass, and their percentages lied within the upper and lower limits suggesting they do not differ significantly.

5.5.1 Fat Free Mass

Fat free mass and fat free mass percentage measured by MF-BIA and DEXA were strongly correlated; $p<0.0001$ and $p=0.005$ respectively. The test of linearity suggested agreement between the two methods (how well they lie on the linearity line). All points were lying along the linearity (agreement) line (Figure 5.1 & Figure 5.2). In Bland and Altman method, plotting the average of the two methods against the difference, all points lied within the limits of agreements (Figure 5.5 & Figure 5.6). Fat free mass measurement using DEXA and MF-BIA can be used interchangeably. Both methods provide similar interpretation of fat free mass constituent in body composition.

5.5.1 Fat Mass

There were also strong significant correlations between MF-BIA and DEXA measurements for fat mass ($p<0.001$) and fat mass percentage ($p=0.006$). All points were lying along the linearity (agreement) line (Figure 5.3 and Figure 5.4) suggesting agreement between the two methods. There were no statistically significant differences between the means of two measurements. In Bland and Altman method, plotting the average of the two methods against the difference (Figures 5.7 & 5.8), all points lied within the limits of agreements. This suggests that the interpretation of the measurement by MF-BIA and DEXA are similar and thus they can both be used interchangeably.

5.5.3 Comparison with other studies:

This study suggests a very good agreement between MF-BIA and DEXA. This is in agreement with previous other studies. Pate johns et al (2006) demonstrated a significantly strong correlation between MF-BIA and DEXA with an r^2 values of fat mass and fat free mass of 0.81 for both ($p<0.001$) (276). The similar strong correlations were also demonstrated in our study with an r^2 values of 0.88 ($p<0.0001$) and 0.78 ($p=0.001$) for fat free mass and fat mass, respectively. In Pateyjohns' study all participants were men (n=43), apparently healthy, between age of 25-60 years, and are either overweight or obese. In my study, only one participant was obese which does not allow me to draw any meaningful conclusion on obese subjects with recent stroke or TIA. However, the correlation between MF-BIA and DEXA measurement was strong for overweight subjects as in Pateyjohns study. It should, however, be cautioned that the overweight population in my study composed only four participants. The agreement between my study results and that of Pateyjohns may suggest that DEXA and MF-BIA provide similar result in overweight subjects.

Body fat percentage was underestimated in a study by Sun et al (277) with MF-BIA measurements of body fat percentage corresponding to 32.89 ± 8.00%, being statistically significantly lower than DEXA measurement of 34.72 ± 8.66%; $p<0.001$. These results were contrary to my study findings, which indicated very good agreement between average body fat percentage measured by DEXA (27.7±9.1) and MF-BIA (27.7±11.5) ($p>0.05$). It should be noted that the study by Sun and colleagues was much larger than my study with 591 healthy subjects. The age range, in the study was not restricted to older population and they study population's age ranged between 19 and 60 years; my study population age range was 50-75 years. The difference in sample characteristics with regard to distribution of age of the population studied may explain differing results observed.

My study as opposed to other studies drew its sample from specific patients population; stroke/TIA population. This may suggest that the agreement seen across fat free mass and fat mass measured by DEXA and MF-BIA in my study is related to the fact that I did not use a wide age range (as in Pateyjohns study), and used a population of similar clinical and health characteristics. The aforementioned studies used apparently healthy volunteer population.

5.5.4 Strengths and Weaknesses:

Strengths of this study include that our patients had variable body mass indexes covering all body mass index ranges albeit with not many patients in the obese category. The DEXA and MF-BIA measurements were carried out consecutively removing MF-MF-BIAs that may occur due to large time scale gap during which body composition may change. I was able to carry out both internal and validation studies of MF-BIA BioScan and the results were consistent.

The main weakness of the study is the relatively small sample size. There was not enough sample size for stratified analyses by BMI categories to allow better comparison

with previous studies. There were difficulties in recruiting older people with a condition such as stroke. Transportation can be a problem for such patients (post stroke) with many living in remote areas.

Financial restraint as a PhD studentship project limited the number of DEXA scans I can carry out. DEXA scans are costly and recruiting a larger sample can be expensive. In addition, specialized personnel must be available with a clinician during the scans making scans only available at certain times and dates. Therefore, the sample size for external validation was conducted in 10 participants.

5.5.5 Clinical Interpretation:

The results of these validation studies indicate strong and significant correlation between MF-BIA measurement and DEXA with regards to fat mass and fat free mass. When observing the limits of agreement in figures 5.5-5.8, it can be seen that all measurements lied within the upper and lower limits (95% Confidence intervals). This suggests that both MF-BIA and DEXA readings do not translate into different clinical interpretation. Only when the measurements are out of the range of limits of agreement by both methods, this suggest two different clinical interpretation (275). Based on these findings both DEXA and MF-BIA can be used interchangeably to measure the body composition indices examined. In addition, MF-BIA also has internal consistency thus it provides a reliable, easy to perform measurement method to assess fat free mass, fat mass, and their percentages in stroke and TIA patient population.

5.5.6 Future work

As indicated in the earlier chapters of the thesis (Chapters 2 and 3) that poor nutritional status have negative prognosis on treatment outcomes in patients in general and stroke patients specifically. Given the reliability of MF-BIA BioScan equipment in assessing

fat free mass and fat mass, this study sets the basis of further research to confirm these findings in a larger sample with various patient populations which are associated with malnutrition. Further work should be aim at the feasibility of using MF-BIA in clinical settings as quick, simple, and easy to use equipment in assessing body composition indices in such patients including stroke patients in long term rehabilitation facilities. In particular relevance to the subject of this thesis, body composition changes after stroke can vary, and that the ability to measure such changes may aid in the nutritional management of stroke patients, allowing clinicians to prevent catabolism commonly seen in stroke patients with long term disability.

What is equally important is to carry out further external validation study of MF-BIA using different BMI categories. My study had only normal weight and overweight group of stroke/TIA patients (only one obese and none were underweight). Thus, firm conclusions cannot be made due to the small number of patients in each category.

Previously one study compared body composition changes after stroke (n = 35) (273). It used the DEXA method in evaluating body composition, indicating that significant losses in lean body mass and bone density loss occurred in the paretic leg compared to the non-paretic leg after stroke; $p<0.05$ (273). This study did show that body composition changes occurred in stroke patients (as in my longitudinal study; Chapter 3). It lacked the validation of MF-BIA by DEXA as only MF-BIA can be readily available in clinical settings and not DEXA. DEXA is relatively expensive, time consuming to perform ranging from 15-20 minutes for one measurement and inconvenient for patients with disability or limited mobility. The authors did not consider at time of the study to examine the utility of MF-BIA or its reliability against DEXA. My study provides new evidence that MF-BIA can be a reliable measurement tool which has excellent agreement with gold standard method, DEXA.

5.5.7 Conclusion

A large number of equipment's with different specification and formulae to calculate body composition indices, many of which were validated, are available at present (183). It is very important that the formulae being used in such equipment are known as in Kyle 2004 paper (183) in order to carry out validation studies and understand if such formulae are useful or not. The formulae programmed in the equipment I used in my study are not known and not revealed by the manufacturer. Nevertheless, my results suggest that MF-BIA BioScan 920-2 is in agreement with DEXA making it an attractive candidate for further research and ultimately for use in clinical care of stroke/TIA patients. MF-BIA BioScan 920-2 was not mentioned in Kyle 2004 literature.

It could be that my study was the first on MF-BIA BioScan 920-2 or it was not reported due to the unknown formulae it used to calculate its estimate of Fat free mass, fat mass, and their percentages. As described in Kyle's 2004 literature review, the validation of the different MF-BIA equipment was carried out against several different gold standard or reference measure. Although it is important to examine MF-BIA against several gold standard methods for assessing body composition, it is equally important to find a universal gold standard method to validate MF-BIA against. The validation against one gold standard method will make it easier for validators to follow one protocol eliminating errors that may cause MF-MF-BIAs when following several different methods. Each gold standard method can have its own errors and may contribute to larger discrepancies in the agreement with MF-BIA than another. Having one method will possibly allow for filtering of MF-BIA equipment's to reach the ones that best provide an agreement with one reference gold standard.

Future work examining the utility of MF-BIA should aim to achieve larger sample size. They should also gather information and evidence on the utility of MF-BIA in other chronic long term disabling conditions including long term management of stroke considering the scarcity of existing evidence using body composition measurement as a monitoring exercise to identify at risk patients and also to monitor progress of the

condition i.e. effectiveness of nutritional intervention in addressing malnutrition associated with long term illnesses. In summary, this validation sub-study suggests an excellent validity of MF-BIA measures for fat free mass, fat mass, and their percentages. A larger sample with wide ranging BMI categories would have been desirable.

Chapter 6: Conclusion

My PhD work described in this thesis aimed to better understand selected body composition changes in acute stroke and how such changes relate to both objective and subjective outcomes. To achieve these aims, I used multi-frequency bioelectrical impedance analysis (MF-BIA BioScan 920-2, Maltron International Limited, Essex, UK) and performed both internal and external validation studies in participants with a recent stroke or TIA. I also examined the utility of MF-BIA in diagnosing dehydration in acute stroke. To better understand the prognostic significance of malnutrition on outcomes in patients with cardiovascular disease I conducted a systematic review and meta-analysis examining the association between various nutritional markers of malnutrition and outcomes in people with a cardiovascular event.

6.1 Malnutrition in acute stroke

In order to put my research in the clinical context of stroke, it is important to re-emphasize the prevalence of malnutrition after stroke. The prevalence of malnutrition including dehydration is well documented at the time of admission with an acute stroke, and the nutritional status of patients with stroke also often deteriorates during the acute hospital stay (17, 20, 61, 65, 70, 71). The prevalence of malnutrition in stroke is due to dysphagia, a common stroke symptom with reported prevalence of at least 40% (120), and other cognitive problems. Dysphagia impedes the ability to swallow while cognitive problems may change eating behaviour thereby affecting dietary intake. Malnutrition has adverse effects on body composition especially in conditions that escalate the stress response in the body and may be associated with immobility such as in stroke.

The evidence I presented in this thesis deepens the understanding of malnutrition in stroke through highlighting its impact on stroke patients. In Chapter 2, I presented evidence on the association between malnutrition on outcomes in people who had a cardiovascular event (post-CVD). In chapter 3, I descriptively presented the impact of stroke on body composition changes and further tried to understand how such changes

may impact objective and subjective outcomes in stroke patients. In Chapter 4, I examined the utility of multi-frequency bioelectrical impedance analysis in diagnosing dehydration after stroke as a non-invasive and swift bedside method, and in Chapter 5 I validated MF-BIA estimates for fat free mass and fat mass against DEXA and also performed internal validation studies.

6.2 Studies findings in the context of the whole thesis

The systematic review and meta-analysis presented in Chapter 2 provided evidence on the association between markers of malnutrition and health outcomes after a cardiovascular (CVD) event. This association was assessed in both cardiovascular and cerebrovascular diseases. Selected nutrition markers examined included body mass index (BMI), weight loss, skin fold thickness, low serum albumin, high serum creatinine, increased serum osmolality, and malnutrition assessed by nutrition assessment tools such as Subjective Global Assessment (SGA) tool. My systematic review and meta-analysis suggest that there was no association between obesity or overweight (both compared to normal weight) and mortality, but there was an association between underweight (compared to normal weight) and higher mortality. Meta-analysis of studies that examined the association between malnutrition assessed using nutrition assessment tools and mortality suggested an association between malnutrition and higher mortality.

The one included study that examined weight loss as a marker of malnutrition suggested that weight loss had no association with mortality, but reduced the risk of recurrent events. If weight loss occurred in obese or overweight patients it could improve their health and post-CVD event outcomes as it can place them within the healthy weight range. It will improve their overall health and reduce their adiposity which is considered a prothrombotic state thus reducing the risk of recurrent event. On the contrary, if patients were already malnourished weight loss could cause further deterioration in their nutritional status thereby increasing the risk of poor outcomes including mortality.

Other nutrition markers examined included low serum albumin, high serum osmolality, and high serum creatinine and my findings suggested that these markers of malnutrition may be associated with higher levels of mortality, but these findings were based on a small number of studies.

The aim of the longitudinal study was to examine body composition changes after stroke and examine whether they have an impact on outcomes. As described in detail in the rationale and hypothesis of the longitudinal cohort study, I hypothesised that body composition changes do occur after stroke due to nutritional inadequacy compounded by the stress response and that such changes may have negative prognosis on outcomes. The findings of the longitudinal study showed interesting observations regarding body composition changes (fat free mass loss, fat mass gain, and protein mass, muscles mass and body cell mass losses) in patients on modified diet, NBM feeding regimen, and those who were classified as suffering from a total anterior circulation infarct stroke (TACI). Due to a small sample size and short duration of hospital stay a firm conclusion on the relationship between body composition changes and type of feeding regimen, type of stroke, and objective outcomes or subjective outcomes cannot be made. Follow up questionnaires were administered at 6 month post discharge from hospital and the response rate was modest with most well participants (with mildest strokes and no post-stroke symptoms) responding to the questionnaire. Furthermore, it was impossible to know what body composition changes occurred over time within the follow up period of 6 months. As a result, no firm conclusion can be made based on the findings.

Nonetheless, I have shown what type of body composition changes occur in stroke and trends in changes occurring in major body components. Fat free mass loss, fat mass gain, protein mass loss, muscle mass loss, and body cell mass loss were observed in patients who were prescribed modified diet (soft mashed diet, pureed diet, or nil-by-mouth), nil-by-mouth, and patients experiencing total anterior circulation infarct stroke subtype (TACI). Further, fat free mass loss, fat mass gain, and protein mass loss were seen mainly in patients with moderate to severe stroke (National Institute of Health Stroke Severity score >10).

Equally important and relevant in the nutritional care of stroke patients is their hydration status; dehydration is prevalent ranging from 30% to more than 60% post-stroke, and has been shown to have impact on post stroke outcomes. The literature presented in Chapter 2, which examined the association of dehydration diagnosed using a serum marker, serum osmolality, and outcomes suggested an association between dehydration and mortality, and an association with complications such as thromboembolism. Therefore, diagnosing and monitoring dehydration in stroke patients should be a priority.

In Chapter 4, I presented the study findings carried out to examine the diagnostic accuracy of MF-BIA BioScan 920-2 in diagnosing dehydration in stroke patients. I found that MF-BIA was not useful to diagnose water-loss dehydration after stroke. Its diagnostic accuracy was far too low to usefully diagnose current or impending dehydration at any selected cut-off point. The caveat is that these findings do not necessarily translate to mean that MF-BIA does not accurately diagnose cellular dehydration, but rather highlight the certain limitations in this study that I will present in the limitation section of this chapter.

In the penultimate chapter of this thesis, Chapter 5, I presented findings of the validation studies of MF-BIA BioScan 920-2. The validation of MF-BIA against DEXA is essential to understand whether fat free mass and fat mass estimated by MF-BIA are in agreement with a reference method (DEXA) which will give confidence to my study findings of the longitudinal study based on MF-BIA estimates. In the validation study of MF-BIA BioScan 920-2 my results suggested that MF-BIA BioScan 920-2 is in agreement with DEXA making it an attractive candidate for further research and ultimately for use in clinical care of stroke/TIA patients and patients with similar situations (e.g. hip fracture patients). The internal consistency of MF-BIA BioScan 920-2 measurement for the selected body composition components was excellent.

6.3 The contribution of this thesis to stroke research

Given the prevalence of malnutrition in stroke patients my study gathered evidence regarding the association between malnutrition and outcomes. The study also presented evidence on the extent of body composition changes that can happen after stroke with regards to the type of feeding regimen, stroke severity assessed by NIHSS score, and stroke subtype assessed using Oxfordshire Community Stroke Project Classification. Whilst these body composition changes occurring during the acute hospital stay can have an impact on longer outcomes in stroke I could not make any firm conclusion based on my results due to relatively small sample size. Although MF-BIA can estimate water fraction body compartment, it does not seem to be useful in diagnosing dehydration after stroke. Nevertheless, MF-BIA may provide valid body composition estimates of fat free mass and fat mass as the validation study suggested its agreement with reference method DEXA. These findings may be helpful in initiating larger validation studies of MF-BIA to examine its agreement with other reference methods.

These findings are relevant to clinicians and health professionals working in the field of stroke management. They may be able to improve the nutritional status of malnourished patients by understanding their nutritional requirement through observing patient body composition changes (e.g. amount of fat free mass loss) and put nutritional management on the list of their priorities to avoid poor outcomes; the evidence from my systematic review suggests that malnutrition after stroke (or a cardiovascular event) is associated with poor outcomes.

Further, my study was novel and provided normative data that can be used for similar stroke related future nutritional research. It can be used in future sample size calculations and to help researchers in the field to determine minimally clinically significant differences for similar research and to be used in further targeted intervention clinical trial.

6.4 Limitations

In the systematic review and meta-analysis, not all studies included used the same comparison group and there were lack of studies using the particular reference category I was interested in i.e. normal nutrition marker parameters, making it impossible to include all studies available in the same meta-analysis. Therefore the evidence synthesis was based on the results from smaller number of studies.

In the longitudinal study follow up data did not lead to any conclusion regarding the association between body composition changes that occurred during acute hospital stay and longer term outcomes. It would be impossible to know what body composition changes occurred during the follow up period that have impacted health differently at final follow up. For example, if fat free mass loss occurred during hospital stay it might have been reversed during the six month follow up period leading to improved strength in a participant. Therefore responses at time of follow up questionnaire administration may not reflect that participant physical health during acute hospital stay and while on the path to recovery. This was evident as participants with fat free mass loss scored higher than those with fat free mass gains or no gains in the Physical Component Summary Scores (PCS) of the short form survey (SF36v2), Barthel Index, and Stroke Impact scale selected items. However, the number of participants who completed follow-up were small and most of them suffered milder stroke and therefore the findings are plausible as they were expected to have relatively good outcome.

I did not find MF-BIA useful in diagnosing dehydration. This can be attributed to the fact that using serum osmolality and serum osmolarity as reference to compare MF-BIA BioScan 920-2 estimated and calculated (from Ritz equations) water fraction values may not have been appropriate and considering that serum osmolality and osmolarity reflect intravascular component rather than cellular dehydration. In addition, the malfunction in the equipment resulted in discarding 40% of my participants' data making the sample small for firm conclusion. When analysed stratified by sex and

using TBW as percentage of body weight, the diagnostic accuracy of MF-BIA improved but remained low. The sample however was further reduced in the stratified analysis.

In Chapter 5, the validation of MF-BIA BioScan 920-2, sample size was relatively small. Although the sample was sufficient to show the validity of the machine used, it would be preferable to have a larger sample in order to validate MF-BIA estimates across a wide range of BMI categories including underweight, normal weight, overweight, and obese participants.

With regard to the device I did not find MF-BIA BioScan 920-2 particularly user friendly. It does not have a keyboard for swift data entry. Data entry (age, sex, etc) and saving of data was therefore slow process prone to result in errors and data loss. Re-running a second measurement for the same participant also required re-entering of all the same information again unless otherwise the new test overwrites previously recorded first examination data. Analysed data were not easily accessible to visual check without downloading the full data set, and there is no warning when unrealistic/implausible readings are recorded. When I carried out MF-BIA measurements I checked initial 20 measurements in the longitudinal and diagnostic accuracy study for any discrepancies and none were observed giving me confidence of the measurements. However as it appeared later I had to discard 40% of participant data from the diagnostic accuracy study and one patient's muscle mass and protein mass data was not estimated in the longitudinal study.

6.5 Future work

If I had the opportunity to carry out the same longitudinal study I would standardize my measurement time points i.e. instead of admission and discharge, measurements can be carried out at two fixed time points, for example day one after admission and day five. During follow up period, it would have been ideal to carry out serial assessment of body composition to monitor changes in body composition after hospital discharge. It would

allow me to observe body composition changes after hospital discharge. For external validation future work examining the utility of MF-BIA should aim to achieve larger sample size. A larger sample with participants who are in a wide range of BMI categories is desirable to understand the usefulness of MF-BIA agreement with DEXA more comprehensively.

Using an alternative device which uses the MF-BIA technique that is more users friendly is also advisable. In addition I would select a machine with known validated equations. In my study, formulae to calculate water compartments are built in the device and are not known to the investigators and I do not know if they used validated formulae and this is why I also used Ritz formulae developed for older people to estimate water compartments. Therefore it is important to note that many BIA machines with different specification and formulae, many of which were validated, are available in the market at present. It is very important that the formulae being used in such equipment are known as in Kyle 2004 paper (183) in order to carry out validation studies and understand whether such devices are reliable to use in clinical practice. The external validation study against DEXA, however, suggested that the MF-BIA machine I used may be reliably used for accurate estimations of fat mass and fat free mass.

Summary

My study was novel as it provided new information with regard to body composition changes in acute stroke while utilizing new validated equipment in estimating body composition component of fat free mass and fat mass. My study also aimed to investigate new non-invasive methods to diagnose dehydration in stroke patients. It contributed new knowledge that can be useful in future research for example sample size calculation and can help researchers in the field to determine minimally clinically significant differences for similar research and further targeted intervention clinical trials.

Appendix I Systematic Review Study Protocol

Malnutrition Markers/assessment tools and their ability to predict long term poor clinical outcomes and mortality in Myocardial Infarction, Transient Ischemic Attack, and Stroke: a systematic review of Prospective Cohort Studies

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Abstract: this is a review protocol not a review and there is not abstract

Justification:

Poor recovery outcomes in acute cardiovascular events, mainly in stroke, are well documented in patients diagnosed with malnutrition. Although poor outcomes stroke are the main acute cardiovascular event reported to be affected by nutritional status acutely. It is an indication that the state of malnutrition plays a major role in other acute cardiovascular event such as Transient ischemic attack and Myocardial infarction. There is a vast array of poor outcomes associated poor nutritional status in acute cardiovascular events ranging from an increased length of stay to increased mortality frequency. Hospitalization duration, acute complications, quality of life, and death are some of the main outcomes affected by poor nutritional status in patients acutely. Malnutrition diagnosed acutely can have a significant influence on recovery outcomes. Understanding the relationship between nutritional status and recovery outcomes associated with acute cardiovascular events can contribute to a better appreciation on the role of nutrition care in acute cardiovascular events. Monitoring nutritionals status acutely can provide valuable information on acute care measures that can improve recovery outcomes.

Objectives:

Our objective to assess nutrition markers from serum albumin measures of hydration, body mass index, body fat, triceps skin fold, and/or serum Creatinine can predict poor outcomes as defined by hospital readmission, disability, functional status and/or mortality after acute cardiovascular event defined as stroke, transient ischemic attack, or myocardial infarction**.**

Methods:

Criteria for considering studies for this review

Types of studies:

Prospect cohort studies examining poor outcomes with evident nutrition markers measured and outcomes evaluated as defined in the objectives.

Type of Participants:

Participants aged 18 years and older who have had a stroke, myocardial infarction, or transient ischemic attack with the nutrition markers serum albumin measures of hydration, body mass index, triceps skin fold, and/or serum Creatinine measured and the outcomes hospital readmission, length of hospital stay, discharge destination, disability, functional status and/or mortality evaluated.

Type of exposure:

Most nutrition markers discussed and analysed in cohort studies are Body Mass Index, Albumin, triceps skin fold, and mid upper arm circumference are reported in several studies, and hydration measures, serum Creatinine (rarely included), and studies using variable malnutrition assessment tools such as Mini Nutritional Assessment (MNA) or Subjective Global Assessment tool (SGA). Further analysis of the data may result in eliminating some of the exposures and elect to focus on those most frequently use to produce a systematic review.

Types of outcomes measures:

Most cohort studies report mortality as all cause mortality. Several studies report other outcomes such as length of hospital stay, functional status as defined by Barthel Index scores. Very few studies report discharge destination and hospital readmission. Later revisions of this review may result in the exclusion of the outcomes that are inconsistently and/or not frequently reported enough to synthesize a systematic review.

Search methods and identification studies:

We conducted a sensitive electronic search of MEDLINE and EMBASE since 1950. Studies abstracts were examined for inclusion in the list of studies to be examined for review inclusion. Existing reviews bibliographies were examined for any relevant studies for the review. Searches were carried out by the PhD student who was trained by an expert systematic review and received systematic review training in a recognized course.

Selection of studies:

Two reviewers independently selected relevant studies with each synthesizing a list of studies with abstracts included. After discussion, agreement, and consensus the two reviews finalized which list of studied meet the inclusion criteria and will be included in the final systematic review.

Data extraction: the search will find the relevant articles. Two reviewers will review and extract data independently using a Cohort data extraction form. This is for data duplication to make sure that no major discrepancy occurs. Details for data extraction will include study population, type of study, measured outcomes, and validity of the results and methods. If possible Validity of studies will be checked through evaluating if the authors used standardized and recognized nutrition assessment markers or tools, if authors diagnosed exposure through medically standardized methods, and if outcomes of functional capacity were assessed using standardized methods such as Barthel Index and SF-36.

Data Analysis:

Descriptive statistics, linear and logistic regression models will be used to describe the relationship between each identified nutrition markers and outcomes including disability, morbidity, mortality, readmission, and discharge destination. 95% Confidence intervals and correlation coefficient will also be presented in the final results.

Appendix II: Search Strategy

Database: EMBASE, MEDLINE, and Web of Science
Date of Search: from inception to October 2010

Search strategy including indexing terms used in MEDLINE

1. BMI.mp
2. body fat distribution/ or body mass index/ or body size/ or body weight/ or waist circumference/ or skinfold thickness/ or waist-hip ratio/
3. weight change or weight loss
4. body weight changes/ or weight loss/ or thinness/
5. adiposity.mp.
6. adiposity/ or body weight/ or waist circumference/ or skinfold thickness/ or waist-hip ratio/
7. Creatinine.mp.
8. *Creatinine/bl, ur [Blood, Urine]
9. Malnutrition.mp.
10. malnutrition/ or deficiency diseases/ or magnesium deficiency/ or potassium deficiency/ or protein deficiency/ or protein-energy malnutrition/
11. Low albumin or Low prealbumin or Low transferrin.
12. Prealbumin/bl [Blood]
13. *Transferrin/bl [Blood]
14. *Serum Albumin/bl [Blood]
15. (hydrat* or dehydrat*).mp.
16. dehydration/ or hypercalcemia/ or hyperkalemia/ or hypernatremia/ or hypocalcemia/ or hypokalemia/ or hyponatremia/
17. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16
18. cohort.mp.
19. cohort studies/ or follow-up studies/ or prospective studies/
20. Myocardial Infarction.mp.
21. myocardial infarction/ or anterior wall myocardial infarction/ or inferior wall myocardial infarction/
22. stroke.mp.
23. Brain ischemia/ or hypoxia-ischemia, brain/ or "intracranial embolism and thrombosis"/ or intracranial embolism/ or intracranial thrombosis/ or intracranial hemorrhages/ or cerebral haemorrhage/ or intracranial haemorrhage, hypertensive/ or stroke/
24. Transient ischemic attack.mp.

25. brain ischemia/ or ischemic attack, transient/
26. 20 or 21 or 22 or 23 or 24 or 25
27. 18 or 19
28. 17 and 26 and 27

Appendix III: cohort data extraction form and validity tool

Cohort Data Extraction Form

Nutrition markers and Stroke, MI, and TIA outcomes

Extractor initials:

Date of extraction:

Author	
Journal	
Year	
Study title	

Study Characteristics:

Country of Origin?		
Language?		
Dates for Cohort Enrolment	From:	To:
Duration of study follow up		
Drop out		
Reason for Dropouts		

Subject Characteristics:

Total population selected	
Total population included in the actual study	
Mean Age	
Females/Males	
Age range	
Inclusion Criteria	

Exposure: Which of the following Exposures assessed in the study?

a. Myocardial infarction b. Transient ischemic attack c. Stroke

Define Malnutrition: How is malnutrition defined in this study? (If applicable)

Nutrition Markers: Which Nutrition markers/assessment tools were measured/evaluated in this study? Tick the space near

Indicator	**Tick below if used**	**Cut off values defined as malnutrition**	**Number below off values**	**Number malnourished**
Body Mass Index				
Weight				
MNA				
Mid Arm Circumference				
Triceps Skinfold				
Other hydration (minerals)				
Serum Albumin				
Serum Creatinine				

Outcome Assessment

Number/percentage in study below cut off values defined as malnutrition	**Outcome measured***	**Outcomes number/percentage** **Malnourished vs. non Malnourished**	**Confidence Intervals, Odds Ratio, Relative risk, p-values, etc….**

***Morbidity, Mortality, Poor outcomes (define below), disability (indicate how it was measured and define), average length of hospital stay, discharge destination.**

Add any definitions, comment in the space below:

Appendix IVa: anthropometric studies included systematic review and meta-analysis description

Study	Median Follow up period	CVD	Age years (mean or range)	Marker	Females/ Males	Outcome assessed	Others variable the Model Adjusted for
Batty 2006	35 years	Coronary Heart Disease	Normal weight: 51.4 Overweight :53.8 Obese: 52.1	BMI	18403 men only 0.9%	Mortality	Age Employment grade Physical activity Smoking habit Marital status, weight loss in the previous year, height adjusted FEV, Blood pressure Diabetes status Cholesterol
Buettner 2007	17 months	Stroke	Under weight: 66.1 Normal weight 65.9 Overweight 64.7 Obese 62.7	BMI	480/1196	Mortality	Age, segment elevation depression, previous MI, elevated cardiac troponin T, elevated white blood cell count, platelet count, kidney function, angiography extent of coronary artery disease, CRP, obesity.
Dagenaise 2005	4.5 years	Coronary heart disease, peripheral artery disease, stroke	66 years	BMI	2182/6620		sex, age, tobacco smoking, previous MI, previous stroke, presence of peripheral artery disease, known micro albuminuria, uses of antiplatelet agents, Diuretics, lipid-lowering agents, h-blockers and calcium-channel blockers, and ACE inhibitors.

							history of hypertension, diabetes, total cholesterol N5.2 mmol/L, HDL b0.9 mmol/L
Domanski 2006	4.8 years	Coronary Heart Disease	Men < 30 years: 64.1 Men > 30 years: 61.8 Women<30 years: 66.4 Women>30 years: 64.1	BMI	1171/5693	Major adverse coronary events including CVD death, non-fatal myocardial infarction, coronary revascularization, and stroke)	Age, history of myocardial infarction, history of angina, history of stroke, current smoking, history of smoking, systolic BP, diastolic BP, total cholesterol, LVEF percentage, Ca channel blockers, lipid lowering drugs, aspirin, beta blockers, history of revascularization, history of hypertension, and for women hormone replacement therapy
Kragelund 2005	8-10 years	Myocardial Infarction	Under weight: 74 Normal weight: 68 Over weight: 66 Obese 63	BMI	2172/4502		Age, smoking, wall motion index, history of diabetes, history of hypertension, cancer, heart failure, previous MI, thrombolysis, in hospital atrial or ventricular fibrillation, previous stroke, WHR
Lopez-Jimenez 2008	186 days	Myocardial Infarction	Underweight :67.7 Normal Weight 63.4 Overweight 31.9 Obese 57.8	BMI	1022/684	Mortality, recurrent myocardial infarction	age, gender, creatinine (≥1.3 vs. <1.3), systolic and diastolic blood pressures, previous MI, CABG, congestive heart failure, peripheral vascular disease, stroke, renal insufficiency, pulmonary diseases, diabetes, BDI scores, CABG treatment after, the index MI, and baseline use of

							vasodilators
Mehta 2007	12 months	Coronary artery disease	<=70	BMI	606/1719	Mortality	Normal BMI, age>70, female gender, diabetes, hypertension, hyperlipidemia, past peripheral disease, family history of coronary artery disease, current tobacco use, family history of coronary artery disease, killip class> I, ejection fraction, baseline heart rate>100,, b-blocker use, systolic BP
Nigam 2006	One year	Myocardial Infarction	51-75	BMI	278/616	Mortality, recurrent Myocardial infarction	Age, gender, diabetes, blood pressure, smoking, family history of CAD, lipid lowering use, beta blocker, aspirin, ACE inhibitor use at discharge
Nikolsky 2006	One year	Myocardial Infarction	49-73	BMI	542/1493	Mortality	Age, sex, diabetes, hypertension, hypercholesterolemia, current smoking, history of prior MI, bypass graft surgery, killip class 2 or 3, creatinine clearance
Rana 2004	3.8 years	Myocardial Infarction	Normal weight 65.3 Overweight 60.6 Obese I 58	BMI	1317/581	Mortality	Age, sex, race, current smoking, former smoking, thrombolytic therapy, tea and alcohol consumption serving/week, education, income, excluding patients with non cardiac morbidity
Rea 2001	3 years	Moyocradial Infarction	61.4	BMI	968/1349	Recurrent Coronary events	age, sex, tobacco use, physical activity, congestive heart activity, and aspirin use

Sierra Johnson 2008	6.4 years	Myocardial Infarction	62	Weight loss	79/311	Mortality	Age, sex, smoking, dyslipidaemia, diabetes, hypertension, myocardial infarction and obesity
Towfighi 2009	14 years	Stroke	> 25	BMI	275/369	Mortality	Hypertension antihypertensive medications, hypercholesterolemia, diabetes, Hyperhomocysteinmia, time from stroke occurence
Wu 2010	16 months median (30 months maximum)	Myocardial Infarction	Group I: 64 Group 2: 62	BMI	1885/4675	Mortality	Age, gender, hypertension, diabetes`
Zeller 2008	One year	Myocardial Infarction	Men by tertile: T1, 67; T2 67; T3 61. Women by tertile: T1, 77; T2, 76; T3 72.	BMI	593/1636	Mortality	Acute therapy, Killip, prior MI, Hypertension, Diabetes, hyperlipidaemia, smoking, CRP, STEMI, LVEF

Appendix IVb: other nutrition markers studies included systematic review and meta-analysis description

Study	Median Follow up period	CVD	Age (mean or range)	Marker	Females/ Males		Model Adjusted
Bhalla 2000	3 moths	Stroke	73.2	Measures of hydration: Osmolality	87/80	Mortality	Age, gender, and stroke severity, stroke subtype, and premorbid Barthel Index
Carter 2007	7.4 years	Stroke	76	Albumin	271/274	30 day mortality post hospital discharge	Age, smoking, stroke subtype, previous stroke/TIA, AF IHD, PVD, and aspirin use
Davalos 1996	3 month	Stroke	66	Variable: MUAC, TSF, and Albumin	37/67	Poor outcomes (Barthel Index<50) or death	Age, Sex, protein energy malnutrition, mean daily value of urinary cortisol, CSS score
Davis 2004	30 days	stroke	<75 or >=75	SGA	87/98	Poor outcomes (Modified Rankin score 2-6)	Mortality Model: NIHSS only. Poor outcome model: NIHSS, age, premorbid MRS variable
Food Trial Collaboration	6 months	Stroke	73.3	Variable	1492/1520	Mortality	Age, gender, prestrike function, living

							condition, and stroke severity
Gariballa 1998	3 months	Stroke	77.9	Albumin	180/81	Mortality and functional status (barthel index scores)	Age, urine incontinence, MRs, gender, previous illness and intake of drugs
Gariballa 1998 (AJCN)	3 months	Stroke	77.6	Albumin	129/96	Mortality and discharge destination	Age, gender, MRs, drug intake previous illness, and smoking status
Hirakawa 2006	During hospital stay	Myocardial Infarction	75.62 (0.3) years for under nutrition 73.44 (0.22) years for normal	Albumin	521/1070	Death during hospitalization.	age, activity of daily living, systolic blood pressure, body mass index, renal failure, bleeding, shock, Killip class, Pulmonary edema, location of myocardial infarction, ejection fraction, angiographic data, vasopressor, intra-aortic balloon pump, mechanical ventilation,

							percutaneous coronary intervention.
Kelly 2004	21 days	Stroke	70.1	Serum osmolality	47/55	thromboembolism	age, Barthel index, leg paresis, incontinence and atrial fibrillation
Sung H Yoo 2008	One week complications and 3 months poor outcomes (	stroke	64.8 (10.3)	variable	47/84	Clinical Complications at one week and poor outcomes (Modified Rankin score 2-6)	vascular risk factors, co morbid diseases, stroke severity, stroke subtypes, and diet methods and amount

Appendix V: PRISMA checklist 2009

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	17
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	18
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	34
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	34
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	35
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	35
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	35
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	326
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review,	35

		and, if applicable, included in the meta-analysis).	
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	36
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	36
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	37-40
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	36
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	40-42

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	37-40
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or sub group analyses, meta-regression), if done, indicating which were pre-specified.	41
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	43
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	43
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	44

Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	53-58
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	62, 63, 66, 68, 72, 75, 76
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	51, 52
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or sub group analyses, meta-regression [see Item 16]).	49, 62
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	78
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	83
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	84
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	NA

From: Moher D, Liberati A, Tetzlaff J, Altman DG, and the PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Appendix VI: Longitudinal Study Protocol

Fat Free Mass and Body Composition Changes after Stroke in Assessing and Monitoring of Nutritional Status, Nutritional Support Adequacy, and Relationships with Long Term Outcomes: an Observational Cohort Study

Table of contents

Abstract

Study Objectives: 1) to describe body composition changes in the acute stroke phase; 2) to examine the effect of different methods of nutritional support on body composition changes after stroke; and 3) to examine the relationships between body composition changes after stroke and long term outcomes.

Background: Stroke complications such as dysphagia may make the maintenance of adequate dietary intake difficult after stroke. As a result, malnutrition after stroke is common. Malnutrition can lead to tissue catabolism and body composition changes. Body composition is readily measurable. The components which can be estimated consist of fat mass, fat free mass, total body water, and mineral contents. In the catabolic state fat mass and fat free mass is the primary energy source for the body. This catabolic state is associated with total body and intracellular water loss and can result in dehydration. Body composition monitoring in the acute stroke phase may help to evaluate the degree of tissue loss mainly through fat free mass to understand energy balance and nutritional status of patients as malnutrition is associated with poor outcomes including death in stroke patients. Other good indicators of energy balance and nutritional status include fat mass, total body water (TBW), and mineral content. These body composition variables can be measured using Bioelectrical Impedance Analysis (MF-MF-BIA). MF-MF-BIA measurement is simple, non-invasive, and can be performed in clinical settings while the patient is lying down.

Methodology: A cohort of stroke patients admitted to Gunthorpe Acute Stroke Unit at the Norfolk and Norwich University Hospital will be prospectively recruited upon consent to the study over 9 months. Body composition variables will be evaluated on admission, a week after enrolment to the study and at discharge using MF-MF-BIA (BioScan 920-2, Maltron International Ltd, and Essex, United Kingdom). Routine haematological biochemical measures including albumin levels will be recorded. Age, gender, stroke type, co-morbidity, pre-morbid status and any episodes of clinician-diagnosed dehydration will also be recorded. Selected patients with feeding regimen change during their acute hospital stay will have their body composition evaluated within 24-48 hours post feeding regimen change for every feeding regimen change. Follow up will be carried out at nine months for each surviving participant using Patient Administrative System (PAS), medical records review, and postal questionnaires. At follow-up the following outcome data will be collected- mortality, hospital admissions, functional status (measured using Barthel and Functional Independence Measure, FIM), health related quality of life (SF-36v2), patient reported outcome (PROM) using Stroke Impact Scale (SIS), discharge destination, acute hospital length of stay and initiation of nutritional support and complication arising from PEG nutritional support.

Outcomes: the primary outcome will be change in average fat free mass stratified by stroke type, severity and predominant feeding regimen. Secondary outcomes include average change in body composition including fat mass, TBW and mineral content. The relationships between these changes and above outcomes at nine months will also be examined.

Background:

Stroke is a chronic condition that can have various effects on the body including dietary intake. Dietary intake in acute stroke is often inadequate, which is usually attributed to high incidence of dysphagia after stroke, and a range of other secondary complications such as cognitive problems affecting eating behaviours, reduced ability to feed oneself independently, disorientation, paralysis, depression, and other sensory related factors (1, 2). Altered dietary intake can lead to weight loss, dehydration and malnutrition in stroke survivors. Weight loss after stroke has been well documented (3, 4). In addition, malnutrition in stroke patients is common.

Malnutrition is commonly defined using Body Mass Index (BMI) cut off points; a BMI of < 18.5 Kg/m^2 in populations aged < 65 years and a BMI of < 22 Kg/m^2 in older groups is considered to be malnourished (5, 6). Deterioration of nutritional status in stroke patients during hospital care is well recognized (7, 8). Malnutrition is thought to be partly contributed by the nature of the stress response instigated in stroke. Stroke patients have been shown to have a great stress response; they have high cortisol levels, resulting in the deterioration of their nutritional status (9).

Body composition is affected in acute medical illnesses including stroke. Furthermore, stroke complications which are associated with altered dietary intake can lead to a negative energy balance. In such circumstances when energy needs were not met, the body will elect to use its own energy reserves resulting in tissue loss leading to subsequent body composition changes. Body composition that can be measured easily consists of fat free mass, fat mass, total body water, and body mineral content. Acute/chronic inflammation instigated during illness leads to catabolism of body tissue with resultant fat free mass loss (6). Fat free mass loss leads to loss of cellular fluids as tissue catabolism results in intracellular fluid loss and expansion of extracellular fluid; cellular dehydration (10). These changes are not uncommon after stroke. Prevalence studies of malnutrition in stroke showed a proportion of stroke patients at the time of the event were already malnourished (10, 11, 12, 14, and 15).

The nutritional status of stroke patients is compounded further by the fact that the physiological changes seen in malnutrition are already happening in a proportion of the elderly population, and stroke accelerates the process. One of the most prominent physiological changes in older people is sarcopenia or fat free mass loss. Sarcopenia is defined as muscle loss that occurs with the aging process leading to general weakness (15, 16). In sarcopenia, fat free mass is replaced by fat mass. The inverse correlation of fat mass with functional status has been well documented; an increase in fat mass was associated with functional limitations in the older people (16, 17).

Dysphagia is one of the commonest complications after stroke. In a recent review, Martino and colleagues (18), reported the incidence of dysphagia as varying from 37% to 78%; using different dysphagia diagnostic criteria including cursory (water swallowing test), clinical (clinical scores), and instrumental (video fluoroscopy) methods. The authors concluded that dysphagia after stroke is common regardless of diagnostic method used. Dysphagia is considered the primary cause of reduced dietary and fluid intake in stroke patients (1, 2). There is also a direct association between dysphagia and malnutrition in stroke patients. The proportion of dysphagic patients suffering from malnutrition, assessed using the Subjective (patient generated) Global Assessment (SGA) tool, was (10/14; 71%) compared to non-dysphagic patients (19/59; 32%) in acute stroke, p=0.007 (11). One week after admission to an acute stroke unit, dysphagic patients were more likely to be malnourished (16/24) 67% compared to non-dysphagic patients (15/67) 24%; p<0.001 (9). The association between dysphagia and malnutrition is prevalent not only in acute settings, but also in care home settings. A study carried out in a Hong Kong care home for stroke patients reported a significantly higher prevalence of malnutrition in dysphagic patients (4/20; 20%) compared to non-dysphagic patients (4/40; 10%); p=0.044 (19).

The prevalence of malnutrition was also higher in dysphagic compared to non-dysphagic patients (62.5% vs. 32.0% respectively) on admission to a rehabilitation unit; p<0.032) (20). There are other reasons why stroke patients may have an altered dietary intake in longer term, the physical and mental impairment and associated disabilities in stroke patients can alter dietary intake; making the eating process physically, socially, and mentally difficult. Hoarding and leakage of food from the mouth, and chewing problems contributed to eating difficulties after stroke in 44% of patients with eating problems (4). Other problems contributing to eating difficulty include food spills, difficulty to sit appropriately for eating, inability to concentrate, prolonged eating time, and inability to control foods in the plate (21).

The eating difficulties that stroke patients experience could make the whole process an unpleasant experience for them (4). There is some evidence to suggest that their new disability and limitations may put stroke patients into a state of depression. In an observational study by Axelssen et al. (4) the authors reported that 65% of the patients in their study entered into a denial phase not accepting their new condition i.e. inability to eat as before. The authors argued that the denial phase caused patients to enter into depression and increased the risk of anorexia (up to 50% in their series) (4). A mean weight loss was reported as 2.6 kg in the 78% of patients with eating difficulties in their study (4). Gariballa et al (22) also reported a decline in average weight in stroke patients at 2 and 4 weeks post admission to acute stroke unit in 48% (96/201) and 25%(51/201); p=0.002. Weight loss may still occur long term after stroke. A more recent

population based study documented weight loss of > 3 kg in 24% and 26% of stroke patients four months and one year post-stroke respectively (3). If weight loss persists for a long duration it can contribute to severe BMI changes that can be classified as malnutrition; BMI < 18.5 Kg/m^2 in < 65 years old population and a BMI < 22 Kg/m^2 in ≥ 65 years old population (5, 6).

Malnutrition is prevalent among stroke patients on admission to a stroke unit. However, malnutrition rates vary between different studies that used different methods to assess malnutrition. Unosson and colleagues reported that 8% of their study subjects (≥70 years old) were protein malnourished on admission; based on serum protein concentrations (7). However, they did not use a validated malnutrition assessment tool such as the Subjective Global Assessment (SGA) or the Mini Nutritional Assessment (MNA) used in other studies (11, 12, 13, and 14). These studies also reported variable malnutrition prevalence rates on admission to an acute stroke unit. The prevalence of malnutrition using SGA was reported to be 19% in one study (11) and 32.1% in another study (12). The two studies that used both SGA and MNA tool reported malnutrition to be at 16% (13) and 26.3% (14) at the time of admission to stroke unit. A consistent finding, however, is that malnutrition seems to be prevalent among stroke patients on admission thereby increasing the risk of further deterioration of nutritional status during hospital stay.

The proportion of stroke patients with malnutrition increases during acute hospital care (8, 9). One study reported a 6% increase in the prevalence of malnutrition from 16% at the time of hospital admission to 22% at the time of discharge measured anthropometrically using Triceps Skin fold thickness (TSF), Mid Arm Circumference (MAC), weight and biochemical parameters including albumin (8). Another study reported that malnutrition prevalence changed from 16.4% at admission to 26.4% and 35% at one and two weeks post admission respectively using MAC, TSF, and serum albumin measurements (9). Another study showed consistent findings reporting a constant decline in BMI ($p=0.006$), Triceps and Biceps skin fold thickness ($p<0.0001$), MAC ($p=0.001$), albumin ($p<0.0001$), and transferrin ($p=0.02$) between week 2 and week 4 post admission in stroke (22).

In a more recent prospective observational study that included 131 patients, malnutrition 24 hours post-admission was diagnosed in 12.2% of patients compared to 19.8% of patients at one week post admission; $p=0.03$ (23). The study used five criteria including a 10% weight loss in the past 3 months and or 6% weight loss one week post admission, weight index (actual weight compared to reference weight) less than 80%, serum albumin <3.0g/dL, prealbumin <10.0 mg/dL, or transferrin < 150mg/dL (23). Malnutrition in the acute phase also increased the risk of malnutrition subsequently for example on discharge to rehabilitation services. The proportion of patients diagnosed with malnutrition on admission to stroke rehabilitation services ranged from 35%

to 67% (5, 20, and 24). The malnutrition diagnosis criteria in previous studies depended mainly on anthropometric measurements such as TSF, weight loss, BMI, and serum albumin.

The positive relationship between malnutrition and poor outcomes in stroke has been well documented. Hospital length of stay was significantly longer in malnourished compared to well nourished stroke patients, nutritional status was evaluated using the SGA tool, by an average difference of 5 days; $p<0.001$ (11). Another study, which evaluated nutritional status using triceps skinfold, madam muscle circumference, serum albumin, and calorimetry, reported a longer duration of hospitalization for malnourished (mean = 28 days) compared to well nourished (mean=17 days) stroke patients; $p=0.001$ (9). The rate of complications were also higher in malnourished stroke patients; 50% in the malnourished group compared to 14% in the well nourished group ($p<0.0001$) (11). Poor outcome, defined as a Modified Rankin Scale ≥ 3 measured 30 days after stroke, was reported in 80% of patients suffering from malnutrition compared to 54% in those with good nutritional status; $p=0.01$ (13). Malnutrition in stroke patients was associated with higher incidence of death compared to non-malnourished stroke patients. Stroke patients with malnutrition had a higher mortality rate (30%) compared to well nourished stroke patients (12%); $p=0.02$ (13). The authors assessed malnutrition using the validated SGA tool (13). A recent study by Yoo et al confirmed these findings; baseline malnutrition at the time of admission was significantly associated with frequent post-stroke complications ($p<0.001$) (23).

The effect of malnutrition on outcome was also reported in stroke rehabilitation services. Length of stay in rehabilitation services was higher for malnourished compared to well nourished patients at admission; $t = -1.88$, df-47, $p=0.033$ (25). Malnutrition in the study was diagnosed by a weight $\leq 90^{th}$ percentile of reference weight or 95% of usual weight or BMI <20 kg/m^2, MAC < 5^{th} percentile, an average of five skinfold measurements < 5^{th} percentile, low circulating lymphocytes, transferrin (calculated from total iron binding capacity, and serum albumin (bromcesrol binding method). These measures of malnutrition were significantly correlated with lower modified Barthel Index (BI). The BI scores for malnourished stroke patients compared to well nourished patients were significantly lower at one month rehabilitation; $p=0.032$ (26).

To date, studies assessing the effects of enhanced nutritional interventions in people who have had an acute stroke have provided variable outcomes. Bath and colleagues carried out a review (26) of the available studies to understand the effect of different enteral feeding methods on stroke outcomes and concluded at the time of the review that further studies were required for a solid conclusion. The authors reported that one study was not completed due to a 58% case fatality (27) and another study (28), only published data n=30, reported a significant improvement in nutritional status extrapolated from albumin levels in those having Percutaneous Endoscopic

Gastrostomy (PEG) compared to Nasogastric (NG) tube feeding at six weeks post feeding administration. Albumin levels improved from 27.1g/l to 30/1 g/l in the PEG group compared to reduction from 31.4 g/l to 22.4 g/l in the NG group; $p<0.003$. The randomised controlled trial reported lower treatment failure in the PEG group (0/16) compared to the NG group (3/14, 21.4%) and reported that six PEG patients were discharged by six weeks after PEG insertion compared to none in the NG group; $p<0.05$. Six week case fatality in the PEG group was 12% compared to 57% in the NG group; $p<0.05$ (28). Despite these reported outcomes it would be difficult to draw any conclusion for several reasons. The sample size is small to make it generalizable and the authors indicate that all patients were in stable condition without indicating the extent before randomizing their patients making it difficult to know if more stable patients were randomized to PEG feeding.

A recent randomized controlled trial by Hamidon et al (29) compared the effects of PEG and NG feeding on patient's nutritional status up to 4 weeks post intervention. In PEG fed patients (n=10) albumin levels were significantly higher than NG tube fed patients (n=12); $p=0.045$. PEG fed patients' albumin levels rose more than those of ($p=0.025$) NG fed patients ($p=0.047$) 4 weeks post intervention indicating better improvement in nutritional status in PEG compared to NG patients (29). However, no statistically significant differences were observed in anthropometric measurements between the two groups (29). Better treatment outcomes were reported in the PEG compared to the NG group: the treatment failure frequency was reported to be 50% in the NG group compared to no failure in the PEG group; $p<0.036$ (29). The authors conclude that PEG feeding improves nutritional status more than NG feeding. This is a small study and such generalizability cannot be made, PEG feeding could have been contraindicated to patient with GI infection which can contribute to lower Albumin count, and PEG fed patients could be in a prefeeding nutritional state than NG fed patients allowing better and more swift nutrition improvement in PEF fed patients as reflected by albumin.

While smaller studies, suggest that PEG feeding provides better outcomes compared to NG feeding in stroke management although smaller studies can generate more MF-MF-BIAs. The FOOD Trial, the largest nutritional intervention trial in stroke patients to date, reported a different outcome. The FOOD trial studied the effect of early vs. none and type (PEG vs. NG feeding) of nutritional support on long term stroke outcomes; up to 6 months post discharge (30). Patients were randomised to either no enteral tube feeding or enteral tube feeding 7 days post-admission to stroke unit, or randomised to PEG vs. NG tube feeding 7 days post admission. Poor outcome (defined as a Modified Ranking Scale (MRS) score of 4-5) and death were evaluated 6 months post discharge. There was no difference in effect between early or no tube feeding on the risk of death (42% mortality for early tube feeding vs. 48% mortality rate for no tube feeding; n=429, OR=0.79, CI 95%

0.60-1.03) or combined death or poor outcome (79% and 80%, respectively; n=429, OR=0.93, 95% CI 0.67-1.30) (30). Similarly, no differences in the effects of the two nutritional support regimens on death and poor outcome were observed. Six months after admission 89% of patients who had been randomised to PEG (n=162) compared to 81% of those given NG feeding (n=159) experienced death or poor outcome (OR=1.86, 95% CI 0.99-3.50) (30). The effect on mortality of the different nutritional regimens was not significant either (49% and 48% for the PEG and NG feeding; OR= 1.04, 95% CI 0.67-1.61) (30).

The effect of early nutritional supplementation on death or poor outcome (Modified Rankin Scale score of 3-5) at 6 months post discharge were also examined in the FOOD Trial (31). Patients were randomly allocated to normal hospital diet or normal diet with additional oral nutritional supplementation (360 ml oral protein supplement of 6.27 kJ/ml and 62.5 g/L in protein daily) during hospital stay until discharge. There was no effect of supplementation on mortality outcome. Death was reported at 13% and 12% for the non-supplemented (n= 2012) and supplemented (n=2000) groups respectively; OR=0.94, 95% CI 0.78-1.13. As for death or poor outcome it was reported at 58% and 59% for the non-supplemented (n=1995) and supplemented (n=2009) groups respectively indicating no effect of supplementation; OR= 1.03, 95% CI 0.91-1.17 (31). Nutrition interventions as reported by the FOOD Trials did not have any important or significant impact on stroke outcomes up to 6 months post stroke.

The FOOD trial adjusted for several prognostic variables including age, gender, premorbid status before stroke (living alone and independence), condition after stroke (ability to talk, lift arms, and walk), and ability to swallow (32). The FOOD Trial while being a multicentre study has its strengths and weaknesses. The strengths as reported by the authors include its large sample size, 10 times larger than any previous trial, and the recruitment of patients from various centres; and thus increased generalizability. There are several weaknesses as suggested by the authors. Weaknesses include informal methods in assessing nutritional status, failing to record the total number of eligible subjects in each centre, and inability to have an onsite source to report change in nutritional status and patient nutrient intake. These could have contributed to not having a universal method in classifying malnourished patients contributing to MF-MF-BIAs in categorizing malnourished patients, inability to report nutritional status improvement in malnourished patients assigned to tube feeding (30) or nutritional supplements (31) initially, and inability to record systematically patients nutrient intake that could be mostly met through oral hospital diet masking the benefits of tube feeding (30) or nutritional supplements (31) initially.

Given the several limitations of the FOOD TRIAL, it remains unclear which is the preferred type of nutritional intervention. These limitations may have influenced outcomes. The

FOOD TRIAL despite being a large multicentre study cannot help in providing raw evidence to help clinicians in decision making considering the inability to record and follow confounding factors that could have contributed for the reported outcomes.

Traditionally weight was used to assess the risk of malnutrition with unintentional loss of 10-15% of body weight as a predictor of malnutrition in disease states, and rapid loss of weight indicating dehydration. Malnutrition can also be evaluated through body mass index (BMI) calculations, with a BMI< 18.5 kg/m^2 and a BMI<22 Kg/m^2 classified as malnutrition for the general and older population respectively (6). However, BMI values cannot predict fat free mass and fat mass values in disease states and even if an increase in BMI occurs it could be attributed to increased fat mass and extracellular water content due to cellular dehydration as indicated earlier (10). Anthropometric measurements such as Mid Arm Circumference (MAC) and Triceps Skin Fold (TSF) have been also used in predicting fat free mass and fat mass respectively. However the disadvantage of TSF and MAC is the requirement for a skilled health professional to carry out these measurements because they require good precision and careful assessment of reproducibility, increasing room for errors and inaccuracy. Biochemical tests can also be used to assess malnutrition and dehydration, including sodium, potassium, phosphorus, urea, serum albumin, and glucose. However, biochemical tests cannot be used to predict fat mass or fat free mass content (6). Body composition measurement using bioelectrical impedance (MF-MF-BIA) analysis is one method that can predict fat mass and fat free mass values.

Total body water is another component that can be assessed by bioelectrical impedance analysis. Total body water can provide information on the degree of dehydration. Physiological changes occurring in the aging process increases the risk of dehydration. These physiological changes are related to reduced capacity in retaining water; such changes include but are not limited to reduced renal filtration rate, increased proximal tubular filtration absorption, and decreased free water clearance (33). Total body water consists of intracellular and extracellular water. Loss of intracellular water is usually defined as dehydration (34 and 35). The diagnosis of dehydration through clinical symptoms and signs can be inaccurate and can lack sensitivity and specificity (36). Physicians misdiagnosed dehydration in third of patients admitted to a hospital (37) despite the dehydration council creating the DEHYDRATION mnemonic listing 12 indicators to be used in dehydration screening (33).

Assessing dehydration using MF-MF-BIA can predict not only total body water, but also specific intracellular and extracellular components. Evaluating intracellular and extracellular water can provide information on the extent of tissue catabolism. As indicated earlier acute/chronic inflammation instigated during illness leads to catabolism of lean body mass loss; fat free mass loss

(6). Fat free mass loss leads to loss of cellular fluids as tissue catabolism results in intracellular fluid loss and expansion of extracellular fluid; cellular dehydration (10). Based on intracellular and extracellular water changes related to lean tissue catabolism caloric and nutritional needs can be modified to allow tissue anabolism and avoid further catabolism. Assessing dehydration through measuring body composition values may provide information on the nutrition and management needs of patients.

There are several methods to assess body composition. Two commonly used methods are dual X-ray absorption (DEXA) and bioelectrical impedance analysis (MF-MF-BIA). DEXA is a reliable method and is used in validating other body composition assessment methods, mainly MF-MF-BIA (38, 39, and 40). DEXA uses x-ray energy to evaluate fat mass, fat free mass, and bone density (6). However, DEXA is expensive, not readily available, and time consuming for patients in clinical settings. MF-MF-BIA on the other hand is convenient. It is simple to perform, non-invasive (41), and quick in providing reproducible results with <1% error (42). Its simplicity lies in the fact that no more than proper operating of the equipment is required by the operator and can be performed at bed-side. It produces results instantly and time efficient.

Bioelectrical Impedance Analysis (MF-MF-BIA)

MF-MF-BIA analysis is based on the resistance imposed by certain components of the human body; body impedance. Body fat is non-conductive to electrical current while lean body mass, consisting of electrolytes and water, is conductive. When a current passes through the human body it faces resistance from the adipose tissue, impedance, while passing through the non-adipose tissue component to complete its circuit. The difference in conductivity, current input and output, is used to calculate fat mass and fat free mass using a validated formula already programmed in the MF-MF-BIA analysis equipment (43). MF-MF-BIA can measure body composition using a single frequency current (SF-MF-MF-BIA) or a multi-frequency current (MF-BIA). In SF-MF-MF-BIA a single current of a known quantity, usually 50 kHz, passes through the body tissue and the difference in current input and output is used to calculate fat free mass and total body water (44). As for the multi frequency MF-MF-BIA, to be used in this study, currents of several frequencies (0, 1, 5, 50, 100, and 200, up to 500 kHz) are passed through the body tissue separately and impedance is generated, currents input and output difference is measured and used in different validated equations already integrated in the equipment to extrapolate body composition variables. MF-BIA gives measurement of fat free mass, total body water, and extracellular and intracellular water (44). Both SF-MF-MF-BIA and MF-BIA use empirical linear regression equations to generate results by the equipment instantly (44). MF-BIA has been used in clinical settings in conditions that includes

but are not limited to older patients (45), patients after coronary artery bypass graft (CABG) (46), patients with HIV (47), and those on dialysis (48).

MF-MF-BIA validation studies to date, mainly conducted in comparison to the gold standard method by DEXA, have produced favourable outcomes. A study to evaluate body composition changes in overweight women on a weight loss program, documented an agreement in the measurements between MF-BIA and DEXA (39). There was no significance difference between DEXA and MF-BIA in measuring fat free mass (r^2=0.87, p<0.001), fat mass (r^2=0.93, p<0.001), and body fat % (r^2= 0.20, p=0.03); MF-MF-BIA did not differ significantly compared to DEXA. The MF-BIA and DEXA showed an agreement in their measurement, trend of body composition changes, although MF-BIA did not give the same exact measurements as DEXA. The study also documented that MF-BIA slightly underestimated fat mass and overestimated fat free mass in lean individuals and overestimated fat mass and underestimated fat free mass in obese individuals compared to DEXA (FM; r2= 0.17, p=0.05 and FFM; r2= 0.16, p=0.05) (39).

A recent study by Schafer et al (49) evaluated the validity of MF-BIA across a range of BMI in healthy subjects compared to DEXA. MF-BIA overestimated fat mass in obese subjects compared to DEXA (p<0.0001); difference 4.11 ± 0.34, and overweight BMI (p≤0.006); difference of 0.95 ± 0.33. Despite MF-BIA overestimation of fat mass the author highlighted that MF-BIA measurements did show body fat percentage agreement with DEXA in the normal and overweight BMI category with a mean difference of -1.56% (limits of agreement -6.7% to 3.6%) and 0.58% (limits of agreement -3.8% to 5.0%) respectively. The agreement is weaker with DEXA with higher BMI values in obese range (i.e. BMI >30); mean difference was 3.50% (-2.2 to 8.8%) (44). MF-BIA overestimated fat free mass in normal and overweight BMI compared to DEXA with a difference of 2.08 ± 0.32 (p<0.0001) and 0.71 ± 0.33 (p≤0.04) respectively. Overall conclusion was that MF-BIA is in agreement with DEXA when measuring normal and overweight subjects although overestimation occurs, and therefore caution should be taken in interpreting MF-MF-BIA results in obese subjects (49).

There is a lack of data on the use of MF-MF-BIA method in evaluating body composition after stroke. One small study compared body composition changes after stroke between Paretic leg and the non-affected leg of patients (n = 35) (50). It used the DEXA method in evaluating body composition, indicating that significant losses in lean body mass and bone density loss occurred in the paretic leg compared to the non-affected leg after stroke; p<0.05(50). The study did not compare body composition changes after stroke at baseline and after the initiation of nutritional support. While DEXA method is considered to be gold standard measurement of body composition,

it is expensive, time consuming to perform, inconvenient for patients and not pragmatic to be used routinely in clinical practice.

In summary, stroke symptoms and complications such as dysphagia, paralysis, and depression can reduce dietary intake, leading the body to compensate for such negative energy balance by utilizing its own energy reserves and increasing body tissue catabolism resulting in body composition changes (51). Body composition changes can have a great impact on treatment outcomes. There is a significant positive association between malnutrition and dehydration and reduced muscle strength, infection resistance, and wound healing in stroke patients (52, 53). Body composition measurement during acute stroke phase may serve to better understand the relationship between these changes and stroke outcome. This may help to gain deeper insight on how such changes can be avoided to improve outcome in stroke.

This study seeks to investigate and describe body composition changes after stroke and their effect on long term post stroke outcomes using Bioelectrical Impedance (MF-MF-BIA), which can be a useful tool in clinical settings when validated in with the standardized DEXA in this population. This study can add significant knowledge to the already existing literature in nutritional aspect of stroke management and improve the understanding of the role of nutrition in stroke recovery.

Objectives:

This PhD research project aims to add to knowledge in the area of nutritional science in stroke. The project will lead to further research to better understand the role of nutrition as a modifiable determinant of long term stroke outcomes. The project will describe body composition changes during acute phase of stroke and investigate the relationship between nutritional and hydration status and several stroke outcomes as outlined below.

Primary Objectives

1. to describe fat free Mass changes after acute stroke by stroke subtype and severity during the course of acute care
2. to examine the effect of different methods of nutritional support on body composition changes after stroke and
3. to examine relationships between body composition changes after stroke and long term outcomes at nine months post stroke

The design of the study will also allow us to examine the following secondary objectives

Secondary Objectives

1. to estimate body composition values that define malnutrition using MAC (for fat free mass), TSF (for fat mass) and BMI (for both) as standard measures

2. To assess the predictive value of individual components of body composition (fat mass, fat free mass, total body water and some minerals) at baseline and changes occurring and during acute care (between baseline and pre-discharge from acute hospital), stratified by predominant feeding regimen and stroke subtype and severity.

3. to assess the effect of hydration status (both at the baseline and change during admission) assessed using Intracellular fluid (ICF) measured by MF-MF-BIA in stroke outcome

4. To validate MF-MF-BIA against DEXA for fat mass, fat free mass, total body water and some minerals in stroke patients using purposeful sampling.

Research Questions:

1. What body composition changes occur after specific types of stroke?

2. What are the effects of different nutritional support regimens on body composition changes after stroke?

3. Do body composition at baseline and their changes occurring during acute stroke phase have an effect on long term outcomes?

Research Questions for Secondary Objectives:

1. What are the magnitudes of changes in body composition values using MF-MF-BIA which define malnutrition benchmarked by standardized MAC, TSF, and BMI values?

2. What body composition changes or values have a significant effect on long term subjective and objective outcomes of stroke?

3. What extent of cellular dehydration occurs in stroke patients as measured using bioelectrical impedance, and what is the relationship between intracellular dehydration and stroke outcomes?

4. How well the body composition values measured using MF-MF-BIA correlate with body composition values measured using DEXA in stroke patients?

Hypotheses:

Hypothesis I: Body composition changes after stroke do occur and the magnitude and proportion of changes occurring in various components of the body (fat mass, fat free mass etc.) are different depending on stroke type and severity.

Rationale: Evidence indicates that a proportion of stroke patients are malnourished on acute admission and their nutritional status deteriorates during acute hospital stay. Malnutrition combined with the stress response in acute conditions results in body tissue catabolism. The human body tries to generate energy from the available energy reserves and this result in catabolic process that result in body composition changes.

Hypothesis II: Negative body composition changes (defined as reduced fat free mass, increased fat mass and decreased intracellular water) after stroke are associated with both objective and subjective poor outcomes. The body composition changes after stroke are influenced by the timing and methods of feeding independently of stroke severity

Rationale: Studies on the elderly populations, main stroke population, suggested that sarcopenia (loss of lean body mass), leads to loss of functional capacity. Nutritional status of stroke patients and stress response in acute stroke phase can result in major body composition changes (hypothesis I) with fat free mass being the most affected component as amino acids are being converted to pyruvate for energy generation.

Objective outcomes hypothesis: Body Composition changes in Fat Free Mass and body water correlate with increased risk of mortality, readmissions to secondary care settings, admission to care homes, and reduced functional capacity

Rationale: It would be reasonable to predict that changes in fat free mass and body water correlate with stroke outcome. Fat free mass or lean body mass loss, results in reduced strength which results in reduced mobility and overall functional capacity. Fat free mass loss, therefore, can result in disability. Fat free mass loss indicates the severity of the illness We hypothesise that fat free mass loss during acute stroke phase controlling for baseline fat free mass will have long term effect after stroke that can be measured by objective outcome measures of readmission to secondary care after hospital discharge location, mortality outcome and functional limitation measured by Barthel Index (BI) controlling for case mix and prognostic indicators..

Subjective outcomes hypothesis: Fat free mass loss is associated with reduced functional capacity and quality of life as indicated by the Stroke Impact Scale, SF-36, and self rated health (5 options) scores/responses.

Subjective outcome: Subjective outcomes to be evaluated in this study are related to the quality of life. Patients who suffer from loss of functional capacity or disability (associated with fat free mass loss as in hypothesis II) will have lower self rated health when evaluating their quality of life and health related QoL (SF-36).

Hypothesis: Cellular dehydration, loss of intracellular fluid volume (ICF)) after stroke as measured by MF-MF-BIA is associated with increased chances of hospital readmission, increased risk of mortality, disability, and reduced quality of life based on patients responses and Stroke Impact Scale and SF-36 scores.

Rationale: Dehydration occurs when intracellular fluid is depleted. Malnutrition is a result of inadequate caloric and nutrient intake leading to a negative energy balance. Both malnutrition and stress cause the body to utilize its own self to generate energy. The outcome is lean body mass loss. Lean body mass loss leads to the release of intracellular fluids into extracellular space causing cellular dehydration; cell mass is lost releasing cells contents (10). We hypothesise that ICF loss after stroke, adjusting for baseline ICF status and controlling for case mix and prognostic indicators, is related to above outcomes long term after stroke.

Null Hypothesis: There is no significant effect of different feeding regimen on long term outcomes after stroke.

The FOOD TRIAL did not show that one feeding method is better than other in terms of poor outcomes after stroke up to 6 months. Therefore, the decision of timing and method of feeding is purely clinical decision, albeit influenced by the FOOD trial results with less PEG insertions offered before 3-4 weeks post stroke. We hypothesise that there is no significant difference in long term outcome up to 9 months post stroke (null hypothesis) between different feeding regimens.

Study Design: Observational cohort study

Study Location: Norfolk and Norwich University Hospital

Inclusion Criteria:

- Age 17 years and over
- Any newly diagnosed stroke (first or recurrent)

- Admitted to the NNUH within 48 hours of stroke onset

Exclusion Criteria:

- Severe stroke NIHSS >30 whose likelihood of survival >7 days is <50% as judged by the stroke physician
- Severe stroke; for palliation only (expected survival of less than 48 hours)
- Very mild stroke or TIA patients who fully recovered within 24 hours of hospital admission
- Life expectancy is less than 3 months prior to the event
- Co-existing terminal illness e.g. advanced cancer, end stage chronic disease such as end stage renal failure and end stage COPD

Patient selection criteria for MF-MF-BIA validation against DEXA

- Eligible to be included in the study as per above inclusion criteria
- Provide consent to attend DEXA assessment after discharge
- Able to walk without aids and attend CTRU for DEXA assessment

Methodology:

Eligible patients will be recruited over a nine month period (June 2010-end February 2011). Follow up data will be collected at nine months (complete follow up in end of November 2011). Data collection will be carried out in four stages: on admission, post-admission for feeding regimen change, at discharge, at nine months follow up.

On **admission**: patients will be recruited within 48 hours of hospital admission. Informed consent will be obtained. Participants' demographic details (age, sex, etc.), weight within 3 days of admission, height mainly as demi span measurement for bed ridden patients, body mass index (BMI), triceps skinfold (TSF), madam circumference (MAC), hand grip strength (non-affected hand) using a dynamometer, presence or absence and degree of dysphagia (routinely assessed by speech and language therapists), type and consistency of allowed food and fluid (e.g. level A, thickened fluid), and body composition measurement (using Bioelectrical Impedance Analysis) will be measured upon consent to the study. Several measurements for triceps skinfold (five skin fold measurements (25) (using a skinfold calliper), midarm circumference (three times), and hand grip strength (using a dynamometer) (three times) will be carried out and mean value will be used for analysis.

From MF-MF-BIA analysis, we will collect data reflecting changes in physical and general health these will include fat free mass (Kg), fat free mass percentage, fat mass (Kg), fat mass percentage, total body water (L), total body water percentage, extra and intracellular water (L), extra to intracellular water ratio, body cell mass (Kg) and percept, extracellular mass (Kg) and percept, creatinine clearance rate (ml/min), glomerular filtration rate (ml/min), protein mass (Kg), mineral mass (Kg), mineral mass percept, total body calcium and potassium (g), muscles mass (Kg), glycogen mass (g), dry weight (Kg), extracellular fluid (L), plasma fluid-intravascular (L), interstitial fluid-extravascular, body volume (L), and body density (Kg/L). MF-MF-BIA data will be collected during hospital stay, post admission; changes in body composition will be captured using MF-MF-BIA upon initiation of feeding regimen; within two days of feeding initiation and on clinician diagnosis of dehydration. The measurements will be repeatedly carried out each time a feeding regimen change is instructed within two days of such change for selected participants. There is no published literature on when best to measure body composition changes after change in dietary pattern and the selection of this time frame is for pragmatic reason; this is based on the research team's clinical experience of required duration to allow the participant to adapt and reflect changes occurred in body composition due to new feeding regimen. At the time of acute hospital **discharges:** baseline measurements described above will be repeated. Progression of MF-MF-BIA changes will be described and differences between baseline and discharge values will be noted.

Selected patients meeting the DEXA-MF-MF-BIA validation study inclusion criteria will be recruited upon their consent to have their body composition measured using DEXA. DEXA measured body composition value will be compared with MF-MF-BIA body composition values including fat mass, fat free mass, total body water, and mineral content for validation purposes prior to discharge.

Other routinely performed test results will be collected at baseline (at the time of enrolment), day 7 (+/- 2 days) and at discharge. These will include FBC, WCC, Platelets, MCV, MCH, Biochemistry data Urea, Creatinine, albumin, total protein, alkaline phosphatase, ALT, GGT, CRP, ESR (if measured), total cholesterol, high density lipoprotein (HDL), triglycerides (TG), low density lipoprotein (LDL) cholesterols, glucose, HbA1C in those with diabetes, MUST and Barthel Index scores. Other prognostic indicators of stroke will also be recorded. These include, stroke type, severity assessed using BAMFORD classification, admission NIHSS, pre-morbid Rankin score, pre-morbid Barthel Index, significant co-morbid conditions. Routinely collected clinical data for stroke register will be collected which includes other prognostic indicators such as time of CT, duration of stay on stroke unit, physiotherapist and occupational therapist assessment, salt assessment, whether or not received thrombolysis, and participation in clinical trials.

Post-discharge follow up at 9 months post-discharge will evaluate long term stroke outcomes. Both objective and subjective outcomes will be measured. Objective outcomes include mortality, cardiovascular events (heart attack, another stroke/TIA), hospital readmission, discharge location, change in residence (residential or nursing home change), nutritional support initiation and change.

Data linkage and retrieval from the Patient Administrative System (PAS) and review of medical records will be carried out to collect these objective outcomes data. Data related to patient activity will also be retrieved from PAS. Medical records will be reviewed to confirm the evidence of initiation of nutritional support, readmission, and change in residence since discharge, other co-morbidity developing post discharge (e.g. attendance to neurology clinic for treatment of contractures). Those participants who are discharged with PEG or NG? Feeding will also be followed up using Dietetic Department's records for any complications arising as the result of PEG.

Subjective outcomes will include patients self-reported health related quality of life using version 2 of the Short Form-36 (SF-36v2), self rated health using a five option poor to excellent scale, Stroke Impact Scale (patient reported outcome measure, PROM) and Barthel Index Scores. The self reported patient outcomes will evaluate variables related to patient's quality of life and mainly disability, functional dependence and independence. Questionnaires will be sent to patients 9 months post enrolment.

Sample size calculation: This is an observational cohort study. The study team has performed thorough literature search and to our knowledge, there are no previous studies of similar nature performed in this field to allow us to do formal sample size calculations. There are no data for body composition values which have been shown to be related to clinically meaningful outcomes such as mortality. Therefore, the objectives of this observational cohort study is to describe the body composition changes that occurred after stroke in the context of stroke severity, patients risk profile and nutritional management to better understand the effect of stroke and its management on changes in body composition (fat free mass, fat mass and dehydration in particular) and to explore the effect of body composition changes on the long term outcomes (both objective and subjective) after stroke.

Data Analysis:

Analysis of all data will be carried out using the latest SPSS version available at the time of analysis. Adjustments will be made for above variables that can have an effect on outcome. Below are the planned statistical tests to achieve study objectives.

Body composition changes in acute care after stroke: to test primary hypothesis I: *Body composition changes after stroke do occur and the magnitude and proportion of changes occurring in various components of the body (Fat mass, FFM etc.) is different depending on stroke severity*. ANOVA will be carried out to test the significance of hypothesis I; $p<0.05$, 95% CI.

Body composition changes after stroke and their effect on long term outcome: to test, *hypothesis II: Body composition changes after stroke results in long term reduced functional capacity. The body composition changes after stroke are influenced by the timing and methods of feeding independently of stroke severity*. In order to test hypothesis II an ANOVA test will be carried out; $p<0.05$, 95% CI.

The effect of body composition changes after stroke and long term outcome (subjective outcomes): to test the hypothesis, *fat free mass loss is associated with reduced functional capacity and quality of life as indicated by the Stroke Impact Scale, SF-36, and self rated health (5 options) scores/responses*, and to evaluate the strength of association between body composition changes after stroke and long term outcome (subjective) a linear regression analysis will be carried out; to understand the probability of the measured outcomes happening (reduced functional capacity and quality of life) when fat free mass loss occurs.

The effect of body composition changes after stroke and long term outcome (objective): to test the hypothesis, *body composition changes mainly fat free mass loss results in increased risk of mortality, readmission to secondary care settings, and admission to rehabilitation services*, and to evaluate the strength of association between body composition changes and long term outcomes. A linear of logistic regression analysis (depending on the outcome) will be carried out to understand the probability of the event happening when fat mass loss occurs. A Cox-regression model will also be designed to take into account the point in time in which an outcome may occur.

The effect of different nutritional support regimen on body composition changes: three means ANOVA will be carried out to test the significance of different feeding regimen on body composition changes. Three means ANOVA will test the difference between Percutaneous Endoscopic Gastrostomy, Naso gastric tube feeding, and a NG feeding with additional oral intake. An unpaired t-test will be carried out for two independently different groups NG vs. PEG to understand their different effect on body composition changes; $p<0.05$, 95% CI.

The impact of the cellular dehydration on long term outcomes: a logistic or linear regression (depending on the outcome) will be carried out to test the hypothesis, *cellular dehydration as measured by MF-MF-BIA after stroke is associated with increased chances of hospital readmission, admission to rehabilitation services, increased risk of mortality, disability, and reduced quality of*

life based on patients responses and Stroke Impact Scale and SF-36 scores using $p<0.05$ and 95% CI. A Cox-regression model will also be carried out to understand the effect of cellular dehydration on long term outcome at a point in time.

Multiple Regression for Model Design: the relationship between body composition and fat free mass changes separately with other variables including age, cellular dehydration, stroke severity, sex, and nutritional support regimen will be all depicted in a model using multiple regression. The model will try to develop a relationship while understanding the strength of association between fat free mass changes and body composition changes (each separately), with age, sex, stroke severity, nutritional support, and cellular dehydration. An example of a regression equation will be

$$\mathbf{Y = a + b_1x_1 + b_2x_2 + \ldots\ldots + b_5x_5}$$

Where Y is fat free mass or body composition and X_1 to X_5 are sex, age, nutritional support, cellular dehydration, and stroke severity regardless of the order.

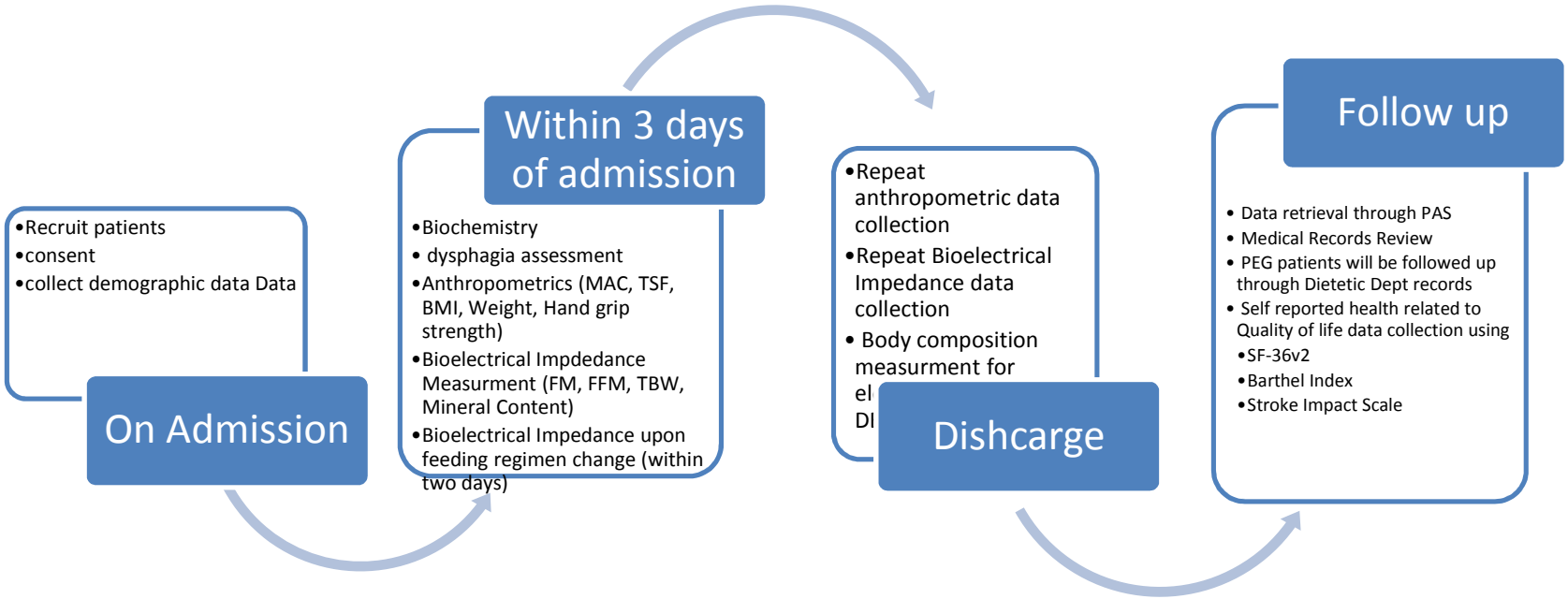
Descriptive Statistics: In addition to the above analytic methods, descriptive statistics such as percentages, median, and means will be calculated to provide a broad understanding and a general conclusion on the reported outcomes.

Study time line: Study Begins: 01/06/2010

Study Ends: 31/12/2011

After ethical approval, data collection will commence on the 1st of June 2010 and ends on 30th February of 2011 (9 months). The stroke team in NNUH looks after about 1000 new strokes per annum. With conservative estimate of 20% consent rate we expect to recruit approximately 120-150 patients over 9 months recruitment period. The follow up period will begin from 1st of March 2011 and end on the 31st December of 2011 (9 months).

Schematic Diagram depicting summary of the project



Patient recruitment period: nine months

Patient follow up period: nine months

Data Analysis: paired or unpaired t-test, Anova, linear regression, logistic regression, and/or Cox-regression model when appropriate.

Multiple regression model will be designed to understand the relationship between Fat Free Mass and Body composition changes and age, sex, cellular dehydration, stroke severity, and nutritional support in stroke patients.

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Appendix VII: Participant Information Sheet

Norfolk and Norwich University Hospital NHS
NHS Trust

PARTCIPANT INFORMATION SHEET

Study title: Changes in body composition after acute stroke

Main investigator: Mohannad Kafri

You are invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of this study?

The aim of this study is to understand what body composition changes occur after stroke. Our body is composed of fat, non-fat (muscle, bone and tissues), water, and its contents (for example salt). There is limited knowledge of the effect of stroke on body composition changes.

Moreover, changes in these components of body have been shown to relate to health in older people. In addition, we do not understand very well the relationship between body composition changes after stroke and the long term outcome on people's life quality. This study therefore also seeks to understand the relationship between body compositions change immediately after stroke and the long term outcome up to one year on stroke patient's quality of life and health.

The findings of this study can assist health care professionals and specialists to understand in what ways we can improve the nutritional care after stroke.

Why have I been chosen?

You have been invited to this study because you recently sustained a stroke and have been admitted to the hospital. You have been invited because we think you are eligible to take part in this study according to our study criteria and you may be interested in helping with the project as a participant.

Do I have to take part?

You do not have to take part in the study if you do not want to. It is **entirely voluntary.** If you decide not to take part in the study, this will not influence your care in any way.

What will happen to me if I take part?

If you decide to take part we would like to assess your body composition values. It is a procedure that is non-invasive, quick, and does not put you in anyway under stress. We will simply attach the machine using sticky patch (which can be easily removed afterwards; similar to sticky patch we use to look at your heart tracing) at your wrist and hand, and leg and foot and take the measurements.

You will not feel anything. It will only take 5 minutes or less. We intend to measure at least twice before you are discharged from the hospital.

If there were a feeding regimen change, we may repeat the measurement. This is only for research purposes and will not affect your treatment in anyway. It is possible that frequent body composition measurements will be carried out after feeding regimen change. Please feel free to ask the nurse for further information and the investigator will visit you to give you a better picture of the measurement if you wish to.

Also we would like to take a measurement of your Skin Fold thickness in upper arm. It does not take more than 5 minutes. It does not cause you any pain. We will take five measurements of the skin fold thickness. We will also want to take three measurements of the circumference of the arm. Again it is a very simple procedure that does not cause any pain and will take less than 5 minutes in total. The last measurement we want to take is your handgrip strength where you simply squeeze a gas filled balloon as hard as you can and hold it while the investigator reads the meter. It does not take more than one minute; we might ask you to repeat the hand grip strength measurement three times. The measures are repeated so as to make sure we record the most accurate measurement.

We would also like to look at your medical records, case notes, and blood biochemical measurements. Looking at your medical records, case notes, and biochemical measurements will help the research team to understand your health status and how it relates to your body composition changes and quality of life. This approach does not require any extra blood test and we will be using available information which is routinely measured and recorded by the clinicians for your care. Please feel free to object and make your decision clear to us if you don't want us to access your data.

After you are discharged from the hospital we will ask you some questions which will be sent to you by mail about 9 months after your stroke. It can be filled in by yourself or with the help of your carer/friend or a family member. You can also refuse to answer all or part of these questions if you decide not to.

You may be asked to attend a special measurement for body compositions (called DEXA scan) at the University of East Anglia if you are appropriate to be included in that part of the study. You will need to lie-down still on a padded bench while taking the scan. It is similar to an X-ray. It is quick, simple, and does NOT require any other procedure other than lying down still for few minutes. The amount of radiation you will be exposed to is minimal and is equal to the amount of radiation you are exposed to everyday from natural resources in the UK in less than two days. We will organise the transport if you require attending the assessment.

We respect your decision and we appreciate your participation. Please feel free to make the decision you feel most comfortable about. Any decision you take will in no way affect the quality of care you receive.

Your results will remain private and no one except the research team will have access to them. These results are only for research purposes and are not for treatment purposes and they will not affect the quality of care you will be getting.

Expenses and payments

Taking part in this research project will not incur any expenses to you. There will be no payment for taking part in this research. The follow-up will be carried out through postal questionnaire in most cases. However, for the follow-up visits if we need to assess your health we will arrange transport for you and provide refreshments.

What will I have to do?

If you agree to participate in the study we will need your consent on the official form. Once consented, we will take measurements. You will be assessed upon consenting, then when feeding changes happen, and at discharge.

What are the risks and nature of taking part?

There is no risk involved in taking part in the study. The equipment we will use to measure your body composition has been checked for its safety by the responsible department of the hospital.

What are the possible benefits of taking part?

It is unlikely that you will directly benefit from the research. However, this is a project examining 1) body composition changes after stroke 2) the influence of body composition change on the long term outcome after stroke 3) and the effect of different feeding regimens on body composition after stroke. Our findings may suggest areas for improvement or intervention which will be of benefit stroke patients and improve stroke care in the future.

What happens if I change my mind?

You are free to withdraw from the study at any time. This will not affect the medical treatment you receive on the ward. Any research data collected from you will not be considered and will be removed.

Will my taking part in this study be kept confidential?

All information, which is collected about you during the course of research, will be kept strictly confidential. All data will be entered into secure computers located in the hospital with limited access measures via a username and password. Your name and any other identifying information will not be included in any study data entered into the computers and your name and address will be removed from any information leaving the hospital/surgery. You will be identified using a specific study code and/or number when entering data into the secure computers.

Research Data collected will be stored on the secure computers for a period of more than three years as this us a PhD research project that takes at least three years to complete.

What will happen to the tests?

The measurements and responses to questions asked will be kept entirely anonymous.

What will happen to the result of the research study?

The study results may also lead to further studies in this particular area. Any information we collect about you will be confidential and used only for the purpose of this study. The information about you will only be available to research staff and the medical staff caring for you. We hope to publicise our findings by submitting the research reports to scientific journals and present our findings at scientific meetings and patient and public forums. Data presented in all medium will be aggregated and anonymised so that no one will be able to identify you based on these publications.

What if something goes wrong?

It is very unlikely that you will be harmed by taking- part in this research project since this project does not involve administration of any drugs or use of any invasive instrument. However, if you wish to complain in the event of any self-perceived harm as a result of this study, the normal National Health Service complaints mechanisms will be available to you.

You can also contact the Patient Advice and Liaison Services (PALS) available in the hospital for support, resolving any problems, suggestions, or concerns. PALS is open weekdays from 9am-5pm and can be contacted on the Telephone 01603 289036 or through email: PALS@nnuh.nhs.uk.

Who is organising and funding the research?

This study is carried out by a research team consisting of Mr Mohannad Kafri, PhD student in Nutritional Epidemiology, University of East Anglia, Dr Phyo Kyaw Myint, Clinical Senior Lecturer/Consultant in Stroke Medicine, Dr Lee Hooper, Senior Lecturer in Research Synthesis & Nutrition, School of Medicine, Health Policy and Practice, University of East Anglia, and Professor John Potter, Professor of Ageing & Stroke Medicine, School of Medicine, Health Policy and Practice, University of East Anglia. The University of East Anglia funded this PhD studentship and the study is supported by the Department of Medicine for the Elderly.

Who has reviewed the study?

This study has been reviewed and given a favourable opinion by the Cambridgeshire I Research Ethics Committee.

Contact for further information If you would like to know more, please contact the principal investigators of the study Mohannad Kafri, Investigator, 01603 286286.

You must be happy about any decision you make and you will be given a copy of this information sheet and signed consent form to keep. Thank you for taking time to read this information sheet.

Thank you for your help.

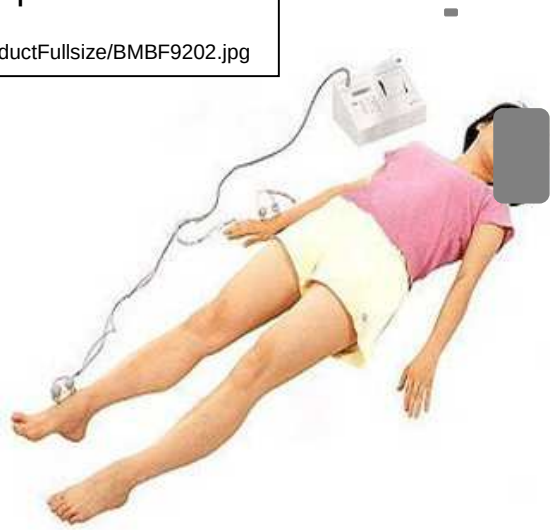
PARTICIPANT INFORMATION SHEET (MEASUREMENT DIAGRAMS)

After you consent to participate we will take the following measurements

1. Body Composition Measurement

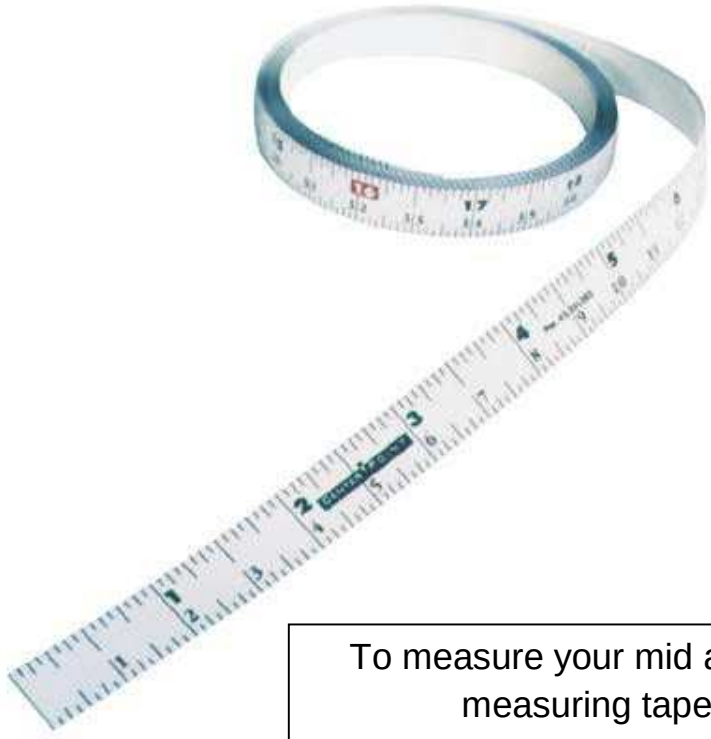
This is the picture of the device (about the size of heart tracing machine (ECG)) we will use to measure your body composition

Image source: http://www.habdirect.co.uk/images/productFullsize/BMBF9202.jpg



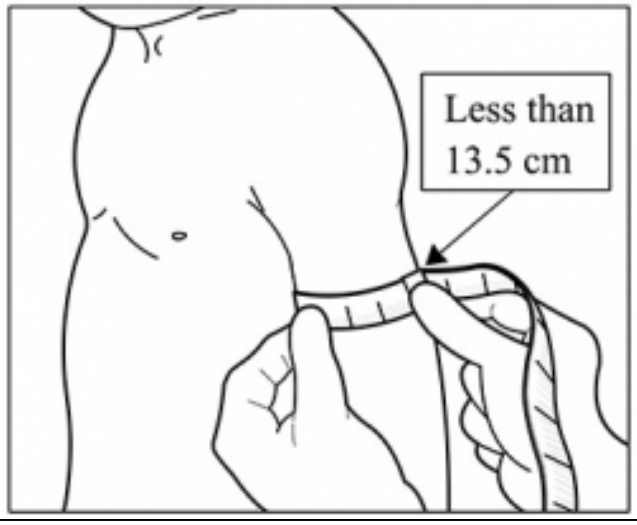
While you are lying down we will take your body composition measurement by attaching sticky patches (like those used in a heart tracing measurement) to your hand and leg

Image source: http://web.tradekorea.com/upload_file/prod/marketing/mkt_files/new_company//giltron/img_en/o_P276050.jpg



To measure your mid arm circumference a measuring tape will be used.

Image source: http://www.northerntool.com/images/product/images/30028_lg.jpg

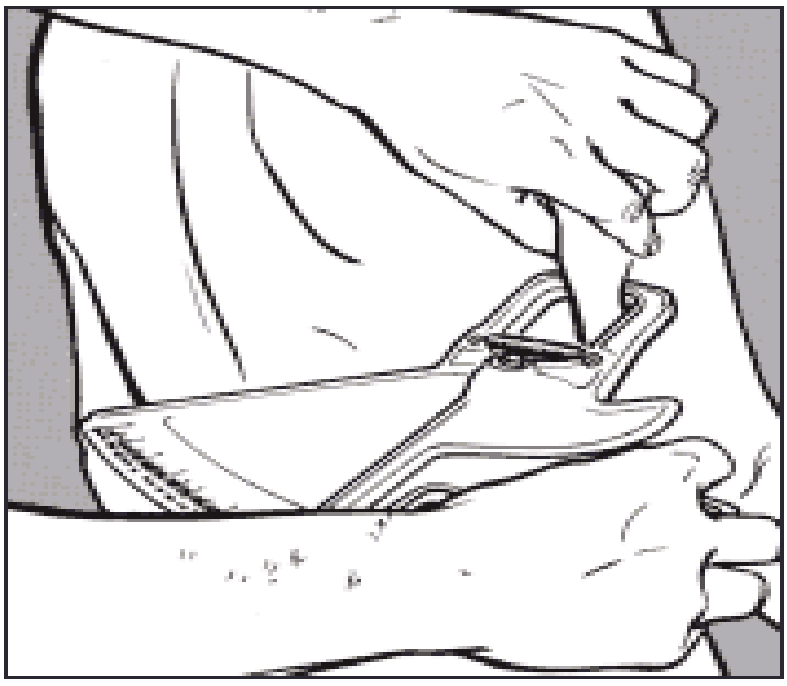


Using the tape we will measure your mid arm circumference as shown in the picture above. We will roll the tape around your arm and take the measurement.

Image source: http://www.squidoo.com/organic-food-eating-right

This is the device used to measure the skinfold thickness. It is called a calliper. The open end of the instrument is used to hold the skin as in the picture below. It will not hurt you.

Image source: http://www.first4shape.com/prodimg/AM3K_1_zoom.jpg

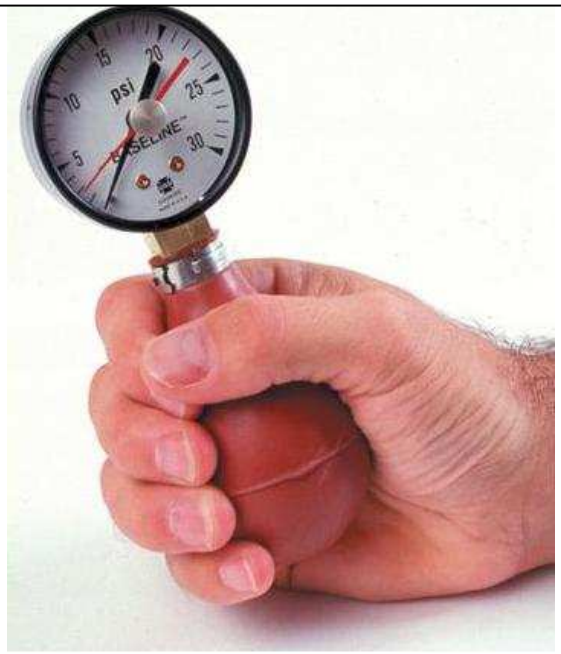


We will take a skin fold from back of your arm (triceps area) and measure its thickness using the calliper.

Image source: http://www.healthgoods.com/v/vspfiles/assets/images/skinfold_caliper_back_arm.gif

This is the example of the device which will be used to measure your hand grip strength. It is called a dynamometer

Image source: http://faculty.washington.edu/kepeter/119/images/muscle_strength_bulb.jpg



To measure your handgrip strength you will be asked to squeeze the balloon while we make the reading

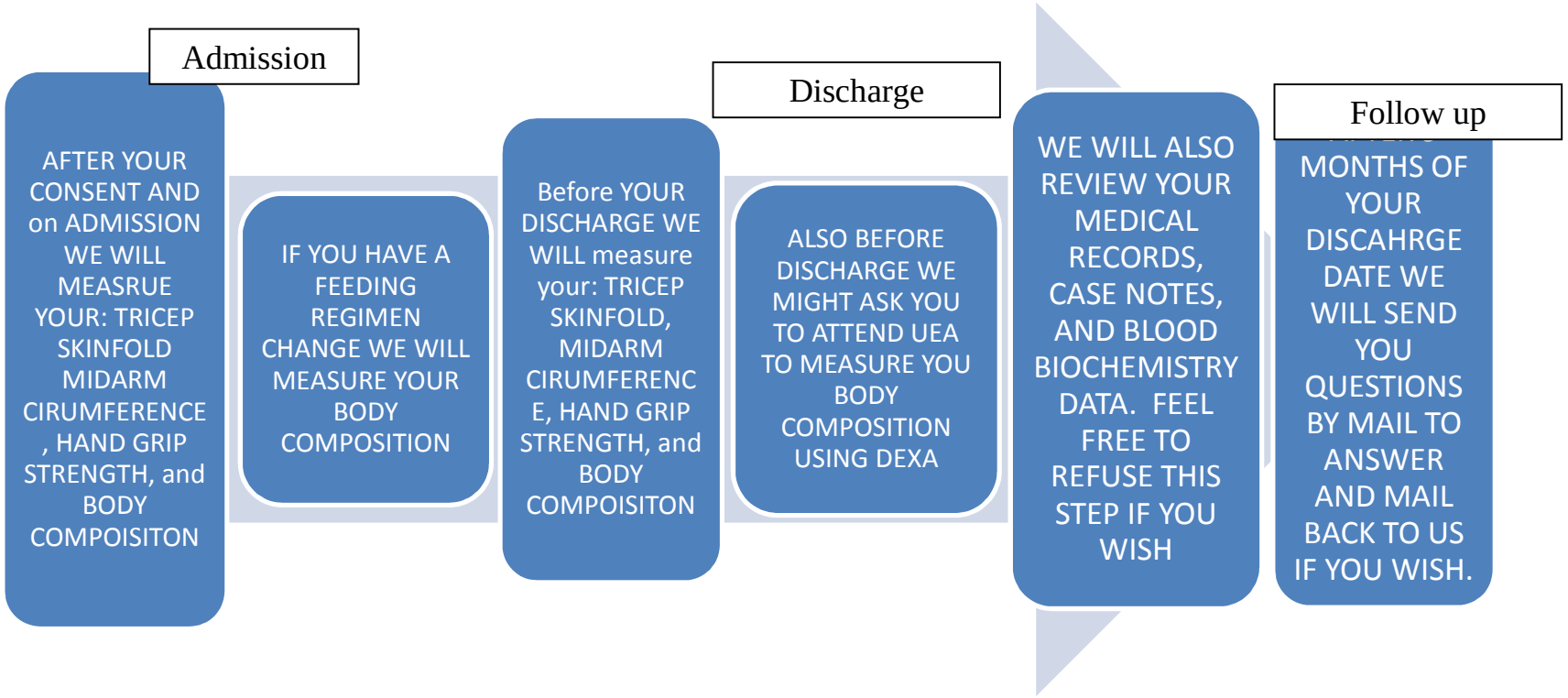
Image source: http://altomedical.com/images/photo_91%5B1%5D.jpg

This the example of the DEXA machine

Image source: http://www.alexanderorthopaedics.com/images/dexa-c.jpg

This is how the DEXA measurement is performed. We will ask you to lie down in a relaxed position on a padded surface while we take the measurement. It is just like an X-ray.

Image source: http://houstonmri.com/Libraries/site_pics/dexa2.sflb.ashx


Admission
AFTER YOUR CONSENT AND on ADMISSION WE WILL MEASRUE YOUR: TRICEP SKINFOLD MIDARM CIRUMFERENCE , HAND GRIP STRENGTH, and BODY COMPOISITON
IF YOU HAVE A FEEDING REGIMEN CHANGE WE WILL MEASURE YOUR BODY COMPOSITION
Discharge
Before YOUR DISCHARGE WE WILL measure your: TRICEP SKINFOLD, MIDARM CIRUMFERENCE, HAND GRIP STRENGTH, and BODY COMPOISITON
ALSO BEFORE DISCHARGE WE MIGHT ASK YOU TO ATTEND UEA TO MEASURE YOU BODY COMPOSITION USING DEXA
WE WILL ALSO REVIEW YOUR MEDICAL RECORDS, CASE NOTES, AND BLOOD BIOCHEMISTRY DATA. FEEL FREE TO REFUSE THIS STEP IF YOU WISH
Follow up
MONTHS OF YOUR DISCAHRGE DATE WE WILL SEND YOU QUESTIONS BY MAIL TO ANSWER AND MAIL BACK TO US IF YOU WISH.

Appendix VIII: Letter to participant's GP

Norfolk and Norwich University Hospital NHS
NHS Trust

Mohannad Kafri
Medicine for the Elderly
Norfolk and Norwich University Hospital, Colney Lane
Norwich NR4 7UY
Tell: 01603 286286
Direct fax: 01603 286428
Email: m.kafri@uea.ac.uk
Trust website:
www.nnuh.nhs.uk

Date: / /20

Dear Dr

Re: The relationship between body composition changes during acute stroke care and long term outcomes study

Your patient has agreed to take part in the above study. The study involves assessing body composition using bioelectrical impedance analysis. It is a simple procedure that is non-invasive, quick, and painless.

The aim of the study is to find out whether there is any relationship between body composition changes during acute stroke care and long term outcomes and it does not interfere with medical care the patient is receiving or involve the administration of any medicine.

If you have any questions please do not hesitate to contact me on above address.

Yours sincerely,

Mohannad Kafri (Investigator),

PhD student, University of East Anglia

Appendix IX: Patients' consent form

Norfolk and Norwich University Hospital NHS
NHS Trust

CONSENT FORM

STUDY ON Body Composition changes after stroke
Name of Researcher: Mohannad Kafri

Trust Project number Please initial each box indicate your agreement

1. I confirm that I have read and understand the patient information document to Trust Study Number-------- dated ----------for the above study and that I have had the opportunity to ask any questions that I may have. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without my medical care or legal rights being affected without giving reasons ☐
3. I understand that sections of any of my medical notes may be looked at by responsible individuals from the Norfolk & Norwich NHS Trust or from the regulatory authorities where it is relevant to me taking part in this research project. I give permission for these individuals to have access to my records. ☐
4. I agree to take part in the above study. ☐
5. I agree to my GP being informed of my participation in this study. ☐

Name of the patient (capital letters) ----------

Signature of the patient ---------- Date:

Name of investigator: ----------

Signature of investigator ---------- Date:

Appendix X: Standard Operating Procedure

Body Composition changes and hydration status after stroke

Anthropometrics: Skinfold measurement

Triceps Skinfold (first choice)

1. Use patient's right arm
2. Determine the midpoint between the top of the shoulder) to the bottom of the elbow.
3. Once the midpoint is determined.....
4. Pinch the skin, the skin fold has to be at 90° to the arm
5. Place Calipers on the skinfold and record measurement
6. Repeat three times

Adopted from: http://www.urmc.rochester.edu/physiology-exercise-lab/equipment/assessment.cfm

Inter ratter reproducibility assessment:

After the first investigator carries the Triceps Skinfold thickness measurement, another investigator must take same measurement at the same time repeating the exact same procedure. Record TSF result on a separate data sheet.

Anthropometrics: Mid Upper Arm Circumference

1. Use patient right arm (if patients suffers from hemiparesis try your best to take the measurement from the right arm unless you cannot, use the other arm however by indicating that you used the other arm; formula is validated for right arm however we can see the difference between MUAC measurement in both sides compared to FFM values from MF-MF-BIA)
2. Identify the midpoint between the elbow and the shoulder (you can measure the upper arm length and determined the midpoint)
3. Record the measurement
4. Repeat three times

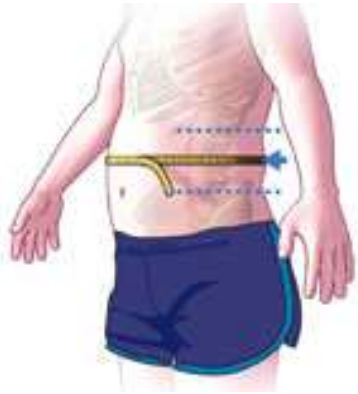
Adopted from: http://www.topendsports.com/testing/tests/girth-arm-relaxed.htm

Inter ratter reproducibility assessment:

After the first investigator carries the Mid Upper Arm Circumference measurement, another investigator must take same measurement at the same time repeating the exact same procedure. Record your measurement on separate data sheet.

Anthropometrics: Waist circumference

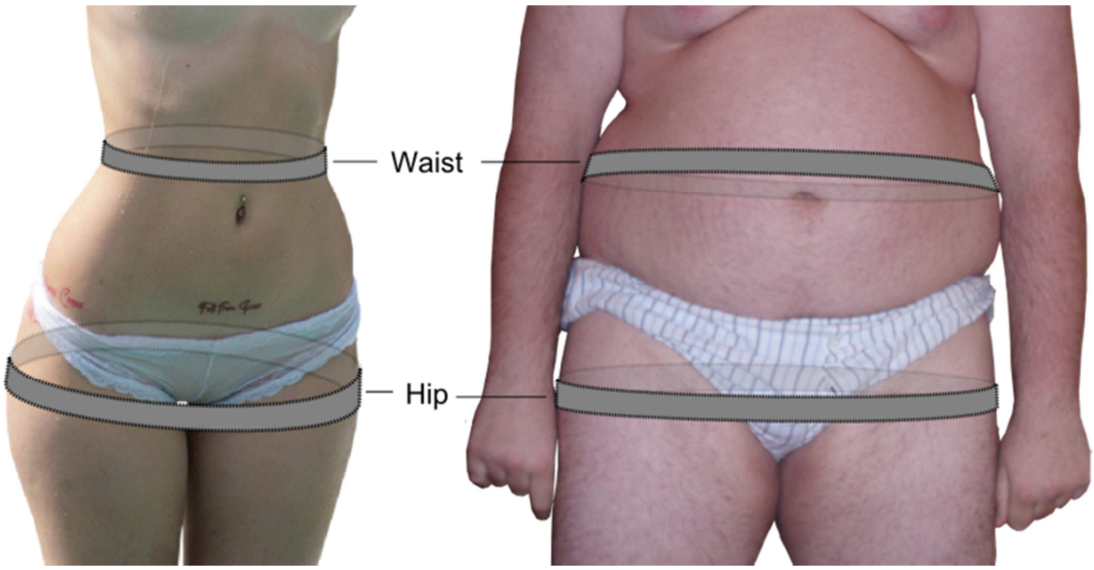
1. As the patient to stand (if the patient cannot stand try measuring while sitting on bed, making sure his back is straight); if the patient cannot stand or sit do not make the measurement; note this.
2. Locate the highest end of the hip bone (the iliac crest)
3. Once located, place the measuring tape horizontally across the waist
4. Record measurement
5. Repeat three times



Adopted from: http://www.drsharma.ca/wp-content/uploads/sharma-obesity-waist-circumference.jpg

Anthropometrics: Hip circumference

1. Ask the patient to stand (if the patient cannot stand try measuring while sitting on bed, making sure his back is straight)
2. Find the widest point on the buttock
3. Once the point is located, place a tap horizontally around the buttocks and measure the hip circumference
4. Repeat three times
5. Round to the nearest 0.1



Adopted from: http://en.wikipedia.org/wiki/File:Waist-hip_ratio.png

Reproducibility assessment:

After the first investigator carries the WC and HC measurement, another investigator must take same measurement at the same time repeating the exact same procedure. Repeat the Waist and Hip circumference measurement each three times and record raw data.

Hand grip strength

1. Use patient's unaffected arm
2. Explain to the patients the measurement process
3. Ask the patient to hold the dynamometer with the unaffected arm after setting it comfortably to suite the grip of each patient
4. Make sure the dynamometer is set to Zero by pressing the on button and the number on the measuring meter is 0.0
5. Ask the patient to squeeze press as hard as possible up to 15 seconds and record the measurement
6. Repeat three times

Image adopted: http://www.fitnessvenues.com/uk/fitness-testing-hand-grip-strength-test

Physical Assessment of Hydration

Tongue Furrows and Dryness

1. Observe the patients tongue
2. Record if Tongue furrows are present (tongue furrows are like small channels that looks somewhat white (due to peeling) present on the surface of the tongue indicating dryness)
3. Classify as yes (furrows) or no (any furrows).
4. Record if the tongue is dry or furred (if dry the tongue will have white dots, patches or will appear white in general due to surface peeling)
5. Classify as furred (tongue furred), dry (tongue dry), both or no (not dry or furrowed)

Reliability assessment: At the same time of this assessment another ratter must carry the same procedure again. Record your result on a separate data sheet. For comparison; carry out a Cohen Kappa test after a collection of several measurements

Skin Turgor

Definition: the ability of the skin to resume its normal form after being pinched or distorted. Delayed ability of the skin to obtain its natural form may indicate a sign of dehydration.

1. Pinch the skin on the back of the hand of the unaffected side holding it for few seconds
2. Release
3. Observe how long the skin takes to return to its natural form (in seconds)
4. Report in seconds

Reliability assessment: At the same time of this assessment another ratter must carry the same procedure again. Record your result on a separate data sheet. For comparison; carry out a Cohen Kappa test after a collection of several measurements

Image adopted from: http://health.allrefer.com/health/dehydration-skin-turgor.html

Capillary refill time

Definition: a test to assess circulation in the hand (usually thumb or any other finger). It can signify dehydration with a host of other conditions.

1. Pressure the nail bed of the middle (longest) finger of the unaffected side of the patient until the natural skin colour is gone (usually takes few seconds)
2. Release the pressure and wait for the natural coloration to return
3. Record the number of seconds taken for the natural colouration to return

Reliability assessment: At the same time of this assessment another ratter must carry the same procedure again. Record your result on a separate data sheet. For comparison; carry out a Cohen Kappa test after a collection of several measurements.

Blood sampling

1. Investigate the presence of an obvious vein
2. Make sure the arm was not used frequently before for blood sampling
3. take a blood sample following hygienic procedure
4. Use the venepuncture system (grey tube top for Glucose and Orange tube top for electrolytes/osmolality).
5. Each tube can hold 5 ml, try to get at least one third full to have enough sample for analysis.
6. If you are using a venepuncture system (use the electrolyte tube (orange colour first) then the glucose tube (grey colour) if you could not get the second sample of blood for glucose try the other arm.
7. If it was difficult to take blood from patient, try first hand, then second...if you still can't STOP and drop this step.

Appendix XI: National Institute of Health Stroke Severity Score

N I H
STROKE
SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

Time: ___ ___:___ ___ [ ]am [ ]pm

Person Administering Scale _____

Administer stroke scale items in the order listed. Record performance in each category after each subscale exam. Do not go back and change scores. Follow directions provided for each exam technique. Scores should reflect what the patient does, not what the clinician thinks the patient can do. The clinician should record answers while administering the exam and work quickly. Except where indicated, the patient should not be coached (i.e., repeated requests to patient to make a special effort).

Instructions	Scale Definition	Score
1a. Level of Consciousness: The investigator must choose a response if a full evaluation is prevented by such obstacles as an endotracheal tube, language barrier, orotracheal trauma/bandages. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation.	0 = **Alert;** keenly responsive. 1 = **Not alert**; but arousable by minor stimulation to obey, answer, or respond. 2 = **Not alert**; requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped). 3 = Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, and areflexic.	_____
1b. LOC Questions: The patient is asked the month and his/her age. The answer must be correct - there is no partial credit for being close. Aphasic and stuporous patients who do not comprehend the questions will score 2. Patients unable to speak because of endotracheal intubation, orotracheal trauma, severe dysarthria from any cause, language barrier, or any other problem not secondary to aphasia are given a 1. It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues.	0 = **Answers** both questions correctly. 1 = **Answers** one question correctly. 2 = **Answers** neither question correctly.	_____
1c. LOC Commands: The patient is asked to open and close the eyes and then to grip and release the non-paretic hand. Substitute another one step command if the hands cannot be used. Credit is given if an unequivocal attempt is made but not completed due to weakness. If the patient does not respond to command, the task should be demonstrated to him or her (pantomime), and the result scored (i.e., follows none, one or two commands). Patients with trauma, amputation, or other physical impediments should be given suitable one-step commands. Only the first attempt is scored.	0 = **Performs** both tasks correctly. 1 = **Performs** one task correctly. 2 = **Performs** neither task correctly.	_____
2. Best Gaze: Only horizontal eye movements will be tested. Voluntary or reflexive (oculocephalic) eye movements will be scored, but caloric testing is not done. If the patient has a conjugate deviation of the eyes that can be overcome by voluntary or reflexive activity, the score will be 1. If a patient has an isolated peripheral nerve paresis (CN III, IV or VI), score a 1. Gaze is testable in all aphasic patients. Patients with ocular trauma, bandages, pre-existing blindness, or other disorder of visual acuity or fields should be tested with reflexive movements, and a choice made by the investigator. Establishing eye contact and then moving about the patient from side to side will occasionally clarify the presence of a partial gaze palsy.	0 = **Normal.** 1 = **Partial gaze palsy;** gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present. 2 = **Forced deviation,** or total gaze paresis not overcome by the oculocephalic maneuver.	_____

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NIH STROKE SCALE

Patient Identification. __ __-__ __ __-__ __ __

Pt. Date of Birth __ __/__ __/__ __

Hospital _____(__ __-__ __)

Date of Exam __ __/__ __/__ __

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(__ __)

3. Visual: Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat, as appropriate. Patients may be encouraged, but if they look at the side of the moving fingers appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if a clear-cut asymmetry, including quadrantanopia, is found. If patient is blind from any cause, score 3. Double simultaneous stimulation is performed at this point. If there is extinction, patient receives a 1, and the results are used to respond to item 11.	0 = **No visual loss.** 1 = **Partial hemianopia.** 2 = **Complete hemianopia.** 3 = **Bilateral hemianopia** (blind including cortical blindness).	____
4. Facial Palsy: Ask – or use pantomime to encourage – the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or non-comprehending patient. If facial trauma/bandages, orotracheal tube, tape or other physical barriers obscure the face, these should be removed to the extent possible.	0 = **Normal** symmetrical movements. 1 = **Minor paralysis** (flattened nasolabial fold, asymmetry on smiling). 2 = **Partial paralysis** (total or near-total paralysis of lower face). 3 = **Complete paralysis** of one or both sides (absence of facial movement in the upper and lower face).	____
5. Motor Arm: The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine). Drift is scored if the arm falls before 10 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in the case of amputation or joint fusion at the shoulder, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	0 = **No drift;** limb holds 90 (or 45) degrees for full 10 seconds. 1 = **Drift;** limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support. 2 = **Some effort against gravity;** limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity. 3 = **No effort against gravity;** limb falls. 4 = **No movement.** UN = **Amputation** or joint fusion, explain: _____ **5a. Left Arm** **5b. Right Arm**	 ____ ____
6. Motor Leg: The limb is placed in the appropriate position: hold the leg at 30 degrees (always tested supine). Drift is scored if the leg falls before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic leg. Only in the case of amputation or joint fusion at the hip, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	0 = **No drift;** leg holds 30-degree position for full 5 seconds. 1 = **Drift;** leg falls by the end of the 5-second period but does not hit bed. 2 = **Some effort against gravity;** leg falls to bed by 5 seconds, but has some effort against gravity. 3 = **No effort against gravity;** leg falls to bed immediately. 4 = **No movement.** UN = **Amputation** or joint fusion, explain: _____ **6a. Left Leg** **6b. Right Leg**	 ____

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NIH STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

7. Limb Ataxia: This item is aimed at finding evidence of a unilateral cerebellar lesion. Test with eyes open. In case of visual defect, ensure testing is done in intact visual field. The finger-nose-finger and heel-shin tests are performed on both sides, and ataxia is scored only if present out of proportion to weakness. Ataxia is absent in the patient who cannot understand or is paralyzed. Only in the case of amputation or joint fusion, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice. In case of blindness, test by having the patient touch nose from extended arm position.	0 = **Absent.** 1 = **Present in one limb.** 2 = **Present in two limbs.** UN = **Amputation** or joint fusion, explain: _____	____
8. Sensory: Sensation or grimace to pinprick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient. Only sensory loss attributed to stroke is scored as abnormal and the examiner should test as many body areas (arms [not hands], legs, trunk, face) as needed to accurately check for hemisensory loss. A score of 2, "severe or total sensory loss," should only be given when a severe or total loss of sensation can be clearly demonstrated. Stuporous and aphasic patients will, therefore, probably score 1 or 0. The patient with brainstem stroke who has bilateral loss of sensation is scored 2. If the patient does not respond and is quadriplegic, score 2. Patients in a coma (item 1a=3) are automatically given a 2 on this item.	0 = **Normal;** no sensory loss. 1 = **Mild-to-moderate sensory loss;** patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched. 2 = **Severe to total sensory loss;** patient is not aware of being touched in the face, arm, and leg.	____
9. Best Language: A great deal of information about comprehension will be obtained during the preceding sections of the examination. For this scale item, the patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet and to read from the attached list of sentences. Comprehension is judged from responses here, as well as to all of the commands in the preceding general neurological exam. If visual loss interferes with the tests, ask the patient to identify objects placed in the hand, repeat, and produce speech. The intubated patient should be asked to write. The patient in a coma (item 1a=3) will automatically score 3 on this item. The examiner must choose a score for the patient with stupor or limited cooperation, but a score of 3 should be used only if the patient is mute and follows no one-step commands.	0 = **No aphasia;** normal. 1 = **Mild-to-moderate aphasia;** some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient's response. 2 = **Severe aphasia;** all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response. 3 = **Mute, global aphasia;** no usable speech or auditory comprehension.	____
10. Dysarthria: If patient is thought to be normal, an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list. If the patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated. Only if the patient is intubated or has other physical barriers to producing speech, the examiner should record the score as untestable (UN), and clearly write an explanation for this choice. Do not tell the patient why he or she is being tested.	0 = **Normal.** 1 = **Mild-to-moderate dysarthria;** patient slurs at least some words and, at worst, can be understood with some difficulty. 2 = **Severe dysarthria;** patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/anarthric. UN = **Intubated** or other physical barrier, explain:_____	____

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NIH STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

11. Extinction and Inattention (formerly Neglect): Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosagnosia may also be taken as evidence of abnormality. Since the abnormality is scored only if present, the item is never untestable.	0 = **No abnormality.** 1 = **Visual, tactile, auditory, spatial, or personal inattention** or extinction to bilateral simultaneous stimulation in one of the sensory modalities. 2 = **Profound hemi-inattention or extinction to more than one modality;** does not recognize own hand or orients to only one side of space.	____

____

____

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Appendix XII: Malnutrition Universal Assessment Tool

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'Malnutrition Universal Screening Tool'

MAG
Malnutrition Advisory G
A Standing Committee of

BAPEN is registered charity number 1023927 www.bapen.

'MUST'

'MUST' is a five-step screening tool to identify **adults,** who are malnourished, at risk of malnutrition (undernutrition), or obese. It also includes management guidelines which can be used to develop a care plan.

It is for use in hospitals, community and other care settings and can be used by all care workers.

This guide contains:

- A flow chart showing the 5 steps to use for screening and management
- BMI chart
- Weight loss tables
- Alternative measurements when BMI cannot be obtained by measuring weight and height.

The 5 'MUST' Steps

Step 1

Measure height and weight to get a BMI score using chart provided. *If unable to obtain height and weight, use the alternative procedures shown in this guide.*

Step 2

Note percentage unplanned weight loss and score using tables provided.

Step 3

Establish acute disease effect and score.

Step 4

Add scores from steps 1, 2 and 3 together to obtain overall risk of malnutrition.

Step 5

Use management guidelines and/or local policy to develop care plan.

Please refer to *The 'MUST' Explanatory Booklet* for more information when weight and height cannot be measured, and when screening patient groups in which extra care in interpretation is needed (e.g. those with fluid disturbances, plaster casts, amputations, critical illness and pregnant or lactating women). The booklet can also be used for training. See *The 'MUST' Report* for supporting evidence. Please note that 'MUST' has not been designed to detect deficiencies or excessive intakes of vitamins and minerals and is of **use only in adults.**

Step 1 – BMI score (& BMI)

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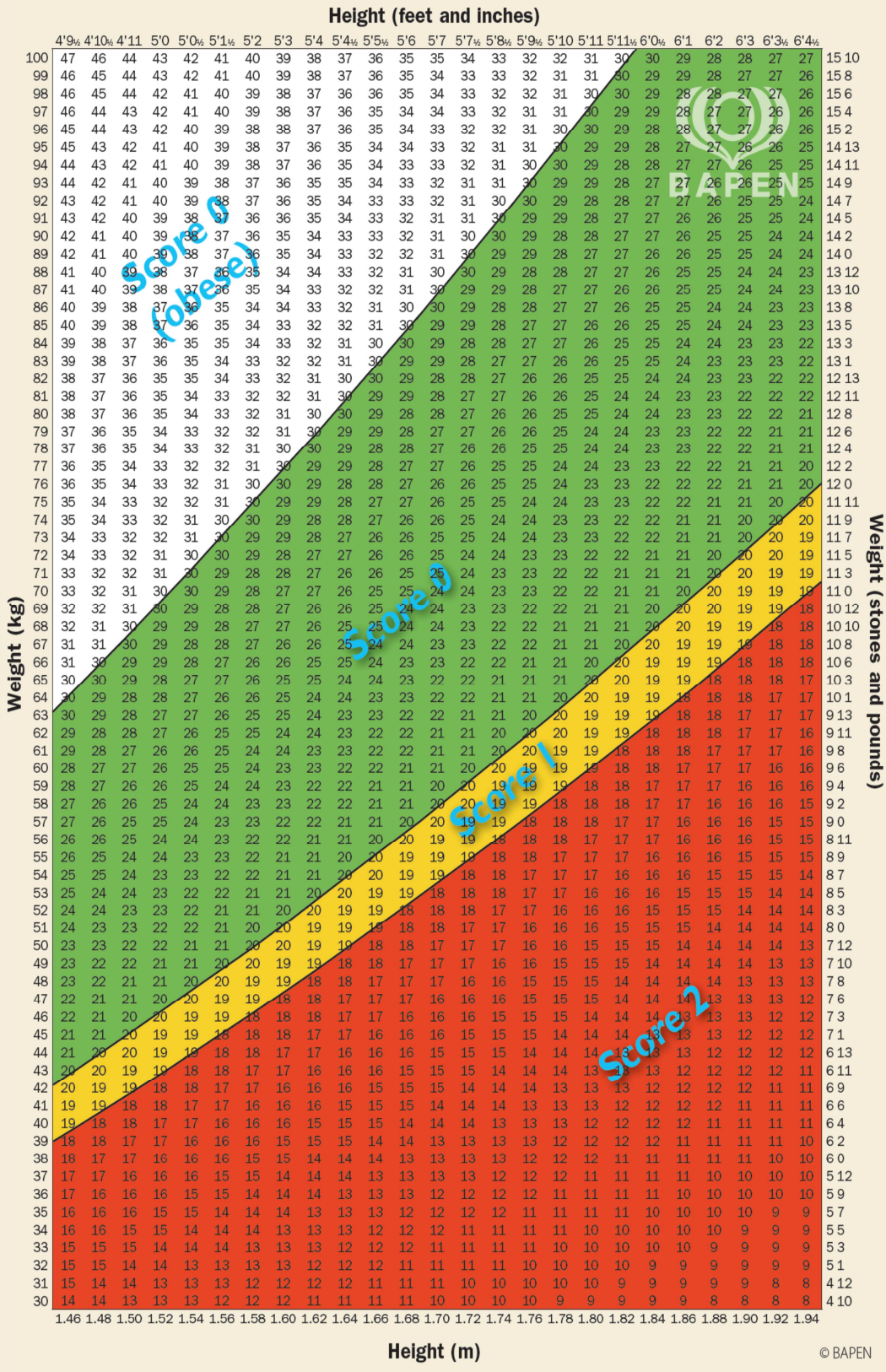
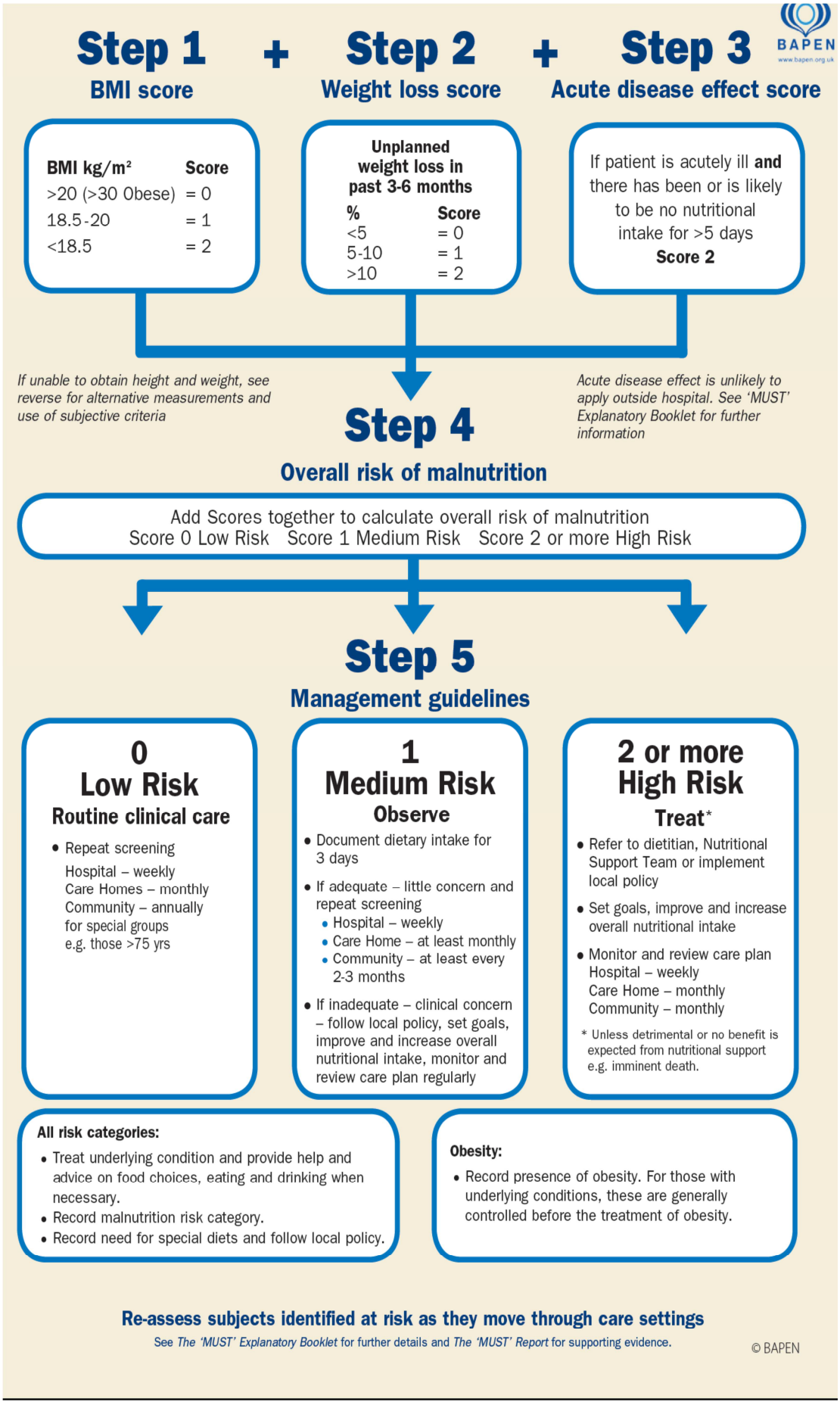


Note : The black lines denote the exact cut off points (30,20 and 18.5 kg/m²), figures on the chart have been rounded to the nearest whole number.


Step 1
BMI score
+
Step 2
Weight loss score
+
Step 3
Acute disease effect score
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BMI kg/m²
Score
>20 (>30 Obese) = 0
18.5-20 = 1
<18.5 = 2
Unplanned weight loss in past 3-6 months
%
Score
<5 = 0
5-10 = 1
>10 = 2
If patient is acutely ill and there has been or is likely to be no nutritional intake for >5 days
Score 2
If unable to obtain height and weight, see reverse for alternative measurements and use of subjective criteria
Acute disease effect is unlikely to apply outside hospital. See 'MUST' Explanatory Booklet for further information
Step 4
Overall risk of malnutrition
Add Scores together to calculate overall risk of malnutrition
Score 0 Low Risk Score 1 Medium Risk Score 2 or more High Risk
Step 5
Management guidelines
0
Low Risk
Routine clinical care
• Repeat screening
Hospital – weekly
Care Homes – monthly
Community – annually
for special groups
e.g. those >75 yrs
1
Medium Risk
Observe
• Document dietary intake for 3 days
• If adequate – little concern and repeat screening
• Hospital – weekly
• Care Home – at least monthly
• Community – at least every 2-3 months
• If inadequate – clinical concern – follow local policy, set goals, improve and increase overall nutritional intake, monitor and review care plan regularly
2 or more
High Risk
Treat*
• Refer to dietitian, Nutritional Support Team or implement local policy
• Set goals, improve and increase overall nutritional intake
• Monitor and review care plan
Hospital – weekly
Care Home – monthly
Community – monthly
* Unless detrimental or no benefit is expected from nutritional support e.g. imminent death.
All risk categories:
• Treat underlying condition and provide help and advice on food choices, eating and drinking when necessary.
• Record malnutrition risk category.
• Record need for special diets and follow local policy.
Obesity:
• Record presence of obesity. For those with underlying conditions, these are generally controlled before the treatment of obesity.
Re-assess subjects identified at risk as they move through care settings
See The 'MUST' Explanatory Booklet for further details and The 'MUST' Report for supporting evidence.
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Step 2 – Weight loss score

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Current weight

kg	Score 0 Wt loss < 5%	Score 1 Wt loss 5 - 10%	Score 2 Wt loss > 10%
	Weight loss in last 3 to 6 months		
	Less than (kg)	Between (kg)	More than (kg)
30	1.6	1.6 - 3.3	3.3
31	1.6	1.6 - 3.4	3.4
32	1.7	1.7 - 3.6	3.6
33	1.7	1.7 - 3.7	3.7
34	1.8	1.8 - 3.8	3.8
35	1.8	1.8 - 3.9	3.9
36	1.9	1.9 - 4.0	4.0
37	1.9	1.9 - 4.1	4.1
38	2.0	2.0 - 4.2	4.2
39	2.1	2.1 - 4.3	4.3
40	2.1	2.1 - 4.4	4.4
41	2.2	2.2 - 4.6	4.6
42	2.2	2.2 - 4.7	4.7
43	2.3	2.3 - 4.8	4.8
44	2.3	2.3 - 4.9	4.9
45	2.4	2.4 - 5.0	5.0
46	2.4	2.4 - 5.1	5.1
47	2.5	2.5 - 5.2	5.2
48	2.5	2.5 - 5.3	5.3
49	2.6	2.6 - 5.4	5.4
50	2.6	2.6 - 5.6	5.6
51	2.7	2.7 - 5.5	5.7
52	2.7	2.7 - 5.8	5.8
53	2.8	2.8 - 5.9	5.9
54	2.8	2.8 - 6.9	6.0
55	2.9	2.9 - 6.1	6.1
56	2.9	2.9 - 6.2	6.2
57	3.0	3.0 - 6.3	6.3
58	3.1	3.1 - 6.4	6.4
59	3.1	3.1 - 6.6	6.6
60	3.2	3.2 - 6.7	6.7
61	3.2	3.2 - 6.8	6.8
62	3.3	3.3 - 6.9	6.9
63	3.3	3.3 - 7.0	7.0
64	3.4	3.4 - 7.1	7.1

kg	Score 0 Wt loss < 5%	Score 1 Wt loss 5 - 10%	Score 2 Wt loss > 10%
	Weight loss in last 3 to 6 months		
	Less than (kg)	Between (kg)	More than (kg)
65	3.4	3.4 - 7.2	7.2
66	3.5	3.5 - 7.3	7.3
67	3.5	3.5 - 7.4	7.4
68	3.6	3.6 - 7.7	7.6
69	3.6	3.6 - 7.7	7.7
70	3.7	3.7 - 7.8	7.8
71	3.7	3.7 - 7.9	7.9
72	3.8	3.8 - 8.0	8.0
73	3.8	3.8 - 8.1	8.1
74	3.9	3.9 - 8.2	8.2
75	3.9	3.9 - 8.3	8.3
76	4.0	4.0 - 8.4	8.4
77	4.1	4.1 - 8.6	8.6
78	4.1	4.1 - 8.6	8.7
79	4.2	4.2 - 8.7	8.8
80	4.2	4.2 - 8.9	8.9
81	4.3	4.3 - 9.0	9.0
82	4.3	4.3 - 9.1	9.1
83	4.4	4.4 - 9.2	9.2
84	4.4	4.4 - 9.3	9.3
85	4.5	4.5 - 9.4	9.4
86	4.5	4.5 - 9.6	9.6
87	4.6	4.6 - 9.7	9.7
88	4.6	4.6 - 9.8	9.8
89	4.7	4.7 - 9.9	9.9
90	4.7	4.7 - 10.0	10.0
91	4.8	4.8 - 10.1	10.1
92	4.8	4.8 - 10.2	10.2
93	4.9	4.9 - 10.3	10.3
94	4.9	4.9 - 10.4	10.4
95	5.0	5.0 - 10.6	10.6
96	5.1	5.1 - 10.7	10.7
97	5.1	5.1 - 10.8	10.8
98	5.2	5.2 - 10.9	10.9
99	5.2	5.2 - 11.0	11.0

Alternative measurements and considerations

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Step 1: BMI (body mass index)

If height cannot be measured

- Use recently documented or self-reported height (if reliable and realistic).
- If the subject does not know or is unable to report their height, use one of the alternative measurements to estimate height (ulna, knee height or demispan).

Step 2: Recent unplanned weight loss

If recent weight loss cannot be calculated, use self-reported weight loss (if reliable and realistic).

Subjective criteria

If height, weight or BMI cannot be obtained, the following criteria which relate to them can assist your professional judgement of the subject's nutritional risk category. Please note, these criteria should be used collectively not separately as alternatives to steps 1 and 2 of 'MUST' and are not designed to assign a score. Mid upper arm circumference (MUAC) may be used to estimate BMI category in order to support your overall impression of the subject's nutritional risk.

1. BMI

- Clinical impression – thin, acceptable weight, overweight. Obvious wasting (very thin) and obesity (very overweight) can also be noted.

2. Unplanned weight loss

- Clothes and/or jewellery have become loose fitting (weight loss).
- History of decreased food intake, reduced appetite or swallowing problems over 3-6 months and underlying disease or psycho-social/physical disabilities likely to cause weight loss.

3. Acute disease effect

- Acutely ill and no nutritional intake or likelihood of no intake for more than 5 days.

Further details on taking alternative measurements, special circumstances and subjective criteria can be found in *The 'MUST' Explanatory Booklet*. A copy can be downloaded at www.bapen.org.uk or purchased from the BAPEN office. The full evidence-base for 'MUST' is contained in *The 'MUST' Report* and is also available for purchase from the BAPEN office.

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Appendix XIII: Short form Survey 36v2 (SF36v2)

Your Health and Well-Being

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.
Thank you for completing this survey!

For each of the following questions, please tick the one box that best describes your answer.

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. <u>Compared to one year ago</u>, how would you rate your health in general <u>now</u>?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

3. The following questions are about activities you might do during a typical day. Does <u>your health now limit you</u> in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	☐ 1	☐ 2	☐ 3
b Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	☐ 1	☐ 2	☐ 3
c Lifting or carrying groceries	☐ 1	☐ 2	☐ 3
d Climbing several flights of stairs	☐ 1	☐ 2	☐ 3
e Climbing one flight of stairs	☐ 1	☐ 2	☐ 3
f Bending, kneeling, or stooping	☐ 1	☐ 2	☐ 3
g Walking more than a mile	☐ 1	☐ 2	☐ 3
h Walking several hundred yards	☐ 1	☐ 2	☐ 3
i Walking one hundred yards	☐ 1	☐ 2	☐ 3
j Bathing or dressing yourself	☐ 1	☐ 2	☐ 3

4. During the <u>past 4 weeks</u>, how much of the time have you had any of the following problems with your work or other regular daily activities <u>as a result of your physical health</u>?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Cut down on the <u>amount of time</u> you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b <u>Accomplished less</u> than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c Were limited in the <u>kind</u> of work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d Had <u>difficulty</u> performing the work or other activities (for example, it took extra effort)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

5. During the <u>past 4 weeks</u>, how much of the time have you had any of the following problems with your work or other regular daily activities <u>as a result of any emotional problems</u> (such as feeling depressed or anxious)?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Cut down on the <u>amount of time</u> you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b <u>Accomplished less</u> than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c Did work or other activities <u>less carefully than usual</u>	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

6. **During the <u>past 4 weeks</u>, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?**

Not at all	Slightly	Moderately	Quite a bit	Extremely
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

7. **How much <u>bodily</u> pain have you had during the <u>past 4 weeks</u>?**

None	Very mild	Mild	Moderate	Severe	Very severe
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

8. **During the <u>past 4 weeks</u>, how much did <u>pain</u> interfere with your normal work (including both work outside the home and housework)?**

Not at all	A little bit	Moderately	Quite a bit	Extremely
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

9. These questions are about how you feel and how things have been with you <u>during the past 4 weeks</u>. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the <u>past 4 weeks</u>…

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Did you feel full of life?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b Have you been very nervous?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c Have you felt so down in the dumps that nothing could cheer you up?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d Have you felt calm and peaceful?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
e Did you have a lot of energy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
f Have you felt downhearted and low?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
g Did you feel worn out?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
h Have you been happy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
i Did you feel tired?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

10. During the <u>past 4 weeks</u>, how much of the time has your <u>physical health or emotional problems</u> interfered with your social activities (like visiting with friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

11. How TRUE or FALSE is <u>each</u> of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
a I seem to get ill more easily than other people	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b I am as healthy as anybody I know	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c I expect my health to get worse	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d My health is excellent	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Thank you for completing these questions!

Appendix XIV: Stroke Impact Scare (SIS)

Stroke Impact Scale

The purpose of this questionnaire is to evaluate how stroke has affected your health and life. We want to know from YOUR POINT OF VIEW how stroke has affected you. We will ask you questions about impairments and disabilities caused by your stroke, as well as how stroke has affected your quality of life. Finally, we will ask you to rate how much you think you have recovered from your stroke.

These questions are about the physical problems which may have occurred as a result of your stroke.

1. In the past week, how would you rate the strength of you're...	**A lot of strength**	**Quite a bit of strength**	**Some strength**	**A little strength**	**No strength at all**
a. Arm that was most affected by your stroke?	5	4	3	2	1
b. Grip of your hand that was most affected by your stroke?	5	4	3	2	1
c. Leg that was most affected by your stroke?	5	4	3	2	1
d. Foot/ankle that was most affected by your stroke?	5	4	3	2	1

These questions are about your memory and thinking capacities.

2. In the past week, how difficult was it to...	**Not difficult at all**	**A little difficult**	**Somewhat difficult**	**Very difficult**	**Extremely difficult**
a. Remember things that people had just told you?	5	4	3	2	1
b. Remember things that happened the day before?	5	4	3	2	1
C. Remember to do things (e.g. keep scheduled	5	4	3	2	1

appointments or take medication)?					
d. Remember the day of the week?	5	4	3	2	1
e. Add and subtract numbers?	5	4	3	2	1
f. Concentrate?	5	4	3	2	1
g. Think quickly?	5	4	3	2	1
h. Solve everyday problems?	5	4	3	2	1

These questions are about how you feel, about changes in your mood and about your ability to control your emotions since your stroke.

3. In the past week, how often did you…	**None of the time**	**A little of the time**	**Some of the time**	**Most of the time**	**All of the time**
a. Feel sad?	5	4	3	2	1
b. Feel that there was nobody you were close to?	5	4	3	2	1
c. Feel that you were a burden to others?	5	4	3	2	1
d. Feel that you had nothing to look forward to?	5	4	3	2	1
e. Blame yourself for mistakes or mishappenings?	5	4	3	2	1
f. Enjoy things as much as ever?	5	4	3	2	1
g. Feel nervous?	5	4	3	2	1
h. Feel that life would be worth living?	5	4	3	2	1
i. Smile and laugh at least once a day?	5	4	3	2	1

The following questions are about your ability to communicate with other people, as well as your ability to understand what you read and what you hear in a conversation.

4. In the past week, how difficult was it to...	**Not difficult at all**	**A little difficult**	**Somewhat difficult**	**Very difficult**	**Extremely difficult**
a. Say the name of someone who was in front of you?	5	4	3	2	1
b. Understand what was being said to you in a conversation?	5	4	3	2	1
c. Reply to questions?	5	4	3	2	1
d. Correctly name objects?	5	4	3	2	1
e. Participate in a conversation with a group of people?	5	4	3	2	1
f. Have a conversation on the telephone?	5	4	3	2	1
g. Call another person on the telephone, including selecting the correct phone number and dialing?	5	4	3	2	1

The following questions ask about activities you might do

during a typical day.

5. In the past 2 weeks, how difficult was it to...	**Not difficult at all**	**A little difficult**	**Somewhat difficult**	**Very difficult**	**Cannot do at all**
a. Cut your food with a knife and fork?	5	4	3	2	1
b. Dress the top part (from the waist up) of your body?	5	4	3	2	1

c. Wash yourself (bath, shower…)?	5	4	3	2	1
d. Clip your toenails?	5	4	3	2	1
e. Get to the toilet quickly?	5	4	3	2	1
f. Control your bladder (not have an accident)?	5	4	3	2	1
g. Control your bowels (not have an accident)?	5	4	3	2	1
h. Do light household tasks/chores?	5	4	3	2	1
i. Go shopping?	5	4	3	2	1
j. Handle money (e.g. count out money)?	5	4	3	2	1
k. Manage finances (e.g. pay monthly bills, manage a bank account)?	5	4	3	2	1
l. Do heavy household tasks/chores?	5	4	3	2	1

The following questions are about your ability to be mobile,

at home and in the community.

6. In the past 2 weeks, how difficult was it to...	**Not difficult at all**	**A little difficult**	**Somewhat difficult**	**Very difficult**	**Cannot do at all**
a. Stay sitting without losing your balance?	5	4	3	2	1
b. Stay standing without losing your balance?	5	4	3	2	1
c. Walk without losing your balance?	5	4	3	2	1
d. Move from a bed to a chair?	5	4	3	2	1

e. Get out of a chair without using your hands for support?	5	4	3	2	1
f. Walk one hundred yards?	5	4	3	2	1
g. Walk fast?	5	4	3	2	1
h. Climb one flight of stairs?	5	4	3	2	1
i. Climb several flights of stairs?	5	4	3	2	1
j. Get in and out of a car?	5	4	3	2	1

The following questions are about your ability to use

your hand that was MOST AFFECTED by your stroke.

---

7. In the past 2 weeks, how difficult was it to use your hand that was most affected by your stroke to...	**Not difficult at all**	**A little difficult**	**Somewhat difficult**	**Very difficult**	**Cannot do at all**
a. Carry heavy objects?	5	4	3	2	1
b. Turn a doorknob?	5	4	3	2	1
c. Open a can or jar?	5	4	3	2	1
d. Tie a shoe lace?	5	4	3	2	1
e. Pick up a small coin?	5	4	3	2	1

The following questions are about how stroke has affected your ability

to participate in the activities that you usually do, things that

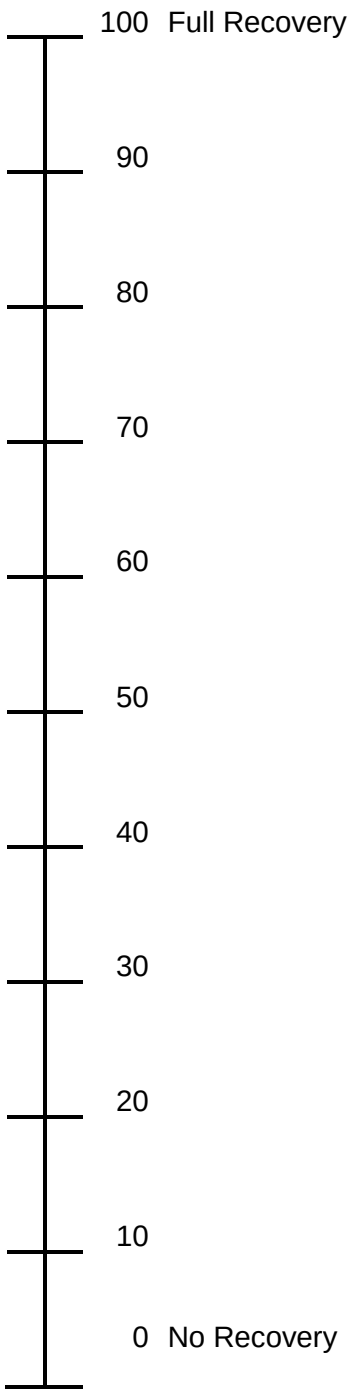
are meaningful to you and help you to find purpose in life.

---

8. During the past 4 weeks, how much of the time have you been limited in...	**None of the time**	**A little of the time**	**Some of the time**	**Most of the time**	**All of the time**
a. Your work (paid, voluntary or other)?	5	4	3	2	1
b. Your social activities?	5	4	3	2	1
c. Quiet recreation?	5	4	3	2	1
d. Active recreation?	5	4	3	2	1
e. Your role as a family member and/or friend?	5	4	3	2	1
f. Your participation in spiritual or religious activities?	5	4	3	2	1
g. Your ability to show your feelings to those close to you?	5	4	3	2	1
h. Your ability to control your life as you wish?	5	4	3	2	1
i. Your ability to help others?	5	4	3	2	1

9. Stroke Recovery

On a scale of 0 to 100, with 100 representing full recovery and 0 representing no recovery, how much have you recovered from your stroke?



Appendix XV: Barthel Index Score

THE BARTHEL INDEX

Date: _____

Activity Score

FEEDING

0 = unable

5 = needs help cutting, spreading butter, etc., or requires modified diet

10 = independent

BATHING

0 = dependent

5 = independent (or in shower)

GROOMING

0 = needs to help with personal care

5 = independent face/hair/teeth/shaving (implements provided)

DRESSING

0 = dependent

5 = needs help but can do about half unaided

10 = independent (including buttons, zips, laces, etc.)

BOWELS

0 = incontinent (or needs to be given enemas)

5 = occasional accident

10 = continent

BLADDER

0 = incontinent, or catheterized and unable to manage alone

5 = occasional accident

10 = continent

TOILET USE

0 = dependent

5 = needs some help, but can do something alone

10 = independent (on and off, dressing, wiping)

TRANSFERS (BED TO CHAIR AND BACK)
0 = unable, no sitting balance
5 = major help (one or two people, physical), can sit
10 = minor help (verbal or physical)
15 = independent _____

MOBILITY (ON LEVEL SURFACES)

0 = immobile or < 50 yards

5 = wheelchair independent, including corners, > 50 yards

10 = walks with help of one person (verbal or physical) > 50 yards

15 = independent (but may use any aid; for example, stick) > 50 yards

STAIRS

0 = unable

5 = needs help (verbal, physical, carrying aid)

10 = independent _____

TOTAL (0–100): _____

Provided by the Internet Stroke Center — www.strokecenter.org

Appendix XVI: Request for Hologic Discovery DXA assessment.

Study name: Body Composition changes after acute stroke and long term outcomes

LREC number: 10/H0304/18

Principal Investigator (requestor): Dr Phyo Myint

Referrer: | **Professional healthcare qualification/registration:**

(by signing referrer hereby confirms that subject meets inclusion criteria and that there is no possibility that female subject could be pregnant)

Subject details:	**Verified by operator (initials)**
Name:	
Study number:	
DoB:	
Address:	
Telephone: **Email:**	
For females, no possibility of pregnancy confirmed	
For all subjects, absence of metal implants etc confirmed	

DXA examination requested (please tick appropriate box):

Whole body	√
Spine	
Hip	
Forearm	

Analyses required (in accordance with LREC):

Bone	
Body composition	√
Segment/region	

Specific/other details

Operator:

(by signing, operator hereby authorizes that the DXA assessment is appropriate)

Appendix XVII: Hologic Discovery dual energy X-ray absorptiometry (DXA) body composition and bone assessments: radiation exposure confirmation of directed dose and appropriate approvals checklist.

(N.B. Radiation directed by the DXA procedure for each scan type is invariable and adherence to scans specified in the Local Research Ethics Committee (LREC) and its approval is mandatory.)

Study details

Study name: Body Composition changes after acute stroke and long term outcomes

Sponsor: Res., Enterprise & Engagement Office for University of East Anglia

Principal investigator and/or local lead: Dr P Myint / Mr M. Kafri

R & D reference number: 2010MFE10S (116-08-10).

LREC number: 10/H0304/18

LREC approval date: 12-10-2010

Confirmations

Medical physics expert: Stuart Yates **Approved signatory** ☐

Clinical radiation expert: Andoni Toms **Approved signatory** ☐

DXA examination indicated on LREC approval:

Whole body ☑

Spine ☐

Hip ☐

Forearm ☐

Radiation exposure appropriate as specified in LREC? ☑

Is the proposed DXA scan appropriate to address the particular research question ☑

Body composition practitioner approval for study to go ahead in Clinical ResearchTrials Unit

Appendix XVIII: Hologic Discovery (Wi) dual-energy X-ray absorptiometry (DEXA) operating procedure for whole body scan

Preliminary DXA set up prior to patient/subject arrival

1 Turn on DXA
2 Run QC
3 Run radiographic uniformity, if indicated

Patient preparation

1 Confirm that identity of patient/subject matches study identifiers and scan(s) required.
2 Obtain and record patient/subject details, address hospital number etc.
3 Patient/subject to undress and put on gown.
4 Obtain and record patient/subject weight and height or ensure that the nurse has measured these on the day.
5 Use checklists to ensure patient/subject suitability and safety

- Pregnancy for females
- Metal objects for all subjects

Whole body DXA scan procedure

1 Click 'Patients' in the main window
2 Click patient's name or, if it their first scan, click 'New patient'.
3 Edit or create a patient record according to 'Patient records' as set out in the Discovery Operator's Manual.
4 Use checklists to confirm patient/subject suitability and safety

- Pregnancy for females
- Metal objects for all subjects

5 Confirm that the subject is below the weight limit of 204 kg.
6 Click 'Perform scan' and check all details
7 Select 'Scan type'
8 Select 'Whole body'
9 Position patient (top of head located at end of midline marker on table, arms at side and toes pointed inwards as in Discovery Operator's Manual)
10 Start scan – runs for about 7 mins.
11 Help patient from table and allow to dress.

Warning: if control panel X-ray indicator fails to shut off within 10 secs of the end of the scan then press the *red emergency stop* button immediately. Call service engineer (Vertec Ltd) before resuming operation.

12 Analyze scan as described in Discovery Operator's Manual.
13 Generate reports and *record patient exposure*
14 Fully complete patient and scan record in log book.

Appendix XIX: Patient safety and DXA operational compliance: checklist questions

For females: ask if there is there any chance that they might be *pregnant*? *If so, postpone scan*.

For all subjects/patients:

Ask if the patient/subject has had any medical procedure within the last 7 days involving:

Contrast media?

Arterial?

Iodine?

Barium?

A nuclear medicine isotope study? If so, contact relevant practitioner/department to establish whether or not the DXA can/should be performed.

Ask if the patient/subject is wearing any metal device or metal objects?

Buttons, zips, belts etc?

Jewellery?

An ostomy device?

Phones, money, in pockets etc?

If so, remove them if at all possible.

Ask if the patient has had any surgery that means they have metal somehow associated with their body?

Pacemaker leads?

Radioactive seeds?

Metal implants?

Hip replacements?

Surgical staples?

Foreign bodies, e.g. shrapnel?

Radio-opaque catheters or tubes?

Bullets?

If so, it is not an issue for the patient but it is necessary to assess the extent that it might interfere with the scan.

Appendix XX: Consent form for adults and children over 16 years of age

Dual energy X-ray absorptiometry (DXA) measurement for project entitled:

Please initial box

1. I confirm that I have read and understood the information sheet dated …./…./20…. for the above study and have been given the opportunity to ask questions. ☐
2. I confirm that I understand that the study involves the direction of a low level of X-ray radiation, exposing me to a level of radiation which is equivalent to about 1 day of environmental or background exposure. ☐
3. I understand that my participation in the DXA measurement part of this study is voluntary and that I am free to withdraw at any time and without giving a reason and without my medical care or legal rights being affected. ☐
4. I agree to my physician being notified of my participation and also being given any findings that may require further investigation. ☐
5. I agree to take part in the DXA measurement as part of the study entitled ' Body Composition changes after acute stroke and long term outcomes '. ☐

_____	_____	_____
Name of Participant:	Date:	Signature:
Name of Researcher:	Date:	Signature:

Researcher:

Supervisor:

- Copy to participant
- Copy to researcher

Glossary of Body Composition terms

B

Body cell mass (BCM): mass of all the metabolically active cells in the body which constitute of muscles cell mass and organs cell mass (278)

E

Extracellular water (ECW): water volume in extracellular space only

F

Fat Free Mass: total mass of skeletal muscles, bones, body organs, and total body water

Fat mass: mass of adipose tissue only

I

Intracellular water: total water available in intracellular space

M

Muscle mass: skeletal muscle mass only

P

Protein mass: total protein mass available in bones, skeletal muscles, and body organs

T

Total Body Water: the sum of extracellular and intracellular water volume

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