The associations of stroke risk and risk factors with dietary intakes and biomarkers of magnesium and protein

by, Lucy Kate Matheson Bain

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Faculty of Medicine and Health Sciences,
University of East Anglia

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Abstract

Background: Stroke is a leading cause of morbidity and mortality, the majority of stroke cases are first events, and therefore primary prevention is essential. Stroke risk and major risk factors, blood pressure (BP) and lipid profile, may be modified by diet. The associations of dietary magnesium and protein intake on these outcomes has been understudied in parallel in a single population and the relationship between diet, nutritional biomarkers and BP is lacking in older populations.

Aim: To determine associations between dietary magnesium and protein intakes and biomarkers with BP, lipid profile and stroke risk.

Methods: This cross-sectional study included 4,443 men and women, a representative sub-sample of the EPIC-Norfolk cohort, aged 39-80 and 234 older men and women, aged 65-79, from the NU-AGE study. Multiple regression analysis and cox-proportional hazards model were used to assess associations between dietary magnesium and protein intakes, with BP, lipids and stroke risk. Dietary and biomarkers of magnesium and protein were assessed in relation to BP in the NU-AGE cohort.

Results: BP was significantly inversely associated with dietary magnesium in men, total and animal protein in women and plant protein (diastolic BP only) for both sexes. Inconsistent relationships were identified between lipid levels and dietary magnesium and protein intakes with discrepancies between sexes. Men with the lowest 10% of magnesium intake were at highest risk of stroke. There was no significant relationship with other dietary intakes and stroke risk. In the NU-AGE study serum magnesium was not correlated with BP in men or women, and urinary urea nitrogen was inversely correlated with diastolic BP in men only.

Conclusions: Dietary intakes of magnesium and protein have the potential to modify stroke risk. A diet higher in plant foods such as wholegrains, green vegetables, nuts, and legumes and quality animal protein may be beneficial in preventing stroke.

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List of abbreviations

%en – percentage of energy
7DD – 7 day diet diary
ABPM – ambulatory blood pressure monitor
AF – atrial fibrillation
ATP – adenosine triphosphate
BMI – body mass index
BP – blood pressure
Ca – calcium
CADSIL - cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CHD – coronary heart disease
CHO – carbohydrate
CI – confidence interval
CRTU – Clinical Research Trials Unit
CsA – cyclosporin A
CVD – cardiovascular disease
DASH – Dietary Approaches to Stop Hypertension
DBP – diastolic blood pressure
DINER – Data Into Nutrients for Epidemiological Research
DM – diabetes mellitus
DRV – dietary reference value
EER – Expected Energy Expenditure
EPIC-Norfolk – European Prospective Investigation into Cancer-Norfolk
FFQ – food frequency questionnaire
GL – glycaemic load
HDL – high density lipoprotein
HLQ – Health and Lifestyle Questionnaire
HR – hazard ratio
HRT – hormone replacement therapy
HS – haemorrhagic stroke
IHD – ischaemic heart disease
IS – ischaemic stroke
IV – intravenous
K – potassium
LDL – low density lipoprotein
MDA – malondialdehyde
Mg – magnesium
Mg Ap-HCL – magnesium-aspartate HCl
MI – myocardial infarction
MP – milk protein
MP+AA – milk protein and amino acids
MUFA – monounsaturated fatty acids
Na – sodium
NC – normal chow
NDNS – National Diet and Nutrition Survey
NEFA – non-esterified fatty acids
NHANES – National Health and Nutrition Examination Survey
NHS – National Health Service
NNUH – Norfolk and Norwich University Hospital
NO – nitric oxide
NS – non-significant
NU-AGE – New dietary strategies addressing the specific needs of the elderly population for healthy ageing in Europe
PA – physical activity
PUFA – polyunsaturated fatty acids
RCTs – randomised controlled trials
RNI – reference nutrient intake
RP – rapeseed protein isolate
RR – relative risk
SBP – systolic blood pressure
SFA – saturated fatty acids
SHRs – spontaneously hypertensive rat species
SPH – soyabean protein hydrolysate
SPI – soy protein isolate
SPSHRs – stroke prone spontaneously hypertensive rat species
sr – self-reported
Supp – supplement
TBARS – thiobarbituric acid reactive substances
TC – total cholesterol
TG – triglyceride
tPA – tissue plasminogen activator
Vit C – vitamin C
Vit E – vitamin E
VLDL – very low density lipoprotein
WBC – white blood cell
WHO – World Health Organisation
WHR – waist-hip-ratio
Zn – zinc

Publications and presentations

Publications

Bain, L. K. M., Myint, P. K., Jennings, A., Lentjes, M. A. H., Luben, R., Khaw, KT., Wareham, N. J. and Welch, A. A. The relationship between dietary magnesium intake, stroke and its major risk factors, blood pressure and cholesterol, in the EPIC-Norfolk cohort. *International Journal of Cardiology* In Press http://dx.doi.org/10.1016/j.ijcard.2015.05.166.

Bain, L. K. M., Myint, P. K., Jennings, A., Lentjes, M. A. H., Mulligan, A. A., Luben, R., Khaw, KT. and Welch, A. A. Contributions to dietary protein intake in a British adult population. *Proceedings of the Nutrition Society* 2015; 74 (OCE2), E179.

Bain, L. K. M., Myint, P. K., Jennings, A., Lentjes, M. A. H., Cassidy, A., Luben, R., Khaw, KT. and Welch, A. A. Dietary magnesium intake and blood pressure in an adult British population. *Proceedings of the Nutrition Society* 2013; 72 (OCE4), E237.

Manuscripts in preparation

A manuscript based on the findings of Chapter Four – Dietary protein intake and contribution of food sources is in preparation.

A second manuscript based on the findings of Chapter Five – The influence of dietary protein intake on stroke risk and risk factors is also in preparation.

Oral presentations

Bain, L. K. M., Myint, P. K., Jennings, A., Lentjes, M. A. H., Mulligan, A. A., Luben, R., Khaw, KT. and Welch, A. A. Contributions to dietary protein intake in a British adult population. *Nutrition Society Winter Meeting 2014, Nutrition and age-related muscle loss, sarcopenia and cachexia,* Royal Society of Medicine, London.

Bain, L. K. M., Myint, P. K., Jennings, A., Lentjes, M. A. H., Mulligan, A. A., Luben, R., Khaw, KT. and Welch, A. A. Relationship between protein as a percentage of energy and stroke risk factors in the EPIC-Norfolk cohort. *Postgraduate Research Student Conference* 2014, University of East Anglia, Norwich.

Bain, L. K. M., Myint, P. K., Cassidy, A., Jennings, A., Khaw, KT. and Welch, A. A. Can eating your 5-a-day keep stroke at bay? *Postgraduate Research Student Showcase* 2012, University of East Anglia, Norwich.

Poster presentations

Bain, L. K. M., Myint, P. K., Jennings, A., Lentjes, M. A. H., Cassidy, A., Luben, R., Khaw, KT. and Welch, A. A. Dietary magnesium intake and blood pressure in an adult British population. *Nutrition Society Summer Meeting 2013, Nutrition and healthy ageing,* Newcastle University, Newcastle upon Tyne.

Bain, L. K. M., Myint, P. K., Cassidy, A., Jennings, A., Khaw, KT. and Welch, A. A. Dietary magnesium intake and blood pressure. *Postgraduate Research Student Conference* 2013, University of East Anglia, Norwich.

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xx

This thesis is dedicated to my Grandmother, Cynthia Corbett (1931-2015), who suffered a stroke in 2004. She was my inspiration for studying the prevention of stroke and I hope this thesis can add to the field and reduce the risk of this preventable disease.

Chapter One

INTRODUCTION

1.0 General overview

Stroke is the second leading cause of death worldwide, and is accountable for 10% of all-cause mortality (2, 3). Stroke is defined by the World Health Organisation (WHO) as; neurological impairment of sudden onset, either focal or global, where symptoms persist for more than 24 hours or result in death within 24 hours of onset and are of presumed vascular origin. The recent trends in stroke incidence have been a decrease in incidence in high income countries and a significant increase in incidence in middle and low income countries (4). However, with the increasingly ageing population, and the higher incidence of stroke with advancing age, it will continue to be a condition that requires investigation and prevention.

The majority of strokes, globally, are ischaemic, accounting for approximately 80% of all cases, whereby there is an occlusion of the blood supply to the brain primarily as a consequence of a blood clot (5). A further 15% of cerebrovascular events are classified as primary intracerebral haemorrhages which occur when an artery within the brain ruptures, the remaining 5% are subarachnoid haemorrhages and result when an artery on the surface of the brain ruptures (5).

The risk of having a cerebrovascular event can be influenced by a number of factors some of which cannot be modified, including age, sex, genetics, and ethnicity (6). However, the majority of risk factors for stroke are modifiable, including; high blood pressure, hypercholesterolemia, atrial fibrillation, type II diabetes, physical inactivity and smoking which combined account for over 65% of first adverse events (7, 8). Some of these risk factors can be strongly influenced by dietary intakes of foods and nutrients, which is discussed in more detail in section 1.3.2 of this Chapter.

Therefore, it may be possible to reduce the risk of stroke occurrence by prevention through lifestyle modification. Previous research has indicated that dietary intake, including

increased consumption of fruit and vegetables and other plant based foods, may have a major influence on stroke incidence or its risk factors, including blood pressure and abnormal lipid profile, with low fruit and vegetable consumption attributing to approximately 11% of strokes worldwide (9-13).

Hypertension, defined as systolic blood pressure (SBP) >140 mmHg and diastolic blood pressure (DBP) >90 mmHg (14), is one of the most important and modifiable risk factors of stroke. It may have a contributory role in up to 70% of all strokes in the UK (6, 15). A number of mechanisms may be involved in the role of hypertension on stroke incidence. These include endothelial dysfunction, increased permeability of the blood-brain barrier and an increase in atherosclerotic plaque formation (16, 17). Likewise, abnormal lipid profile, another major potentially modifiable risk factor for stroke, can influence stroke risk via a number of possible mechanisms, which are yet to be fully elucidated. However it appears that increased risk may in part be due to atherogenic effects and clotting abnormalities (18). These mechanisms of action will be discussed in more detail later in this chapter.

This chapter provides a narrative review of relevant literature related to stroke risk and risk factors for stroke based on keyword searches using MEDLINE (Ovid) and PubMed. Additional relevant articles were identified from article reference lists.

Research has indicated that dietary habits can have an effect on blood pressure and serum lipid levels, for example substantial evidence supports a strong association between increased sodium intake and increased blood pressure and hypertension (19, 20). However, to date, research into the association between intakes of other dietary components including the impact of plant based foods, any food derived from plant material including fruit, vegetables, grains and their products such as bread, pasta, and their influence on blood pressure has provided less consistent findings (20-23).

With regard to diet and serum lipid levels, the primary focus of research has been on dietary fatty acid intakes which influence lipid levels both positively and negatively depending on

the type of fat consumed (24-26). The consumption of polyunsaturated fatty acids (PUFA), omega-3 fats and plant-derived monounsaturated fatty acids (MUFA) affect serum lipid levels favourably, leading to increases in high density lipoprotein (HDL) and decreases in low density lipoprotein (LDL) (24). Whilst diets high in saturated fats may negatively influence lipid profile; including higher LDL and lower HDL levels.

Most evidence surrounding plant based nutrients and cholesterol levels is related to the consumption of flavonoid rich foods including nuts, cocoa, wine, soy products and fruit beverages, with mixed findings (27-30). There is some evidence to indicate a beneficial effect on serum LDL and total cholesterol levels from soy protein isolates and green tea, but no effect was illustrated on HDL levels (31-33). A number of studies have reported an increase in circulating HDL following the consumption of flavonoid rich fruit beverages including orange, cranberry and grape (13, 34-36).

It is also not known, whether dietary intakes or biological markers of intakes are more highly correlated with differences in blood pressure, and thus may be more accurate predictors of change, particularly in older populations.

Previous research has utilised a number of different methods of dietary assessment. These methods are described in more detail in the following section and their respective strengths and limitations are discussed.

Bias and measurement error in dietary assessment methods

The self-reported measurement of dietary intake is inherently prone to bias (37, 38). Bias can be introduced at any point of the study including during recruitment, data collection, analysis and interpretation but is broadly split into selection and information bias (38). Selection bias is related to the recruitment process and can occur if the recruited population has a different relationship with the exposure of interest than the general population they are sampled from. Information bias, which includes recall bias, is the result

of either random or systematic differences in how exposure and outcome are reported in the study sample. Bias in a study can ultimately result in drawing inaccurate conclusions.

Self-reported measures include FFQs, 24hr recall and dietary records, with FFQ being the most commonly used due to its low relative cost, ease of administration and data entry (37). All the methods aim to determine the usual habitual diet of the individuals and study population. Each method is prone to its own biases and mis-reporting. For example FFQs and 24hr recalls rely on memory; particularly so for FFQs which may be aiming to establish approximate consumption of food items over long periods of time. Dietary records aim to avoid this problem by being completed by the participant at the time, or soon after, eating. However, they are more burdensome for the volunteer and require a greater input during data entry by the research team. Another common difficulty for all methods is in reporting portion size accurately. FFQs and 24hr recalls are often based on standard portion sizes, images may be used to help participants to select the most appropriate portion but differences from actual intake will occur. Weighed food records may most precisely report portion size, however, it is not always possible for participants to weigh all food items, such as when eating away from the home. And as such, discrepancies in portion size consumed may also occur (37, 38).

Measurement error can be systematic or random. Systematic error includes person-specific, constant additive and intake-related bias, these types of systematic error rarely occur in isolation (37). Person-specific bias is related to particular characteristics of the individual which lead to over or under-reporting such as age, sex, or weight. An average of observed intake in this case would not give representation of true intake (as is the case with within-person error in random error). Constant additive error is consistent for all participants, but still leads to over or under estimation of intake (compared with true intake). Intake related bias on the other hand differs between individuals. For example there may be greater under-reporting in those with true higher intakes compared with individuals with true lower intakes. Random error includes within person error, which

includes day to day variability in intake or reporting of intake in relation to true intake. With sufficient repeats the average of intake can be representative of true intake, minus bias (37).

FFQs are mostly influenced by systematic error, which is not reduced or influenced by repeats and averaging. Whereas 24hr recalls and food diaries are mostly affected by random error, which can be reduced with repeated measurements, although they may still be additionally affected by systematic error (39).

There is not yet a method which reports true intake 100% accurately. However, gold standards, recovery biomarkers, are available for some nutrients, including total energy, protein intake and micronutrients such as sodium and potassium, which can be used to validate observed intakes from self-reported measurements. Recovery biomarkers are objective measurements which are directly related to nutrient intake in the general population. Validation studies, utilising a reference measurement such as a biomarker of intake, can be used to determine how well the dietary assessment method, observed intake, estimates true intake (37).

1.1 Stroke incidence and prevalence

1.1.1 Global data and burden

Cerebrovascular disease accounts for 8.6% and 11.0% of worldwide deaths for men and women respectively, equating to more than 5.5 million deaths annually (40). It is estimated that by 2020 global stroke burden will account for more than 61 million disability-adjusted life years (DALYs) (2). This in part is due to the increase in the proportion of the population aged over 45 years as the incidence of stroke increases significantly with age, and doubles each decade after 45 years (41-44).

In the developed world the incidence of stroke is declining although, with the ageing population the crude number of strokes is increasing (2). In contrast in many developing countries the incidence of stroke is rapidly increasing as depicted in **Figure 1.0** which illustrates worldwide mortality for both sexes from cerebrovascular disease. It is estimated that by 2030 stroke mortality in the developing world including Latin America, the Middle East and sub-Saharan Africa will have tripled (45).

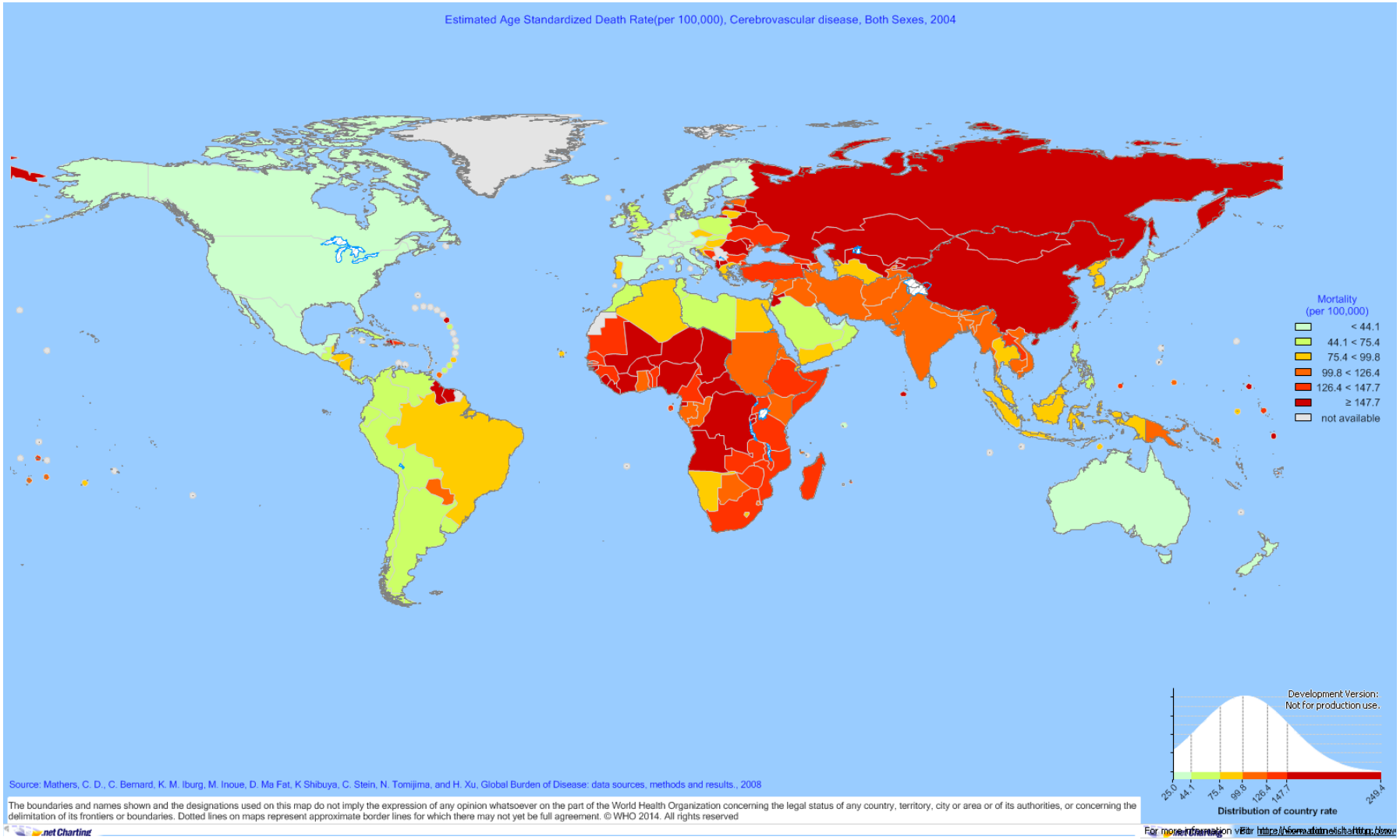


Figure 1.0 Global cerebrovascular mortality for men and women from 2004 (1)

1.1.2 Western population data

In Europe, stroke is the second most common cause of death, accounting for 1.28 million deaths per year, which is approximately 11% and 18% of all deaths in men and women respectively (46). The incidence and mortality rate from stroke is greater in Central and Eastern parts of Europe compared with Northern, Southern and Western areas as depicted in **Figures 1.1 and 1.2** for men and women respectively (47, 48). This largely correlates with the age of populations in the majority of European countries which is increasing and has been reflected in a 45% rise in stroke related hospital discharges since 1990 (47). The majority of strokes in Western populations, including Europe, are ischaemic, accounting for 85% of all strokes (8).

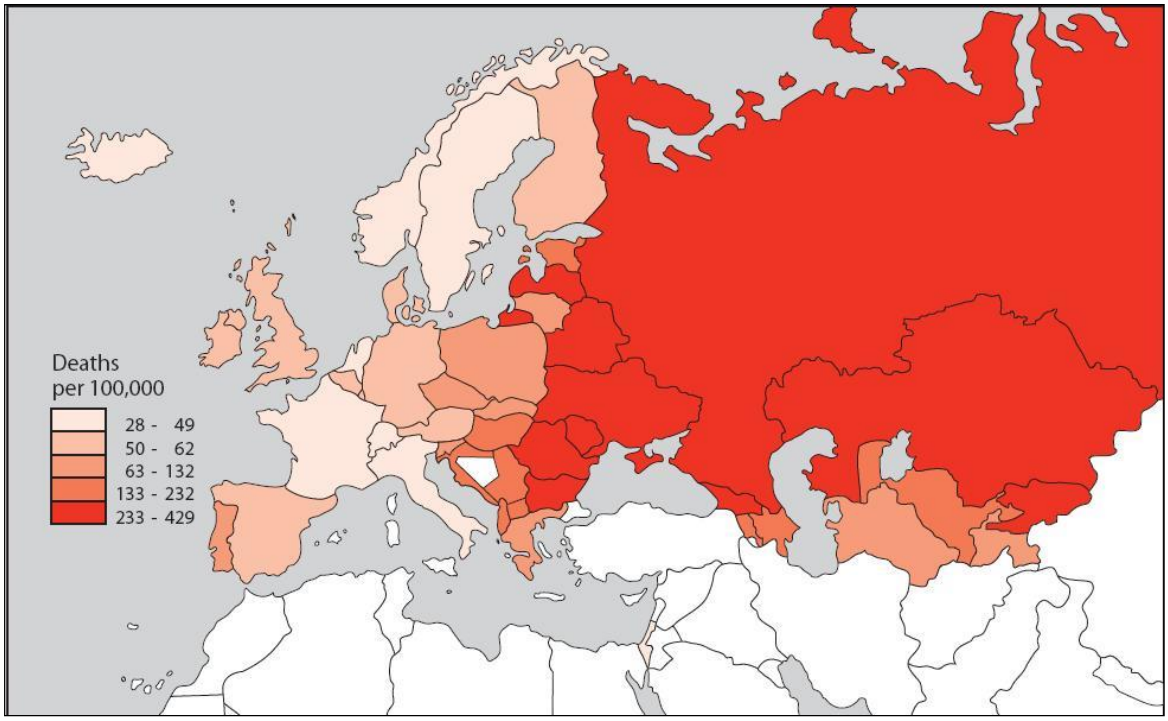


Figure 1.1 Age-standardised stroke mortality, men aged 35-74, in 2001 (46)

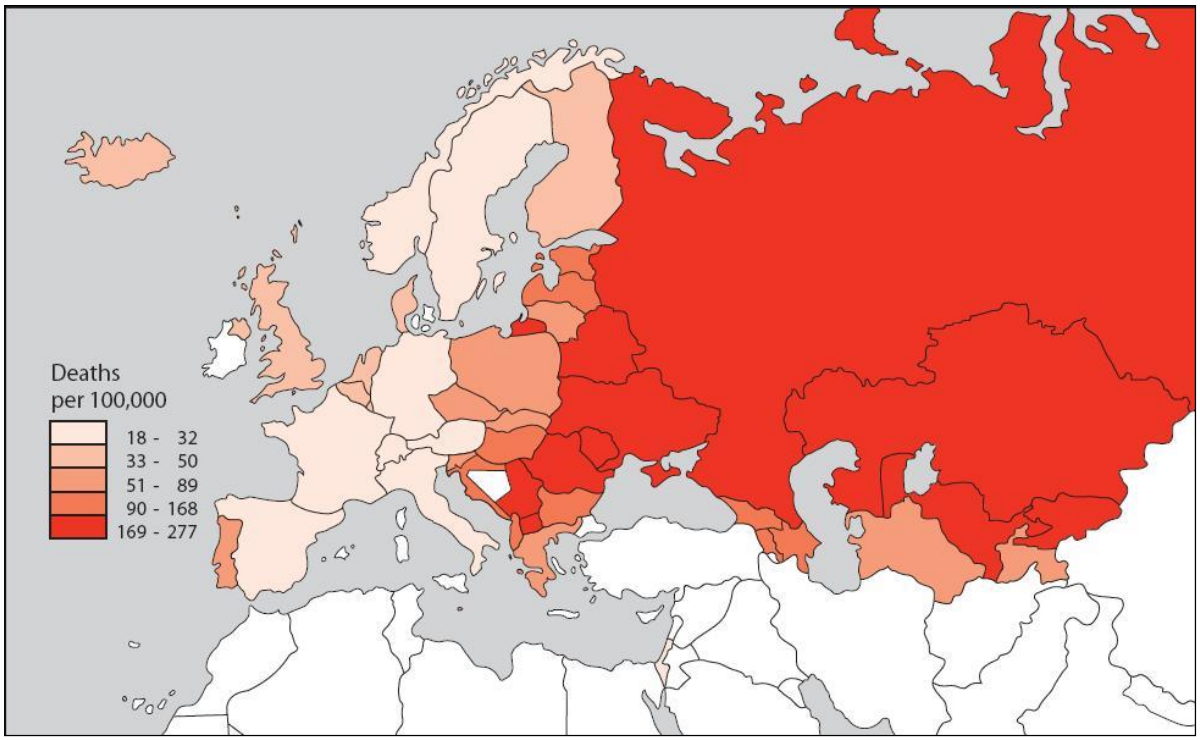


Figure 1.2 Age-standardised stroke mortality, women aged 35-74, in 2001 (46)

1.1.3 UK data

Stroke is one of the three leading causes of mortality in the UK, accounting for approximately 9% of all deaths, and it is the primary cause of adult disability in the UK (8, 47). Each year over 110,000 individuals experience a first stroke and a further 30,000 have a subsequent event (49). With an increasingly ageing population profile (**Figure 1.3**) the number of people experiencing a first stroke each year will be greater, raising the burden on the NHS. At present, stroke is estimated to cost the NHS a total of £8.9 billion per annum (p.a) (50). This is inclusive of diagnosis, inpatient and outpatient treatment, informal care and loss of productivity (50).

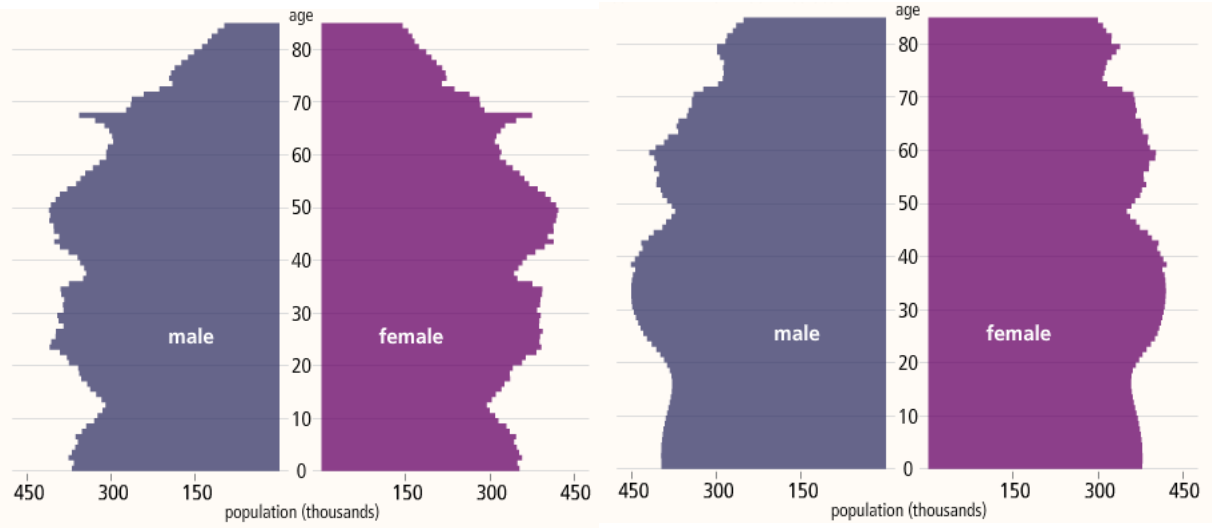


Figure 1.3 Age structure of England and Wales, 2014 (population 57.3 million) and 2050 (population 69.0 million) (51)

1.2 Stroke background

1.2.1 Classification of stroke

Stroke is a neurological impairment of sudden onset, either focal or global, where symptoms persist for more than 24 hours or result in death within 24 hours of onset and are of presumed vascular origin. Strokes are broadly classified as either ischaemic or haemorrhagic in origin. Ischaemic strokes result from the occlusion of an artery supplying part of the brain. This is the more common type of stroke accounting for 80-85% of all stroke cases. Ischaemic strokes can be either thrombotic, where a blood clot forms in situ in an artery supplying the brain, or embolic which is the result of a more mobile clot that has formed elsewhere in the body, often originating from cardiac or carotid arteries, and travels through the bloodstream and causes a blockage in a medium or small sized vessel.

Haemorrhagic strokes, caused when an artery leading to or on the surface of the brain ruptures, account for a smaller proportion of stroke incidences. They can be further subdivided into intracerebral haemorrhage when a vessel within the brain ruptures and subarachnoid haemorrhage which leads to bleeding on the surface of the brain. Intracerebral haemorrhage leads to the release of blood into the brain, thus increasing the pressure which damages surrounding cells in addition to damage resulting from subsequent lack of oxygenated blood supply. Following subarachnoid haemorrhage blood accumulates between the skull and brain (the subarachnoid space) which damages surrounding cells. In addition leaked blood may also combine with cerebrospinal fluid, this can cause reduced circulation of cerebrospinal fluid which leads to further fluid build-up and additional increase in pressure.

Transient ischaemic attack, is a short lived ischaemic event with symptoms typically lasting only a few minutes. In lay terms it is often referred to as a mini-stroke and frequently precludes a ‘full’ stroke. The present thesis does not cover this type of cerebrovascular event.

1.2.2 Stroke aetiology

Atherosclerosis

Atherosclerosis is the formation of plaques in arteries, which are comprised of a number of materials including fatty deposits, cholesterol and calcium ions (52). It is often referred to as hardening or furring of the arteries.

Atherosclerosis begins with damage to the endothelium. A number of factors can lead to endothelium damage including mechanical force, bacterial and viral infections, hypertension, raised cholesterol, smoking, raised glucose and homocysteine levels (53). This initial damage then allows LDL to infiltrate the subendothelium where it is oxidised by macrophages (54). This stimulates the release of adhesion molecules, which attract additional monocytes and T lymphocytes to the site by acting as receptors. Monocytes migrate into the intima and transform to macrophages. Macrophages then ingest the previously oxidised LDL and develop into foam cells (54). On death the macrophages release stored lipid which then forms the basis of the lipid-core. T lymphocytes and foam cells form a fatty streak which is the beginnings of an atherosclerotic plaque (54). The plaque grows due to smooth muscle cell proliferation in addition to the localised accumulation of macrophages and foam cells. The plaque can be stabilised by the formation of a thick fibrous cap, however, if this cap degrades or is weakened the result is rupture and release of the tissue factor lipid core. When this comes into contact with blood, the clotting process begins (54).

Atherosclerotic plaques can be a causal factor in strokes in one of two ways; they may lead to the occlusion of an artery leading to or within the brain or a plaque in a larger vessel in the body may rupture initiating the formation of blood clot which may then break off and travel to the brain and occlude the micro vessels (52). Early stages of atherosclerotic plaque formation can also influence stroke risk factors including blood pressure. As the build-up of plaque progresses the arteries narrow, this leads to an increase in pressure due in part to reduction in size but also in the reduced flexibility of the artery walls (52).

Thrombus and embolus

A thrombus forms locally within a vessel, the formation of a thrombus can be initiated at the site of a ruptured atherosclerotic plaque or at any point where there is damage to the endothelium. An embolus is a mobile clot, and is usually a fraction of a thrombus (52).

Cerebral aneurysm

Cerebral aneurysms, restricted to vessels in the brain, usually form at branching points in arteries and result from pressure of blood flow. Over time the artery walls are weakened allowing for a 'ballooning' effect. A large aneurysm can exert pressure on surrounding brain or nervous tissues which may present with symptoms. However, most commonly symptoms are only present after an aneurysm has ruptured.

1.2.3 Stroke treatment

Treatment for each type of stroke differs as does the medical outcome, with a higher proportion of haemorrhagic strokes being fatal.

Acute therapy

It is important to identify and diagnose a stroke rapidly as delay in treatment leads to less favourable outcomes. The most widely used treatment for ischaemic strokes is thrombolysis using Alteplase, which is a tissue plasminogen activator (tPA). tPA catalayses the reaction leading to the conversion of plasminogen to plasmin. This then initiates fibrinolysis as the plasmin degrades fibrin which breaks down the clot. These fragments can be removed from the bloodstream by proteases or during filtration in the liver and kidneys (55). The aim is to disperse the blood clot, allowing restoration of normal blood flow (56). This treatment is only recommended to be used where the onset of symptoms was less than 4.5 hours ago (56). In addition anti-coagulants and/or aspirin may be given to reduce the likelihood of subsequent clots forming depending on the aetiopathology.

Intracerebral haemorrhage may be treated with both medical and surgical interventions. In instances where the intracerebral haemorrhage is caused by hypertension, the most common cause in up to 60% of all cases, then medication to normalise blood pressure is given. After which action may be taken to reduce pressure including the use of surgery. Surgery may also be employed in the treatment of ischaemic strokes. Subarachnoid

haemorrhage, which are frequently caused by an aneurysm, often require emergency surgical procedures, in order to repair the damaged vessel and to drain the excess fluid which can otherwise cause cranial pressure (57).

1.3 Risk factors

1.3.1 Non-modifiable

Age

Although a stroke can occur at any age, three quarters of all strokes occur in individuals aged over 65 years, making age the most important risk factor for stroke (43). In addition the incidence of stroke has been reported to double with each advancing decade after 45 years (58). The effect of age can be attributed to the accumulative impact of ageing on the cardiovascular system, such as the formation of atherosclerotic plaques and changes to vascular structure, in addition to gradual rise in blood pressure (59, 60).

Sex

Despite the incidence of stroke being greater in men, albeit to a lesser extent than for cardiovascular disease, the overall prevalence of stroke is greater in women. This is in part due to their increased longevity and the associated exponential increase in stroke risk with age. In women the onset of menopause has been associated with increased risk of stroke, in part due to the effect of changes in hormonal balance on stroke risk factors, such as acceleration of atherosclerosis (61). In addition hormone replacement therapy (HRT) use has been indicated to increase the risk of stroke by up to 30%, however there is limited evidence as to whether risk is dose dependent or related to specific routes of administration (transdermal vs oral) (61-63).

Genetics

There is evidence to suggest that genetic predisposition may play a contributory role to an individual's risk of stroke. A family history of stroke may increase risk by up to 75% (64).

To date there is no one single gene associated with stroke risk. However, the genetic role in stroke is in relation to risk factors such as hereditary predisposition to high blood pressure (BP), diabetes and vascular abnormalities such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADSIL). CADSIL is a result of genetic mutation of the *Notch 3* gene and causes migraine-like headaches, strokes and dementia (64).

1.3.2 Modifiable

Blood pressure and stroke

High blood pressure is the single most modifiable risk factor for stroke. There is an approximate fourfold increase in stroke risk in hypertensive individuals (SBP >140 and DBP >90 mmHg) compared with the normotensive population (65). It is acknowledged that even those with prehypertension, defined as SBP 120-139 mmHg and DBP 80-89 mmHg, are at increased risk of stroke (16, 66, 67). It has been identified that up to 40% of individuals fall into the prehypertensive category depending on sex, age and ethnicity (66, 68, 69). Often individuals in the prehypertensive stage progress to being hypertensive within 4 years, this is particularly the case for individuals aged over 65 (68). Therefore reducing their blood pressure at this earlier prehypertensive stage may potentially decrease stroke risk. Evidence suggests that even a modest reduction of 1-3 mmHg in systolic blood pressure can lead to a 20-30% reduction in stroke risk and a 4 mmHg reduction in diastolic blood pressures equates to a 23% reduction (16, 70, 71). Hypertension increases the risk of stroke incidence via a number of mechanisms including, the promotion of atherosclerotic plaque formation. Over time this can lead to occlusion of blood vessels restricting blood flow (16). It also affects smooth muscle cells by inducing hypertrophy leading to narrowing of the arteries.

There is a large body of research to support the benefits of lowering salt intake on blood pressure reduction (19, 20, 72). Changes similar to this with other nutrients remain more controversial and therefore further research to elucidate the potential beneficial role between increased consumption of plant-based dietary components and a reduction in blood pressure is required.

Abnormal lipid profile and stroke

The relationship between total cholesterol and stroke risk is inconsistent, despite a significant amount of research being conducted in the field, the findings remain contradictory (6, 73-78). This may in part be due to total cholesterol (TC) being formed primarily from HDL cholesterol and LDL cholesterol which have opposing effects on stroke risk. Increased HDL concentration may infer a protective effect against stroke whilst the

converse is true of LDL (74, 77, 79). There is additional evidence to support different effects of serum cholesterol on stroke subtypes. Lower serum TC has been associated with an increased risk of haemorrhagic stroke incidence, whilst concentrations above 7.23 mmol/L have been associated with an increased risk of ischaemic stroke incidence (80).

Where evidence is in support of increased total stroke incidence associated with higher TC concentration the association is weak or non-significant, and is more commonly related to younger populations aged ≤ 45 years (80-83).

Diabetes and stroke

Both type I and type II diabetes mellitus (DM) have been associated with an increased risk of stroke incidence, both total stroke and subtypes, and less favourable outcomes (78, 84-87). A greater risk of incidence has been identified in those with insulin dependent DM and also in association with duration of disease. Therefore those diagnosed at younger ages have greater risk than individuals diagnosed later in life (85, 88).

The increased risk of cerebrovascular events associated with DM may in part be due to co-morbidities that also frequently occur in conjunction with DM including dyslipidaemia, and hypertension. Individuals with DM also commonly present with increased carotid intima-media thickness, a significant independent predictor of stroke risk (86, 89) and enhanced incidence of atherosclerotic plaque formation (90).

Hyperglycaemia associated with DM has been linked to endothelial dysfunction including inhibition of nitric oxide (NO) production which otherwise has a vasodilatory effect (90). In conjunction with this there is upregulation of vasodilators including endothelin-1 which is required for the activation of endothelin-A receptors located on vascular smooth muscle cell (90).

Atrial fibrillation

Atrial fibrillation (AF) is predominately associated with increases in ischaemic stroke risk, with approximately 1 in 6 stroke patients having AF (91). There is a five-fold increase in risk of stroke in patients with AF compared with the general population (92). The increase in risk may also be sex specific with women who had untreated AF having a 40-70% greater risk of having a stroke compared with males with AF (93). As with DM, AF is also associated

with more severe events and less favourable outcomes (91). The associated increase in risk with atrial fibrillation may in part be due to the increased likelihood of individuals also having further cardiovascular diseases including hypertension, coronary heart disease (CHD) and prior myocardial infarction (MI) (92).

Smoking

Cigarette smoking is one of the main modifiable risk factors for stroke along with high blood pressure. When compared with non-smokers, current smokers have twice the risk of stroke (94, 95).

Former smoking remains a risk factor for stroke. Both the Nurses' Health Study and Physicians Health Study indicated an increased risk associated with former smoking. However, this risk may return to baseline levels after 5 continuous years of cessation (96, 97). Smoking may also indirectly increase the risk of stroke by influencing stroke risk factors including blood pressure. Long term smoking is linked to increased risk of developing hypertension (98, 99). Other mechanisms by which smoking can increase stroke risk include acceleration of atherosclerotic plaque formation as illustrated in animal models. There is also evidence that smoking leads to increased vascular stiffness, and has procoagulant affects including enhanced platelet aggregation and increased fibrinogen concentrations (58, 100, 101)

Diet and stroke

Dietary habits and intakes can have a significant impact on general health and wellbeing. Inappropriate consumption of a range of foods and nutrients has been associated with increased risk of developing a number of non-communicable diseases such as CVD and stroke. **Table 1.0** adapted from Lim et al (102) illustrates the influence of certain dietary habits on global deaths. Additionally within the top ten risk factors attributing to burden of disease in Western Europe specifically were diets low in fruit, nuts and seeds, high sodium intakes and alcohol use (102).

Table 1.0 Global all-cause mortality (in millions) attributable to dietary risk factors (2010)

Dietary risk factor	Men	Women
Low fruit intake	2.84	2.06
Low vegetable intake	1.02	0.78
Low wholegrain intake	0.96	0.76
Low nuts and seeds intake	1.39	1.08
Low fibre	0.44	0.30
Low calcium	0.08	0.05
Low omega-3 fatty acids	0.79	0.60
Low PUFA	0.31	0.22
High red meat	0.02	0.02
High processed meat	0.47	0.37
High sugar-sweetened beverages	0.16	0.14
High trans fatty acids	0.29	0.22
High sodium	1.73	1.37

Table adapted from Lim et al (102).

In relation to stroke risk specifically, a number of studies have identified an inverse association between increased fruit and vegetable consumption and stroke risk in both male and female populations (103-107). It has been suggested that this is likely to be due to the high levels of beneficial constituents such as fibre, flavonoids, micronutrients and other phytochemicals that are found in plant based foods. These constituents exhibit a range of beneficial effects including increased oxygen scavenging – reducing oxidative stress, reduction and/or prevention of endothelial dysfunction, and reduction in blood pressure and improved lipoprotein profile (104, 108, 109). It may also be that increased consumption of fruit and vegetables displaces the consumption other foods such as those high in saturated fat which is associated with increased risk of CVD. There is also evidence that a higher fruit and vegetable consumption is associated with other beneficial dietary and lifestyle habits such as lower prevalence of smoking, and increased physical activity.

To date, research has produced equivocal results with regard to reducing stroke risk and dietary patterns. One study did however report an estimated risk reduction of 46% between the highest and lowest quartiles of serum vitamin C levels, used as a marker of high fruit and vegetable consumption (110, 111). Whilst others found that after adjusting for dietary vitamin C intake the association between fruit intake and stroke risk was non-significant (112). For vegetable intake a similar effect was shown after adjustment for β-

carotene intake (112). This may suggest that any potential protective effects are likely to be limited to specific plant foods or combinations of nutrients only when consumed in certain quantities (113). However, the characteristics of these studies' cohorts are different which may in part account for this disparity in findings. Current UK government guidelines recommend the consumption of a minimum of five portions of fruit and vegetables per day as part of a healthy diet. High fruit and vegetable intake is estimated to reduce the risk of stroke by 26% in those consuming more than 5 servings of fruit and vegetables per day compared with individuals consuming less than 3 portions per day (104, 114-116).

Diet and blood pressure and lipid profile

Elevated blood pressure and the development of hypertension can be modified by diet because a number of dietary factors have been associated with the risk of hypertension including; salt, alcohol, saturated fat and high cholesterol (19). Other dietary components including; higher fruit and vegetable, protein, potassium, magnesium and calcium intakes may exert a beneficial effect on blood pressure (19, 20).

Favourable lipid profile may be achievable by dietary modification. A number of dietary components have been implicated with influencing blood lipid profile including most commonly total fat intake and fat subtypes (saturated (SFA), MUFA and PUFA), alcohol intake and whole grain consumption.

Dietary patterns

The Dietary Approaches to Stop Hypertension (DASH) diet is the most widely known dietary strategy to reduce BP and prevent hypertension. It is characterised by high intake of fruit and vegetables, low-fat dairy products, whole-grain, nuts, fish and poultry whilst reducing total and saturated fat intakes, and limiting consumption of red meat and high sugar foods (including sweets and sugar-sweetened drinks) (72). This dietary pattern lead to significant reductions in BP in both normotensive and hypertensive populations (117). Additionally reductions in LDL and TC have been reported in those consuming a DASH diet, however, a decrease in HDL levels was also noted (118).

The Mediterranean diet, like the DASH diet, is rich in fruit and vegetables and cereal products but encourages higher fat intake in the form of MUFA, from nuts, olive oils and

seeds. It is also characterised by an overall low intake of meat and meat products, and moderate consumption of alcohol and dairy products. There is evidence that following this dietary pattern may reduce blood pressure.

Those consuming a vegetarian diet have been reported to have lower blood pressure and LDL cholesterol levels compared with their non-vegetarian counterparts (119-121). This may be attributable to a combination of a number of factors, including the tendency for those on a vegetarian diet to have a lower body mass index (BMI) than those consuming a non-vegetarian diet. In addition a vegetarian diet is often associated with higher fruit and vegetable intakes than the omnivorous diet. Interventions involving following a vegetarian diet have also led to a decrease in TC as well as produced beneficial effects on LDL profile of participants in the intervention arm. Vegetarian diets are typically higher in a number of food groups and compounds which may exhibit beneficial effects on serum lipid profile. This includes higher intakes of fruit and vegetables, overall fibre intake, and greater consumption of nuts which has been shown to significantly reduce circulating total and LDL cholesterol levels (13). Vegetarian diets may also contain higher levels of soy protein and plant sterols which are present in small quantities in many of the previously mention foods. Mechanisms are likely to be a combination of factors rather than attributable to one specific component of the diets and may also relate to other lifestyle factors associated with more health conscious vegetarian diet.

Fruit and vegetables

Previous studies have investigated the effects of increased consumption of whole grains, fruit and vegetables (122-124). The specific mechanisms of effect are not well understood, and may in part be attributable to other components of these foods, including higher fibre, vitamin or mineral content, and typically lower fat contents. However, further research is required to elucidate the potential mechanistic effects.

Previous studies investigating the effects of specific groups of fruit and vegetables and stroke risk have yielded inconclusive findings. A significant trend was reported with increased total fruit and vegetable intake and increased fruit intake, but not with increasing vegetable intake (125). There was a greater increase in number of portions of vegetables

than fruit across the quintiles (0.9-5.1 portions of vegetables compared with 0.4-3.1 portions of fruit) (125). Therefore it is not necessarily that the effect of fruit is due to concurrent higher intakes of vegetables. This would suggest that there are additional benefits of fruit consumption, it may be that fruit is often consumed in the raw state unlike vegetables which are often cooked. Cooking can lead to degradation of beneficial compounds such as vitamins, which may explain the non-significant effect of higher vegetable intake, despite the number of portions being greater than that for fruit intake (126).

A prospective study of 74,961 Swedish men and women, with 4,089 stroke cases, reported a RR of 0.87 (95% CI 0.79-0.97) for quintile 4 of total fruit and vegetables intake, which had a median intake of 5.2 portions of fruit and vegetables (125). This finding may be relevant in terms of UK government guidelines which recommend consumption of at least 5 portions of fruit and vegetables per day, which many people do not reach.

Specific foods

Specific foods such as consumption of fish, nuts or soy products may also benefit serum lipid profile. This could be due to a number of factors including their fatty acid composition, vitamin and mineral content, specific proteins and the presence of other beneficial constituents such as flavonoids in these foods (24). Specifically studies have indicated potential inverse associations between TC and LDL levels and the consumption of soy protein products. Research in relation to the potential impact on HDL and triglyceride (TG) levels has produced more inconsistent findings (27, 28, 127, 128).

Plant stanols and sterols

In the UK diet the main sources of plant sterols and stanols were cereals and bread, vegetables and vegetable fats (129). Average intakes in the Western diets are between 200-400 mg/d, higher intakes are seen in those with predominantly plant based diets (130). Plant stanols and sterols help to reduce the absorption of cholesterol in the intestine, leading to decrease in circulating LDL and total cholesterol levels (131). They may also have additional beneficial effects including reducing inflammation, and coagulation and improved endothelial function.

Alcohol

Moderate alcohol intake, estimated at 1-2 drinks/d, may be beneficial for both blood pressure and lipid profile, specifically HDL, compared with no alcohol consumption. However, high alcohol intakes are associated with increased blood pressure and overall increase in risk of CVD mortality (12, 22, 132, 133). Despite this, a systematic review by Brien et al (133) reported no significant associations either positive or negative between alcohol intake and TC, LDL or TG levels. The relationship with blood pressure is dose-dependent above ≈2 drinks/d and independent of obesity, salt intake and age (72). The mechanisms of alcohol consumption and blood pressure are unclear. A number of potential mechanisms have been proposed including the effect of alcohol on; renin-angiotensin-aldosterone axis which has a role in arterial vasoconstriction and maintaining extracellular fluid volume, heart rate variability, cortisol levels and fluctuations in ion levels including calcium (134, 135). Other explanations include a mild withdrawal effect due to overnight fast that is often prior to blood pressure measurements. There is evidence that this may even be the case in individuals who drink moderately but over a long period of time, and therefore not exclusively affecting those with heavy consumption (136). Alcohol consumption along with social class, education and physical activity status, may also be indicators of other dietary habits that influence blood pressure.

Macronutrients

What many of the above dietary patterns have in common is a high intake of fruit and vegetables. There is evidence that some specific macro and micronutrients may influence BP and lipid profile including fat intake, sodium, potassium and calcium.

A number of macronutrients including carbohydrates, fibre, and fat intake have been well studied in relation to risk factors for stroke, BP and lipid levels (24, 72, 137, 138). For example a meta-analysis of placebo controlled RCTs increasing fibre intake suggests that an average 11.5 g/d increase in fibre intake was associated with lower SBP and DBP (approximately -1.13 and -1.26 mmHg respectively) (139). Twenty four trials were included in the meta-analysis, comprising a total of 1,404 men and women aged 23-63 years. The trials ranged in duration from 2-24 weeks and fibre dosage varied from 3.5 – 42.6 g/d, in a range of formats; soluble, insoluble, mixed and through dietary increase. In 8 of the trials

hypertensive participants were included (139). In relation to dietary fat intake, the type and amount of fat consumed is important for both BP and lipid levels (24). Blood pressure and lipid levels are adversely affected by high intakes of SFA. However, higher intakes of unsaturated fatty acids may be inversely associated with BP and beneficially influence lipid profile, decreasing LDL and TC and increasing HDL levels (25, 72, 138).

Fibre consumption has been indicated to be a more accurate predictor of BP and plasma cholesterol levels than 'other dietary components' (139).

Protein

Increased dietary protein intakes, have previously been inversely associated with BP (140, 141), however the influence of different sources of protein, and more specifically whether different amounts of dietary plant or animal protein has an effect on blood pressure has been less conclusively studied.

In the UK, on average, protein intake accounts for between 14-16% of an adult's daily energy intake (142). The primary sources of protein are meat and meat products, attributing approximately 37% of daily protein intake. A further ~23% is obtained from cereal and cereal products, whilst milk and dairy products accounts for ~14% of daily protein intake (142).

Previously, vegetarians eating largely plant protein have been shown to have lower BP than their omnivorous counterparts (143). However, this does not necessarily illustrate that it is the protein content of their diet that has beneficial effects, but it may be that the vegetarian diet is, in general, lower in total and saturated fat, and higher in fibre which as previously stated can both have a significant influence on risk factors for stroke, BP and lipid levels (25, 72, 138). In addition the vegetarian diet may also be indicative of other more healthful dietary and lifestyle factors (144). In addition to this, cultures where protein intake is primarily obtained from plant sources such as Asian populations, where ~47% of protein intake is from plant, tend to have lower BP than Western populations whose consumption of animal protein is greatly increased, with ~36% of protein from red meat sources and only ~33% from plant sources (145, 146).

Protein has a key role in satiety, and this may therefore also indirectly influence cholesterol levels, as weight loss can favourable influence lipid profile (147). Randomised controlled trials (RCTs) have also investigated the effects of high protein and low carbohydrate diets. Findings have indicated that plasma lipid levels can be modified by higher consumption of protein (148). They found that a diet higher in protein, in this instance beef protein, led to a reduction in plasma triglyceride levels after 2 weeks of intervention, compared to baseline measurements. This effect was independent of saturated fat intake. However, the saturated fat content of the diet appeared to influence plasma total, LDL and HDL cholesterol as greater reductions in total and LDL cholesterol were reported in those in the low saturated fat group (8% of energy intake, compared with 13% of energy intake). The consumption of the low saturated fat diet also led to a reduction in HDL concentrations. A smaller reduction was also shown in those consuming the higher saturated fat diet, which may reduce the potentially beneficial effects of reduction in total, LDL and triglyceride levels previously indicated. To further assess this, the ratio of HDL-LDL cholesterol could be calculated, as a higher ratio of HDL to LDL cholesterol may be beneficial for disease risk reduction.

Mechanisms related to dietary protein intake, include regulation of blood pressure through changes in plasma amino acid concentrations. Higher circulating levels of cysteine, glutamate, arginine, leucine, taurine and tryptophan have been indicated to have potential BP lowering effects (149). This is thought to be through their influence on a number of metabolic processes including insulin resistance, oxidative stress, renin-angiotensin aldosterone system and renal function. The effects on renal function include influencing proximal sodium resorption and cell permeability. This in turn increases glomerular filtration rate via increased renal plasma flow (149). Amino acids may also influence nitric oxide (NO) bioavailability. L-arginine is specifically associated with increasing production of NO, which reduces BP through its vasodilatory effects (149, 150). Soy protein has been associated with lower blood pressure; this is believed to be largely due to the presence of isoflavones and other beneficial compounds rather than the protein constituents. However, it is a good source of arginine, which, as previously detailed, influences NO and

BP (151). These relationships have also been highlighted in studies investigating the relationship of biomarkers of protein intake and BP.

Micronutrients

Calcium, potassium, sodium

The literature surrounding dietary calcium intakes and cardiovascular disease and stroke risk has been inconsistent (152, 153). Wang et al (152) reported no significant association between dietary calcium intake and incident stroke in prospective studies but report a RR of 0.86 (95% CI 0.69-1.06) for pooled results, of 254,876 men and women from 8 studies, comparing the highest vs lowest dietary calcium intakes. The studies included participants from US, Finland and Japan with ages between 34-99 years (152). More recently Larsson et al (153) reviewed the relationship between dietary calcium intake and stroke risk specifically and noted a potential for dietary calcium to be inversely associated with stroke risk in populations with low calcium intake.

There is also emerging evidence of a potential influence of the Ca:Mg ratio on overall mortality including specifically stroke risk (154). It has been suggested that the Ca:Mg ratio may also influence the effects of the two nutrients individually on stroke risk. This may be due to the way in which these nutrients antagonise each other, and the close routes of absorption they share. For example, recent work has highlighted that TRPM7, a magnesium ion transporter also has an affinity for calcium (155) although the majority of magnesium is absorbed in the small intestine and thus only small amounts via TRMP7 channel (156). These two ions may also compete for absorption in the intestine, with research suggesting a higher calcium intake reduces the absorption magnesium (157). Therefore the ratio of intake may be important.

Dietary potassium intake has been inversely associated with stroke risk in a meta-analysis of over 240,000 men and women (including 7,066 incident strokes) (158). A higher potassium intake of 1064 mg/d was associated with a 21% decrease in stroke risk ($P \leq 0.001$). The UK dietary reference value (DRV) for adults (over 18 years) is 3,500 mg/d. This reduction in stroke risk may in part be due to the effects of dietary potassium intake on blood pressure (159) a key risk factor for stroke.

The mechanism of increased dietary salt adversely influencing blood pressure is linked to restrictions in the kidney's ability to handle high dietary salt intakes, as humans have adapted evolutionary to be able to consume and excrete only <1g salt/d which is far less than the average consumption in the Western world (160).

Magnesium

Two recent meta-analyses have investigated the effects of dietary magnesium on stroke risk and a third on CVD risk (161-163). Both meta-analyses found a significant inverse association between dietary magnesium and stroke risk (161, 163). However, the study on CVD endpoints, rather than stroke specifically, found no significant association with diet but a significant inverse association with circulating magnesium and CVD risk (162). Dietary magnesium intake may influence stroke risk via effects on risk factors including blood pressure however, results are inconsistent (22, 164-166). In addition, the effect of dietary magnesium intake on lipid profile is a currently under-researched area (167-170). There has also been a distinct lack of studies conducted in European, and specifically UK populations, or those which concurrently assess the associations with risk factors and stroke risk in one population of men and women. Furthermore few studies have assessed the influence of biomarkers of magnesium, how they relate to dietary intake and stroke risk factors.

Most recently Adebamowo et al (171) investigated the associations of intakes of magnesium, potassium and calcium on stroke risk in two Nurses' Health Study cohorts. The women in cohort I were aged 30-55 years and in cohort II 25-42 years at baseline. Follow-up was 30 years and 22 years for cohorts I and II respectively, with a total of 3,780 incident stroke cases reported. The two cohorts were pooled for analyses. The authors sought to investigate the relationship of these nutrients individually and as a combined score with stroke risk. After adjusting for a number of relevant confounding factors which included, age, calendar year, total energy intake, BMI, parental history of heart disease ≤60 years, alcohol intake, physical activity, smoking, HRT use, oral contraceptive, menopausal status, aspirin use, multivitamin, hypertension, hypercholesterolemia, DM baseline, and thiazide use the RR for total stroke between extreme quintiles of total magnesium was 0.87 (95% CI 0.78-0.97) P trend = 0.007. However, this was attenuated with the addition of potassium

and calcium intakes to the model; RR 0.93 (95% CI 0.79-1.08) P trend = 0.69 for total stroke and total magnesium intake. A stronger association was seen between dietary magnesium and total stroke, RR 0.81 (95% CI 0.73-0.90) P trend = 0.001 before adjustment for potassium and calcium. After additional adjustment for calcium and potassium the RR of extreme quintiles of dietary magnesium intake and total stroke was 0.82 (95% CI 0.69-0.97) P trend = 0.08. They also investigated the relationship of a mineral score combining mineral intakes of magnesium, potassium and calcium. This was achieved by assigning each quintile of each mineral a point, 1 for the lowest quintile through to 5 for the highest quintile, and summing the total score. The score ranged between 3 and 15 points. Pooled analysis of the two cohorts indicated a significant trend toward lower stroke risk with higher intakes of these three minerals P trend = 0.003 across quintiles of the score. A RR of 0.81 (95% CI 0.72-0.91) was reported between the extreme quintiles after adjustment for the confounding variables previously mentioned.

Research gaps

It is therefore evident that there are dietary components, including intakes of plant-based foods, which need to be further explored in terms of associations with stroke risk and risk factors, including blood pressure and lipid profile. Large scale population studies are a useful way to assess these potential associations between diet and biomarkers and stroke risk and risk factors

There have been inconsistent findings in relation to blood pressure (22, 164-166) and lipid profile, two risk factors for stroke, with dietary magnesium intake (167-170). There is more evidence to support an association between dietary total protein intake and blood pressure (140, 141) but less in relation to lipid profile (172, 173). In addition the association of different types of protein, animal and plant based, is an understudied area, particularly in relation to lipid levels. Some work has been conducted investigating the differences in association of types of protein with blood pressure, but these have not been conducted in UK population and the protein sources of the diet differ between countries (123, 124, 141, 174).

For stroke risk recent work suggests an inverse association between stroke risk and dietary magnesium intake, and there is less evidence in relation to protein intake (175-177). These studies have often not focused on European, and specifically UK populations. In addition previous studies have not concurrently assessed the associations of magnesium and protein with risk factors and stroke risk in one population of men and women. Few studies have sought to assess the influence of biomarkers of magnesium and protein, and how they relate to dietary intake and BP in older populations.

In addition research into the composition and contribution of foods and food sources to protein intake in the UK diet is limited and there has been a lack of previous research into how different sub-types of protein, plant and animal based, contribute to total protein intake and if they differ between men and women (178).

This thesis aims to address these gaps in the current literature.

1.4 Aims and objectives

The overall aim of this thesis is to elucidate a gap in the current literature around the associations between plant-based nutrients and their associations with major modifiable stroke risk factors as well as risk of stroke. More specifically two areas were identified that are currently understudied, specifically in the UK population. The first was the influence of dietary magnesium intake and dietary protein intake on major stroke risk factors, blood pressure and lipid profile, and stroke risk. And the second was the use of biomarkers of magnesium and protein and their relationship to dietary intakes and blood pressure.

Hypothesis: increased dietary magnesium intake will be associated with reduced stroke risk and lower blood pressure and beneficial lipid profile compared with lowest intakes. With regard to proteins, I hypothesised that a higher plant:animal protein ratio would have beneficial effects for stroke risk and risk factors. In terms of biomarkers it is hypothesised that urinary urea will correlate well with dietary protein intake, and have a similar relationship with blood pressure. Serum magnesium may correlate less strongly with dietary intake than protein and urinary urea, but may be related to blood pressure.

Research questions this thesis aims to answer are:

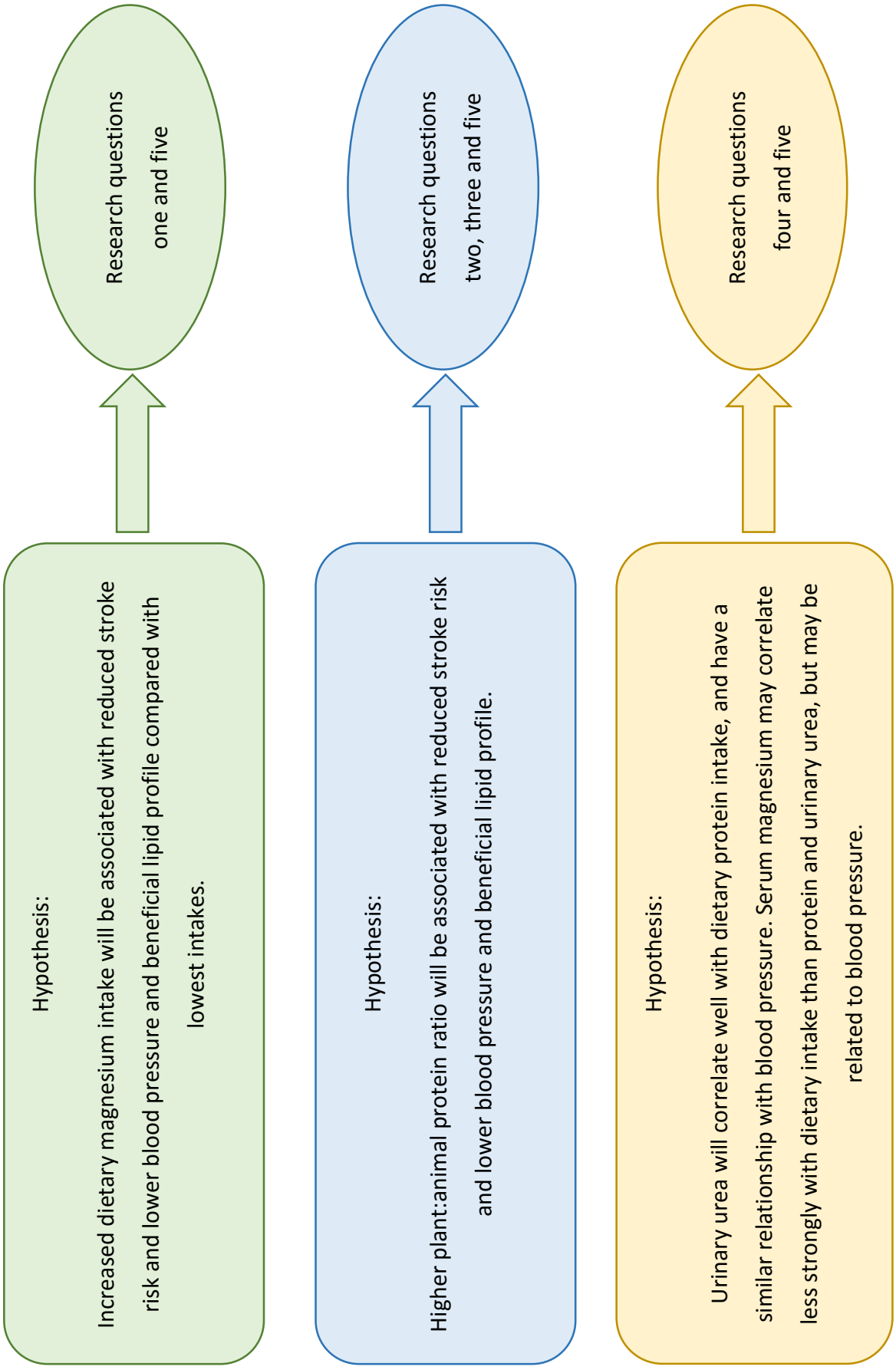
1. What is the relationship between dietary magnesium intake and stroke risk factors, blood pressure and serum lipid levels and the risk of stroke in middle and older aged men and women?
2. What are the main contributing sources of dietary protein intake, and how do the subtypes of protein (animal, plant and ratio of plant:animal) and food group sources of protein differ between men and women?
3. What are the relationships between dietary protein intake, including that from different types (animal and plant) and stroke risk factors, blood pressure and serum lipid levels and the risk of stroke in middle and older aged men and women?
4. How do biomarkers of magnesium and protein intake compare with dietary reported intakes in relation to blood pressure in older men and women?
5. What are the implications of the findings of this thesis for public health nutrition

The relationship between dietary intakes of magnesium and protein with stroke risk factors and risk of stroke (questions 1 and 3) was determined through statistical analyses of a case-cohort of 4,443 men and women aged 39-80 years part of EPIC-Norfolk. The results are presented in the following chapters; Chapter Three – The influence of dietary magnesium intake on stroke risk and risk factors and Chapter Five – The influence of dietary protein intake on stroke risk and risk factors.

The main contributing sources to dietary protein intake and how subtypes of protein and food sources differ across intakes and between men and women was determined across quintiles of intake using linear regression to assess trends. The results are presented in Chapter Four – Dietary protein intake and contribution of food sources.

The correlations between biomarkers of magnesium and protein intake and dietary intakes of these nutrients were assessed to identify which method was most strongly related to blood pressure (question 4). A cohort of 234 older men and women, aged 65-79 years, in the New dietary strategies addressing the specific needs of elderly population for an healthy ageing in Europe (NU-AGE) study was analysed. The results are presented in Chapter Six – Associations between dietary intakes and biomarkers of magnesium and protein intake on blood pressure in the NU-AGE study.

The implications of this thesis in regards to public health (question 5) are primarily discussed in Chapter Seven – Discussion.


Research questions one and five
Hypothesis:
Increased dietary magnesium intake will be associated with reduced stroke risk and lower blood pressure and beneficial lipid profile compared with lowest intakes.
Research questions two, three and five
Hypothesis:
Higher plant:animal protein ratio will be associated with reduced stroke risk and lower blood pressure and beneficial lipid profile.
Research questions four and five
Hypothesis:
Urinary urea will correlate well with dietary protein intake, and have a similar relationship with blood pressure. Serum magnesium may correlate less strongly with dietary intake than protein and urinary urea, but may be related to blood pressure.

Chapter Two

SUBJECTS AND METHODS

2.0 General overview

This chapter will begin with a review of cohort studies in nutritional epidemiology before going on to outline the study methods of the two cohorts (EPIC-Norfolk and NU-AGE) analysed in this thesis. The statistical methods employed for data analysis will also be detailed.

Nutritional epidemiology aims to study and identify relationships and associations between dietary intakes and disease (179). A number of study designs can be employed in order to assess relationships between diet and disease including case-control, cohort (prospective and retrospective), and cross-sectional. Prospective cohort studies in nutritional epidemiology initially begin with the recruitment of a healthy population who are then followed up for a defined period of time whilst incidence of disease occurrence and dietary intake are recorded. In this way, by recording dietary habits and intakes before the onset of disease, cohort studies may be free from recall bias or changes that an individual may have made to their diet or lifestyle following diagnosis of disease. Repeated measures of dietary intakes may also be obtained throughout the study period allowing for changes in habits to be recorded. This is not the case for other study design methods, such as case-control and cross sectional. Large prospective cohort studies are often limited by practicalities in obtaining detailed and repeated measures of dietary intake due to their large sample size, and long duration of follow-up which are essential in order to contain sufficient cases to conduct robust analyses. Financial implications can also affect prospective cohort studies for the same aforementioned reasons (179).

2.1 Study design EPIC-Norfolk

The European Prospective Investigation of Cancer-Norfolk (EPIC-Norfolk) is part of cross European EPIC study spanning 10 countries **(Figure 2.0)** and comprising of approximately 450,000 participants. The initial aim of EPIC, developed in 1989, was to determine potential associations between dietary intakes and the risk of developing cancer. Detailed information was collected on a number of variables including lifestyle factors, dietary habits, anthropometric measurements and biological samples.

Figure 2.0 Map of participating centres of European Prospective Investigation into Cancer

The EPIC-Norfolk cohort comprises over 26,000 men and women recruited between 1993 and 1997. The EPIC-Norfolk arm of the study was co-ordinated in Cambridge. Participants were however, resident in the Norfolk area including the city of Norwich and surrounding small towns and rural areas. This area was chosen for two reasons; the first being that it is

primarily served by one principal hospital. The second reason was that there is little migration out of this geographical area which would allow more consistent follow-up of participants (Day et al. 1999). A total of 35 General Practices (including 121 General Practitioners) in the specified area agreed to be part of the study. The participants who were aged between 39-80 years at the start of the study were identified from the registers of these participating General Practices (180). Initially 77,630 individuals were invited to participate in the study via GP mail-out. Approximately 40%, 30,445, of those invited consented to be part of the study.

Ethical approval for the EPIC-Norfolk arm of the study was obtained from Norwich Local Research Ethics Committee before the start of recruitment in 1993.

Initially the sole aim of EPIC-Norfolk was to elucidate the potential associations between dietary habits and the risk of developing cancer. However, EPIC-Norfolk has since been expanded and in addition to diet-cancer associations it also investigates a number of other endpoints. This included coronary and cardiovascular outcomes, and collection of data on a number of non-dietary exposures such as blood pressure, physical activity and smoking status.

The cohort characteristics were compared with the most relevant Health Survey for England data (181) which indicated that the cohort was representative of the general UK population, with the exception of smoking status. In the EPIC-Norfolk cohort there was a lower percentage of current smokers compared with the national average. The mean SBP and DBP of the EPIC-Norfolk cohort was also slightly lower than reported in the Health Survey for England but other anthropometric measures including height, weight and BMI were similar.

The cohort analysed during the present study (n=4,920) is comprised of a random sample of 4,000 men and women aged 39-80 years, representative of the sample of 25,639 men and women with data for food diaries from EPIC-Norfolk cohort, and remaining stroke cases. The cohort includes 1,102 stroke cases, of which n=920 were not part of random sample (n=182 stroke cases were included in the random sample). After exclusion of participants whom reported baseline prevalent stroke, or had missing data for one or more

variables of interest (n=477), a sample size of 4,443 men and women remained for analysis and a total of 982 incident stroke cases were included.

Anthropometric measures

During the baseline health check, which took place either at a research clinic or participant's GP surgery, a number of anthropometric measurements were taken by trained staff according to standardised protocols (180). This included height to the nearest mm, using a free-standing stadiometer. Weight measured to nearest 0.2 kg with participants wearing light clothing and no shoes was also recorded. From these measurements BMI was calculated. Waist, hip and chest measurements were also recorded to the nearest mm (Day et al. 1999).

Clinical and biological measures

In addition to the anthropometric measures a series of clinical measures and biological samples were also obtained from participants. This included blood pressure, which was taken after participants had been seated for 3 mins. Two readings were taken using Accutorr Sphygmomanometer (Datascope, UK) with the participants arm in the horizontal position in line with the mid sternum (180). A mean of these two values was calculated and used in analysis. At the clinic visit, non-fasting venous blood (42ml), and urine samples were taken. These were later analysed to determine circulating concentrations of a number of compounds including TC and associated fractions. Samples were stored in liquid nitrogen at -196 °C for later analysis including of micronutrients such as vitamin C (Day et al. 1999).

Classification of stroke cases

Fatal and non-fatal stroke incidence was ascertained using death certificate data and linkage with hospital records. Stroke was defined as ICD-9 430-448 or ICD-10 60-69. The current study is based on follow-up to 31st March 2008. Numbers of stroke are given in the tables, total person years was 42,557 years.

Lifestyle factors

Information was also obtained from participants in relation to a number of potential lifestyle risk factors via a Health and Lifestyle Questionnaire (HLQ). This included smoking status which was categorised as current, former, or never and physical activity which was

assessed with the use of a short physical activity questionnaire based on typical activity from the previous 12months. Physical activity status took account of work and leisure related activities and participants were ranked into one of four categories. These were 'Inactive' representing a sedentary job and no leisure activity, 'Moderately Inactive' representing either a sedentary job and <0.5 hours of leisure activity/d or standing job but no leisure activity, 'Moderately Active' a sedentary job and 0.5-1.0 hours of leisure activity/d, standing job and <0.5 hours of leisure activity/d, or a physical job and no leisure activity, 'Active' sedentary job and >1.0 hours of leisure activity/d, standing job and >1.0 hours of leisure activity/d, physical job with some leisure activity or a heavy manual job.

Education level was determined from the HLQ and was defined as the highest qualification obtained at that time. Participants were ranked into one of four categories depending on their highest qualification: 'degree or equivalent', 'A-level or equivalent', 'O-level or equivalent', and 'less than O-level or equivalent'.

Previous medical history

The presence of a number of existing underlying medical conditions was ascertained through HLQ. Conditions of interest included; stroke, cancer, myocardial infarction and diabetes amongst others at baseline. In conjunction with this, participants were requested to detail any medication that they were taking. Medications of particular relevance to this thesis include use of aspirin, antihypertensives and statins.

Dietary assessment method

The dietary habits of participants in the EPIC-Norfolk cohort were measured in multiple ways: 24hr recall, semi-quantitative food frequency questionnaire (FFQ) and 7 day diet diary (7DD).

24hr recall

All potential participants were sent a 24 hour recall to complete, to report all that had been consumed, food and drinks, over the previous 24h period, and example of what was expected was provided.

Semi-quantitative FFQ

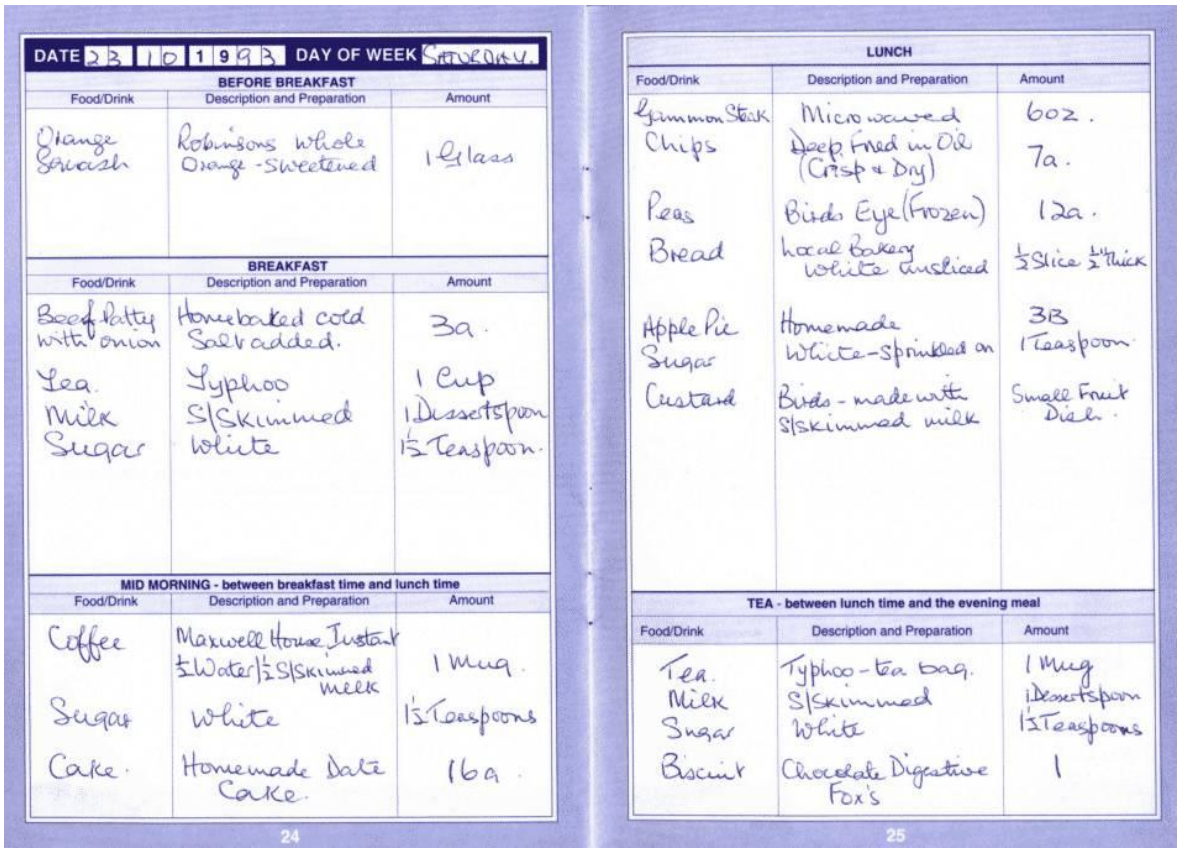
Prior to the first health check participants completed a semi-quantitative FFQ. The FFQ, which largely followed a standard format, was comprised of 130 food items. Some amendments to the FFQ were made to improve precision of information recorded, including measuring milk intake to the nearest ¼ pint, and questions regarding the type of fat used during different cooking methods. Frequency of intake was reported by ticking one of nine categories, this ranged from no consumption to ≥6/d. If there were ≥10 missing responses for food items the FFQ participants were excluded from analysis as the FFQ was considered incomplete. In the case of multiple choices per food item or omission of selection of frequency this data was also excluded from analysis (Bingham 2001). This was the standard method of dietary assessment across all EPIC cohorts, although the format of the FFQs differed between cohorts.

7 day diet diary (7DD)

In addition to the first 24hr recall and semi-quantitative FFQ, in EPIC-Norfolk, during the first health check an interviewer conducted a second 24 hour dietary recall. During this appointment the interviewer also explained the format of the 7DD and provided instructions to the participant on how to complete the remainder of the diary. The 24h recall, a copy of which was retained by the interviewer, then formed the first day of the participant's 7DD which was the chosen method for EPIC-Norfolk. The 7DD was chosen after validation studies indicated the diet diaries provide a more accurate representation of dietary intakes, over FFQs (182-184). For example FFQs were reported to overestimate dietary intakes of a number of food groups including fruit and vegetables, milk and cheese. Whilst dietary components such as total energy and a number of micronutrients including potassium, carotene and vitamin C were also more accurately represented by the 7DD compared with either FFQ or 24h recall (180, 183, 185). In addition dietary protein intake recorded from 7DD was more highly correlated with 24hr urinary nitrogen than from the FFQ and 24hr recall. A Correlation of 0.65 between 24hr urinary nitrogen and dietary nitrogen intake was reported for all participants, a sample of 156 women from EPIC-Norfolk, whereas for the FFQ and 24hr recall the correlations were 0.24 and 0.10 respectively (183).

Participants were requested to record all food and drink items consumed within the 7 day period in a 45-page full colour food record diary. Included in each food diary were colour photographs of 17 foods, each with three incremental portion sizes, small, medium and large, an example of one set of portion size photographs is shown in **Fig 2.1** on the following page. Participants were requested to indicate which photograph best represented their portion size for each of the items. In addition participants recorded the weight of food items or used household measures to describe the portion size. Participants were also requested to provide a description of the food eaten, including the preparation methods involved, and where appropriate the recipe for the item, type of fat used, and the brand names of products. This was requested for all meals, snacks and drinks consumed during the 7 day period, an example of a page from the EPIC-Norfolk food diary is shown in **Fig 2.2** on the following page. Instructions on how to record this information was provided in the front of the booklet for participants' reference.

Figure 2.1 Series of colour photographs showing guide to different portion sizes of breakfast cereals in the EPIC-Norfolk 7DD.



DATE 23 10 1993 DAY OF WEEK Saturday

BEFORE BREAKFAST

Food/Drink	Description and Preparation	Amount
Orange Squash	Robinsons Whole Orange - Sweetened	1 Glass

BREAKFAST

Food/Drink	Description and Preparation	Amount
Beef Patty with onion	Homebaked cold Salt added.	3a.
Tea.	Typhoo	1 Cup
Milk	S/Skimmed	1 Dessertspoon
Sugar	White	1½ Teaspoon.

MID MORNING - between breakfast time and lunch time

Food/Drink	Description and Preparation	Amount
Coffee	Maxwell House Instant ½ Water/½ S/Skimmed Milk	1 Mug.
Sugar	White	1½ Teaspoons
Cake.	Homemade Date Cake.	16a.

24

LUNCH

Food/Drink	Description and Preparation	Amount
Gammon Steak	Microwaved	6oz.
Chips	Deep Fried in Oil (Crisp & Dry)	7a.
Peas	Birds Eye (Frozen)	12a.
Bread	Local Bakery White Unsliced	½ Slice ¼" Thick
Apple Pie	Homemade	3B
Sugar	White - Sprinkled on	1 Teaspoon
Custard	Birds - made with S/Skimmed milk	Small Fruit Dish.

TEA - between lunch time and the evening meal

Food/Drink	Description and Preparation	Amount
Tea.	Typhoo - tea bag.	1 Mug
Milk	S/Skimmed	1 Dessertspoon
Sugar	White	1½ Teaspoons
Biscuit	Chocolate Digestive Fox's	1

25

Figure 2.2 Example of one page from EPIC-Norfolk 7DD.

A specific program, DINER (Data Into Nutrients for Epidemiological Research), was developed for entry of dietary information from the 7DD (186). DINER allows the detailed information provided in the diet diaries to be translated into structured data files for nutritional analysis. The program is more flexible than other software available on the market and enables the detail of the diary, including cooking method, type of fat used and commercial brand names of products, to be retained (187). Due to the classification structure used to code food items, DINER is also able to adapt to changes in food items available on the consumer market (186). The input of items from the food diaries requires a high level of detail, this in itself greatly reduces the risk of bias between coders influencing the data, and analysis of consistency has echoed this (186).

Development of protein variables

In my thesis I aimed to determine if the source of protein, whether plant or animal based influenced stroke risk and risk factors. In order to investigate the relationships between dietary sources of protein intake and stroke risk and risk factors in the cohort additional information was required. This involved determining the types of protein of ~11,000 food items in the existing EPIC-Norfolk food database. Another PhD student and I categorised food items into one or more of the following four types of protein: animal-land, animal-marine, animal-derived and plant.

Animal-land was defined as meat and meat products from land based animals including beef, pork and poultry. Animal-marine was defined as protein from water dwelling animals including fish and seafood. The category: animal-derived, is comprised of animal products and includes dairy products, eggs, and animal fat used during cooking. The plant category is comprised of anything that is of plant origin including grains, fruit and vegetables. An additional category ‘unclassified’ was also created this includes items where the protein content was minimal and thus were deemed to not contribute highly to daily protein intake. Food items included in this category include those such as honey, jellies and gravies made up with water, artificial sweeteners and yeast.

Food items could be assigned to more than one category, for example in the case of mixed dishes such as breaded fish in a white sauce. This item would be assigned to the ‘animal-

marine' 'plant' and 'animal-derived' categories. In the instance where description was lacking for example, "cod in sauce non-descript", assumptions were made based on standard recipes from McCance and Widdowson (188). In this instance it was assumed that for "sauce non-descript" the base would be a white sauce made with semi-skimmed milk and therefore contain components that were animal derived.

This information was then utilised by the nutritionists at EPIC-Norfolk to create a set a variables relating to different types of protein. A total of 21 groups were created which included plant, animal land, animal marine, animal derived, unclassified and a number of groups for individual food items or clusters of products. The groups of food items or clusters of products were not differentiated by the type of protein and therefore included a mixture of different protein sources. For example one grouping was 'Pasta in tom sauce, rice dishes, fish in oil, coated fishes, sandwich filling with vegetable and fish' which therefore includes a mixture of at least plant and marine protein. In order to decide which type of protein to assign each grouping to a series of calculations were made using standard recipes in McCance and Widdowson (189) to identify the component contributing most highly to protein intake and the group was assigned to this type of protein. For example in the grouping 'Pasta in tom sauce, rice dishes, fish in oil, coated fishes, sandwich filling with vegetable and fish' it was deemed that marine components would provide a higher amount of protein than the plant based items included in the group and thus this grouping was assigned to animal-marine. Variables shown later in the thesis represent protein in grams/d as a continuous variable.

For statistical analysis the 21 groups were combined together to form 5 groups. The combined groups were as follows; plant, animal-land, animal-marine, animal-derived and unclassified. It should be noted that the unclassified category was not included in any analysis. The contents of these groups are detailed in **Table 2.0**.

Protein intakes were then expressed as a percentage of total energy intake. Variables of protein intake as a percentage of total energy were created by multiplying the protein intake in g/d by 4 (the number of kcal in one gram of protein), divided by total energy intake and multiplying by 100:

$$(\text{protein}*4/\text{total energy})*100$$

The ratio of plant:animal protein intake as a percentage of total energy was calculated by dividing plant protein intake as a percentage of energy by animal protein intake as a percentage of energy.

Table 2.0 Contents of five protein groups.

Protein group	Content
Plant	Fruits, vegetables, grains, concentrated squashes Coffee beverages with soya, sweetened tea, sugared drinks, sweetened made up squash, beer/shandy, liqueur, gravy with veg juices
Animal-land	Beef, pork, chicken, turkey, game etc. Gravies with meat juices Gravies with meat & vegetable juices egg dishes with meat/ham, meat in milk/cheese sauce, soups with meat and milk/cheese, sandwich fillings with meat and mayo/cheese Heinz sandwich filler non-specific and sandwich filling non-specific Rice dishes, pasta dishes in tom sauce, veg dishes, coated chicken, kebab, meat/offal dishes, sandwich fillings Pasta dishes, savoury pies, pizzas, egg dishes, coated meat products and dishes with cheese/milk/cream Paella, rice dishes
Animal-marine	Fish or seafood Fish in milk/cheese sauce, omelette with fish, sandwich fillings with fish & mayo Pasta in tom sauce, rice dishes, fish in oil, coated fishes, sandwich filling with vegetable and fish Pasta dishes, pizzas, fish dishes Canapés, coated fish with ham & cheese
Animal-derived	Butter, lard, milk, eggs, cheese, yogurt, cream Pasta dishes, breads/biscuits with milk/cheese/garlic butter, porridge made up with milk, shortbread/homemade biscuits, cakes, savoury pies/quiches, sweet pastries with cream/milk, custard/milk pudding, pancakes, pizzas, Yorkshire pudding, milk beverages, cheesecake, mousse, omelettes/soufflés, vegetable/potato dishes, dairy spreads, (chocolate) confectionary, soups with veg and dairy, milk based sauces, sandwich fillings with veg and egg/cheese, meal replacement products Sweetened coffees with milk, meal replacement products
Unclassified	Water, water based drinks such as tea and coffee, alcoholic beverages (with the exception of those containing the addition of sugar, which would also be classified under the 'plant' category), water used in made-up products such as jellies and gravies, artificial sweeteners, yeast (including marmite and Bovril), honey, raising agents and stock.

2.2 Statistical methods

All statistical analyses in this thesis were conducted using the statistical package Stata; version 11 (StataCorp. College Station Tx. 2009). Continuous variables are presented as mean with standard error and categorical variables are presented as number and percentage. A two-sided P-value of ≤0.05 was considered statistically significant. The independent samples *t test* was used to assess differences in baseline characteristics between men and women.

Only participants with complete data for all the variables studied were included in each of the analyses. Participants with missing values for smoking status were recoded and classified as 'current smoker' (n=37). Those with missing data on the use of aspirin continuously for 3 months or more were recoded and categorised as 'no' (n=710). This was decided because smoking status is likely to be underreported whereas medication use is less likely to produce recall bias. The multivariable models used and justification of covariates adjusted for are detailed in section 2.2.1.

Sex-specific analyses were conducted. This was to account for differences associated with a number of variables of interest including BMI, waist-hip-ratio (WHR) and energy consumption which may influence blood pressure, lipid profile and stroke risk. Participants were stratified by sex-specific quintiles of magnesium (mg/d) or protein intakes as percentage of energy (%en) respectively. Participants with the lowest intakes of these nutrients were in quintile 1 through to those with the highest dietary intakes in quintile 5. Quintiles were chosen as this approach enables greater analysis of observational data than with tertiles or quartiles, including the ability to identify U or J shaped relationships where present. Additionally for the association between dietary magnesium and stroke risk hazard ratio (HR), sex-stratified groups of data-derived categories of dietary magnesium intake were created. The first group contained those with the lowest 10% of dietary intakes and the subsequent 3 groups contained a third of the rest of the population with increasing magnesium intake. The lowest 10% of dietary intakes of magnesium, Group 1, was used as the reference category. This approach was used as it was hypothesised that those with the

very lowest intakes of dietary magnesium would be at the greatest risk of stroke compared with higher intakes.

Multiple regression analysis with multivariable adjustment was employed to assess differences in SBP, DBP, and TC, LDL, HDL, and TG levels with sex specific quintiles of dietary intakes of magnesium and protein (including different types of protein). Analyses were adjusted for relevant confounding variables, which are detailed in the following section. Confounding variables are those factors which influence both the independent and dependent variables and if not accounted for may lead to bias in the relationships studied (dietary intakes with stroke risk and risk factors). Adjusting for these factors can reduce the influence of confounding and provide a more accurate representation of the actual relationship being studied; dietary intake with stroke risk and risk factors. A modified Prentice weighted cox proportional hazards model with multivariable adjustment was used to estimate HR and 95% confidence intervals (CIs) for the potential associations between dietary magnesium and protein intakes and stroke risk in men and women separately.

2.2.1 Justification of covariates – blood pressure analyses

Confounding variables included in the statistical models were identified through review of current literature on factors shown to be associated with both the independent and dependent variables.

Multivariable model 1

In analyses of dietary magnesium and total protein intakes in relation to blood pressure covariates incorporated in the statistical models included in model 1 were: age, due to the significant increases in the incidence of hypertension with advancing age (190). Since every 5 kg/m^2 increase in BMI is associated with 5 mmHg and 4 mmHg increase in SBP and DBP respectively (191), BMI was included per 1 unit increment. Also included was smoking, as long term smoking has been linked to increased risk of developing hypertension, most specifically in individuals smoking ≥15 cigarettes/d (98, 99).

Conversely being routinely physical active has been related to a decrease in BP with a number of studies illustrating this (192-194). Physical activity level was categorised as one of the following; inactive, moderately inactive, moderately active or active. A higher

educational attainment has been associated with more healthful dietary and lifestyle habits, therefore this was included in the model (195). Education level was ranked into one of four categories depending on their highest qualification: 'degree or equivalent', 'A-level or equivalent', 'O-level or equivalent', and 'less than O-level or equivalent'. The use of antihypertensive medication was also included as a covariate, a binary variable (yes or no).

Multivariable model 2

In this model further adjustment was made for previous incident MI or DM at baseline, family history of stroke or MI. Individuals with diabetes, specifically those with type II diabetes, are more likely to have high blood pressure than the non-diabetic population (196-198). A family history of stroke or MI was also included. A family history of stroke specifically has been estimated to increase stroke risk by approximately 30% (199, 200). Dietary factors were also added, including total energy intake in order to demonstrate the effect of magnesium intake independent of total caloric intake as a continuous variable. It is important to adjust for total energy intake for a number of reasons. This includes that total energy intake is, often positively, associated with dietary intake of individual nutrients and may also be directly related to disease outcome on the causal pathway (37). Additionally where total energy is perhaps associated with disease but not necessarily on the causal pathway, not adjusting for total energy may attenuate associations between other nutrients and disease outcome (37). Alcohol intake, as a continuous variable, was adjusted for, as daily consumption above approximately 3 units is associated with increases in SBP and DBP. Dietary potassium intake was included due to its inverse association with BP in a dose dependent manner and dietary sodium is positively correlated with BP and prevalence of hypertension, so was also used in the model (159, 201). The use of calcium and magnesium supplements and the ratio of calcium to magnesium intake was included, these two ions antagonise each other and may also compete during intestinal absorption. The Ca:Mg ratio, calculated by dividing dietary calcium and magnesium intakes, may also influence stroke risk (154). The use of magnesium supplements was included as a covariate in analyses rather than combined with dietary intake as the independent variable. The reasons for this include differences in the formulation of magnesium supplements that participants may have been taking, the bioavailability of these different formulations is

inconsistent. In addition evidence from the NDNS (202) indicates that the inclusion of use of dietary supplement data does not substantially change the mean magnesium intake as a percentage of RNI or the percentage of people with magnesium intakes below the LRNI. Therefore magnesium supplement use was adjusted for in analyses. In analyses for protein source, the sources of protein to be analysed were incorporated in the multivariable model.

2.2.2 Justification of covariates for lipid profile analyses

Multivariable model 1

Covariates incorporated in model 1 include age, as both LDL and TC increase with age (203-206). BMI was also included in model 1 because positive associations between BMI and LDL and TC, and inverse associations with HDL have been reported (Schroder et al. 2003). Smoking status was also included in the model as LDL and TC levels tend to be higher in smokers than non-smokers. Whilst the opposite is true for HDL levels (207). Conversely physical activity may have a beneficial impact on lipid levels, with increases in HDL and reduced levels of LDL cholesterol reported (12, 208). Education level was included in the model as there is evidence to suggest that higher educational attainment is associated with an overall more health conscious lifestyle and dietary habits (195, 209). Baseline reported MI or DM, as typically type II diabetes is associated with an abnormal lipid profile (210). Family history of stroke or MI was also adjusted for, justification of which is as detailed previously. Finally the use of statin medication was included in this model.

Multivariable model 2

Model 2 additionally adjusted for dietary factors including total energy, alcohol intake, total fat intake, the ratio of dietary Ca:Mg intake and the use of calcium and magnesium supplements. Diets high in saturated fats negatively influence the protective effects of HDL cholesterol. Whilst diets lower in saturated fat and higher in MUFA and PUFA reduce LDL concentrations but do not affect HDL levels therefore increasing the HDL:LDL ratio (12). Moderate alcohol consumption, 1-2 drinks/d, positively influences HDL levels, however, heavy alcohol intake is associated with overall increase risk of CVD mortality (12). Additionally for analyses by type of protein, the sources of protein not being analysed were

included in the multivariable model, for example plant protein analyses were adjusted for animal protein intake and vice versa.

2.2.3 Justification of covariates for stroke risk hazard ratio analyses

Stroke risk, model 1 comprised of age, BMI, smoking, physical activity, educational attainment and alcohol intake. Model 2 additionally adjusted for serum total cholesterol, baseline reported MI or diabetes and family history of stroke or MI. Model 3 included the addition of SBP and DBP, use of aspirin medication >3 months, use of antihypertensive medication, the ratio of dietary Ca:Mg and the use of calcium and magnesium supplements. For protein source specific analyses, the other sources of protein were also included in the model as a covariate.

Prentice weighted cox regression analysis was used to calculate hazard ratios and 95% CIs for the risk of incident stroke in association with dietary magnesium intake. Analyses were conducted by sex stratified quintiles of dietary magnesium intake with lowest intake (Q1 <242 mg/d and <204 mg/d for men and women) as the reference category. Additionally due to the appearance of higher risk in the lowest intakes further analyses were conducted stratified by the bottom 10% of intakes (<214 mg/d and <180 mg/d for men and women) and subsequent 30% groups of magnesium intake. This approach was taken based on the hypothesis that the highest risk of incident stroke would be in those with the very lowest dietary magnesium intakes. In this instance the lowest intakes (<214 mg/d and <180 mg/d for men and women) were used as the reference categories.

The multivariable models described in the preceding section and used in Chapter Three – Dietary magnesium stroke risk and risk factors and Chapter Five – Dietary protein stroke risk and risk factors, are summarised in the following tables **(Tables 2.1, 2.2 and 2.3).**

Table 2.1. Multivariable models magnesium or protein and blood pressure

Model 1	age, BMI, smoking status, physical activity, education level and use of antihypertensive medication.
Model 2	model 1 + baseline reported MI or DM, family history stroke or MI, dietary total energy, alcohol, potassium, sodium intakes, ratio of dietary Ca:Mg intake, calcium supplement use (including contribution from medication), magnesium supplement use (including contribution from medication) protein source*

*****in analyses of type of protein sources of protein not being analysed were included in multivariable model

Table 2.2. Multivariable models magnesium or protein and lipid profile

Model 1	age, BMI, smoking status, physical activity, education level, baseline prevalent MI or DM, family history of stroke or MI and statin medication used.
Model 2	model 1 + dietary total energy, alcohol, fat, ratio of dietary Ca:Mg intake, calcium supplement use (including contribution from medication), magnesium supplement use (including contribution from medication) protein source*

*****in analyses of type of protein sources of protein not being analysed were included in multivariable model

Table 2.3. Multivariable models magnesium or protein and stroke risk

Model 1	age, BMI, smoking status, physical activity, education level and alcohol intake
Model 2	model 1 + serum cholesterol levels, baseline prevalent MI or DM. Family history of stroke or MI.
Model 3	Model 2 + SBP and DBP, use of aspirin medication, use of antihypertensive medication, dietary total energy, ratio of dietary Ca:Mg intake, calcium supplement use (including contribution from medication), magnesium supplement use (including contribution from medication), protein source*

*****in analyses of type of protein sources of protein not being analysed were included in multivariable model

2.2 NU-AGE New dietary strategies for healthy ageing

The overall aim of the NU-AGE project, a cross European collaboration, is to improve the quality of life and health of the ageing European population and to elucidate the role of the whole diet in the process of ageing (211). There are several objectives of the NU-AGE study including the development of a food pyramid specifically for the ageing population, aged 65 years and older. The overall design of the NU-AGE study was as a randomised controlled trial to assess the influence of a whole diet intervention on healthy ageing. However, as this analysis is cross-sectional based on baseline data only, the methods detailed relating to the NU-AGE study will only be those relevant to the analysis in this thesis. More detailed information on the NU-AGE study can be found in the published study design (211).

Analyses on data from the NU-AGE study aimed to compare the relationship between dietary intakes and biomarkers of magnesium and protein intake with blood pressure in older men and women. The NU-AGE study was chosen for several reasons including that the study participants had a smaller age range, of older participants a population where this relationship has previously been understudied. For urinary markers, in this instance urinary urea, this may have importance as renal function decreases with ageing. The NU-AGE study enabled analysis of the associations between dietary intake and biomarkers of magnesium and protein in an exclusively older population, who are at greater risk of stroke than younger individuals.

Ethical considerations

NU-AGE received ethical approval from the local ethics committee, Norwich, and the Research and Development department of NNUH. All research was conducted in accordance with the Declaration of Helsinki and following Good Clinical Practice guidelines. The study is also registered on clinicaltrials.gov, NCT01754012.

Participant Recruitment

A total of 272 men and women aged between 65-79 years at baseline were recruited to the Norfolk arm of the study, and were randomised into either the control or intervention arm. Participants were resident in Norwich and the surrounding towns. Recruitment was via a number of methods including advertisements, flyers and posters targeted towards elderly

communities. Volunteer recruitment was also conducted via local GP practices utilising patient databases to target postal recruitment packs to relevant potential participants.

Potential participants initially expressed interest by telephone or email and were sent an information sheet and response slip. In the case of recruitment via GP mail-outs participants' interest in taking part in the study was ascertained by the return of a response slip. After this participants were invited to attend an initial meeting to further discuss the study and its eligibility criteria. The aim of this visit was to provide more information about the study in general and allow the opportunity to ask any questions. Participants were given at least 48 hours to consider their involvement in the study before attending a screening appointment.

Participant Screening

At the initial screening appointment those interested, and eligible participants, gave informed consent and signed a medical declaration form as well as completing admission questionnaires, to ensure the inclusion criteria detailed below were met, completed by a member of the study team.

Both men and women who were independent and free living were invited to be part of the study. Additionally participants must not have been suffering from chronic disease for the previous 2 years and they also had to be willing to comply with the requirements of the dietary intervention of the study.

Potentially eligible volunteers were excluded from taking part if they were suffering from severe heart disease, kidney disease, respiratory insufficiency, liver cirrhosis, or Type 1 Diabetes Mellitus. Those with significant disease (such as aggressive cancer, or dementia) or unstable organ failure (or organ failure necessitating a specific diet) were also excluded from the study. If potential participants had taken a course of antibiotics within the preceding 2 months or had changed their habitual medication within the past 3 months there were ineligible to be part of the study, additionally participants taking anti-inflammatory medications were also excluded. Participants were not allowed to be taking part in a another study at the same time which involved changes to the habitual diet, or which required blood sampling that in combination with the current study would exceed

the maximum allowed volume of 500 mL within a 4 month period. Those with malnutrition, defined as a BMI <18.5 kg/m^2 or those who reported weight loss of more than 10% of body weight within the past 6 months were also ineligible. If participants were diagnosed as frail during screening, using the criteria detailed by Fried et al (212) which takes into account measurements of walking speed, grip strength and physical activity levels, they were not eligible to take part in the trial. Those unable to provide informed consent were also excluded from taking part. During the screening appointment participants were requested to sign consent forms. The screening visit included two questionnaires which assessed both lifestyle habits such as alcohol intake, medication history and medical history to ensure eligibility for the study. Several anthropometric and physical performance measures were also taken including height and weight, to calculate BMI, grip strength and speed of gait.

If participants were eligible to take part in the study, the interviewer would provide instructions on the completion of 7DD, 7 day activity monitor, 24h urine collection and faecal collection (in a sub-sample of consenting participants only). These are described in more detail below.

Dietary intervention

The overall design of the NU-AGE study was as a long term whole-diet intervention. However, as this thesis is related to the cross-sectional analysis of baseline data only the intervention will not be described in length. Briefly the intervention was 12 months in length. Participants who were randomised to the control group were requested to maintain the normal habitual diet for the duration of the trial, they were additionally provided with an information leaflet about healthy eating in general from the British Dietetic Association (2011). Participants who were randomised to the intervention arm were requested to consume a diet which complied with the NU-AGE Food Based Dietary Guidelines. Participants received individualised dietary advice according to their 7DD in order to assist them in achieving the dietary goals. This support was ongoing throughout the study period. Participants on the intervention diet were also provided with vitamin D supplement as it was deemed to be extremely difficult to achieve the recommended levels through dietary intake alone. Participants were provided with several products to aid compliance with the

intervention diet, this included whole wheat pasta, olive oil, low sodium cheese and margarine spread.

Dietary intake

Dietary intake was recorded using 7 day weighed food diaries which the participant completed prior to first study day, and thus before randomisation to one arm of the intervention had taken place.

During the screening appointment, the interviewer went through the food diary with the participant and provided instructions on how to complete the diary. Participants were requested to record all food and drink consumed over the 7 day period in the booklet provided. Participants were given guidance on how to report the portion size of the food items recorded. This was asked to be done by weighing the item in its raw or cooked form (and stating which). Where this was not possible they were asked to use household measures, such as cups, spoons and jugs or other approximates such as half a packet. Information on the time and location of the eating occasion were also requested. There was a section at the end of each day in which a participant was able to include details of recipes for home cooked dishes. The food diary was completed within 2 weeks of the date of the study day, the majority of food diaries were carried out in the week preceding the study day.

After the 7DD was completed a member of the study team conducted a 'home-visit' to the participant. During the visit any queries on the food diary were made, such as omissions of information such as brand names of margarine/butter spreads. Measurements of the typical household items used to denote portion size in the food diaries were made. For example, measuring the amount of liquid held in mug used for hot drinks, and glasses for other drinks, in order to improve the accuracy of dietary coding. Participants were also requested to keep packaging of food items finished during the food diary period to aid with data entry of the diaries. Food diaries were entered in Tinuviel WISP software.

Physical activity

Physical activity levels for the purpose of this analysis were measured using ActiGraph accelerometer. The Actigraph device **(Figure 2.3)** was worn for 7 consecutive days (the

same 7 day period as the food diary was completed). Participants were instructed, by a member of the study team, on how to wear the ActiGraph device and provided with an information sheet and activity monitor log. For continuity all participants were requested to wear the ActiGraph on their left hip.

Participants were informed to wear their ActiGraph device at all times except from when washing or swimming and overnight. Participants were requested to record these occasions using the provided activity monitor log, noting the time taken off and replaced and reason for removing e.g. Swimming, sleep.

Figure 2.3 ActiGraph accelerometer device.

Study Day

Participants generally attended for their first study day 2 weeks after their screening appointment. This time frame allowed for relevant documents to be sent to the participants GP, and the completion of the 7DD and physical activity monitor using accelerometry (carried out during the same 7 day period). Participants also began their 24hr urine collection on the proceeding day up to and including the first urine on the morning of their study day. On the morning of the study day participants arrived at the clinical trials unit having fasted for 8 hours. Participants were advised to drink plenty of water. The structure of the study visit is illustrated in **Figure 2.4**.

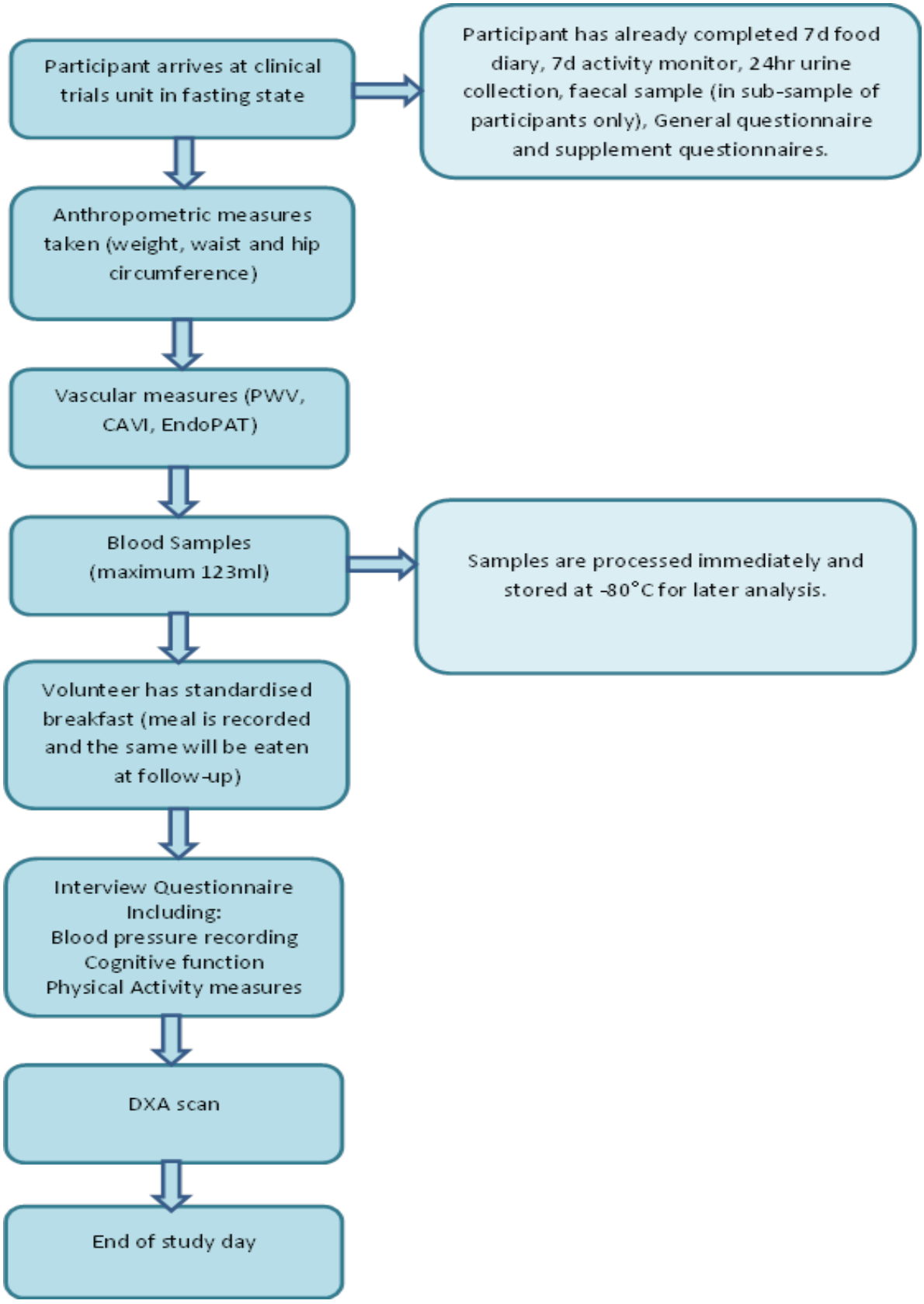


Figure 2.4 Procedures carried out on the study day

Anthropometric Measures

At the start of the study day a member of the study team carried out some anthropometric measures. The participant's weight was measured and along with their height, previously recorded at the screening visit, were used to calculate BMI. Waist and hip circumference were also measured following standard protocol.

Blood pressure

Participants attended the CRTU at the University of East Anglia for the study day. Blood pressure measurements were taken by a trained member of staff according to standard protocol with the participant rested, for 5 minutes, and seated in the upright position. The measurement was carried out a total of three times, with 1 minute interval between each reading. The mean of each of these three readings was calculated and used in analyses.

Blood samples

A qualified nurse, following standard procedure withdrew a total of either 100 mL or 123 mL of whole blood (additional 23 mL taken from participants volunteered for further characterisation of immune function). A maximum of 33 mL of whole blood was further processed for serum, and plasma, samples. Samples were stored at -80 °C until baseline recruitment was completed. Samples were then sent to the laboratory at the NNUH for analysis of serum magnesium. Where there was insufficient sample to conduct analysis data for this variable is missing. Serum magnesium was analysed using Abbott Architect analysers and reagents (213). The analysis is based on magnesium acting as a cofactor in reaction with isocitrate dehydrogenase (**Fig 2.5)**. Magnesium concentration is determined by detecting changes in absorbance at 340nm, where an increase in absorbance is as a result of the formation of NADPH. Magnesium concentration is directly proportional to NADPH (213).

$$\text{D-isocitric acid} + \text{NADP} \xrightarrow[Mg^{2+}]{\text{Iscocitrate dehydrogenase}} \text{2-oxoglutarate} + CO_2$$

Figure 2.5 Equation of determining magnesium concentration of serum sample.

Urine

All participants were requested to complete a 24 hour urine collection, following standard procedure, in the proceeding 24 hour period prior to the study day. Briefly this involved discarding the first urine of the 'first' day and collecting all subsequent urine that day and the first urine of the following day. Participants were given opaque containers in which to store the urine, these containers contained 2.7 ml of 1% concentration sodium azide as a preservative solution, containing 16.2 mg nitrogen. Participants were instructed to store the containers in a cool environment, but were not specified to refrigerate them.

On receipt of the urine samples at the CRTU, the container was weighed. If multiple containers were returned the contents were combined in a bucket, reserved specifically for this use, to ensure urine was thoroughly mixed and the bucket was weighed. The urine was then aliquoted, and stored at -80 °C for future analysis. A 20 ml sample of urine was aliquoted and sent to the laboratory at the Norfolk and Norwich University Hospital (NNUH) for the analysis on the same day, including urinary urea using Abbott Architect analysers and reagents. The analysis is based on the hydrolysis of urea to ammonia and carbon dioxide by urease. Ammonia and α-ketoglutarate are then converted to glutamate and water, a reaction catalysed by glutamate dehydrogenase during which NADH is also oxidised. These are two moles of NADH per mole of urea. Urea concentration is determined by detecting changes in absorbance of NADH at 340nm, decrease in NADH is proportional to urea concentration (213).

To obtain values for urinary urea nitrogen and total urea nitrogen, a series of steps outlined below were conducted:

1. laboratory values of total urinary urea mmol/L were converted to total urinary urea g/d using the formula:

   ((urea mmol/L * volume of 24hr urine collection) / 1000) * 60

2. urinary urea nitrogen content was calculated by multiplying urinary urea g/d by 0.46 as the molecular weight of nitrogen within urea is 28.013 comprising 46% of the molecular weight of urea:

   urinary urea * 0.46

3. Total urinary nitrogen was calculated by dividing urinary urea nitrogen by 0.84 (214):

   urinary urea nitrogen / 0.84

4. Protein intake was estimated by multiplying total urinary nitrogen by 6.25 (215):

   total urinary nitrogen * 6.25

Education level

Number of years in education was used as a measure of education level, with the assumption that those undertaking further years in education would be obtaining higher qualifications. Years in education was ascertained through the question “How many years did you go to school for (starting from primary school)?” in the General Questionnaire completed at baseline.

Smoking status

Smoking status was manually coded into one of three categories; current smoker, former smoker, never smoked. Smoking status was ascertained from the following questions: “Have you ever smoked regularly, i.e. almost every day for at least one year?”, “If yes, for how many years did you smoke regularly?”, and “Do you smoke regularly, now?” in the General Questionnaire completed at baseline

Generation of dataset

The current dataset of 272 men and women was generated by linking data from respective questionnaires and biological sample data files by participant ID code. The dataset was manually checked for discrepancies and mismatched ID codes. Some variables were manually created based on existing data, for example use of antihypertensive medication yes/no categories (1=yes, 0=no).

Statistical methods

All statistical analyses were conducted using the statistical package Stata; version 11 (StataCorp. College Station Tx. 2009). Continuous variables are presented as mean with standard error and categorical variables are presented as number and percentage. A two-sided P-value of ≤0.05 was considered statistically significant. The independent samples *t test* was used to assess differences in baseline characteristics between men and women.

Pearson's correlation was used to assess correlations between dietary intake and biomarker and each measurement independently with blood pressure.

Regression, with adjustment for confounding variables, detailed below, was used to assess the relationship between dietary intake and biomarkers of magnesium and protein intake and blood pressure.

Model 1 included age, BMI, smoking status, moderate physical activity, school years, use of antihypertensive medication, total energy intake and dietary sodium intake. Model 2 (magnesium analysis only) additionally adjusted for dietary potassium intake and calcium supplement use.

In addition sensitivity analysis was conducted excluding those taking antihypertensive medication and adjusting for confounding factors (model 1 for protein and model 2 for magnesium intake and biomarkers respectively).

Contribution to the study

I undertook several roles including in early stages of participants expression of interest and screening for the study. This included tasks such as contacting participants whom had returned a response slip of expression of interest in the study to invite them to an initial

meeting to learn more about the study and to arrange a screening appointment. I was also involved in undertaking screenings of some participants. In addition I had some experience of sample preparation on study days, preparing biological samples for storage. Contacting participants in the control arm of the intervention at 4 and 8 month intervals of the study to complete a telephone based follow-up questionnaire. I also had a role in data entry. Entering study data, including questionnaires, anthropometric and biological measures into the central NU-AGE database following standardised study-wide protocol. Finally I generated the dataset used in the analyses presented in Chapter Six – Associations between dietary intakes and biomarkers of magnesium and protein intake in the NU-AGE study as described in the section above.

Chapter Three

THE INFLUENCE OF DIETARY MAGNESIUM INTAKE ON STROKE RISK AND RISK FACTORS

3.0 Introduction

Magnesium is one of the most abundant cations in the human body. Magnesium has a number of important roles, including development and maintenance of skeletal bone, regulation of glucose homeostasis, and metabolism of calcium. It is also involved during the synthesis of adenosine triphosphate (ATP), and acts as a co-factor for over 300 enzymes (216-219). The majority of magnesium is stored in bone and skeletal muscle with serum concentrations accounting for <1% of total body magnesium (218). Intestinal absorption of dietary magnesium is dependent on the magnesium status of the body rather than the amount consumed. Therefore, in individuals with depleted magnesium status intestinal absorption is likely to be greater than individuals with saturated magnesium levels (156). Extracellular magnesium concentrations are tightly regulated, primarily by the kidneys, with intestine and bones also having a role. It is estimated that approximately 25-75% of dietary magnesium is absorbed daily, with the remainder excreted in urine and faeces (156).

One of the highest sources of magnesium is nuts, other good sources include seeds, unprocessed grains, tofu, pulses and leafy green vegetables due to magnesium being a central part of chlorophyll (156, 188, 219) **(Table 3.0)**. It is also believed that approximately 10% of daily magnesium intake is from drinking water. However, this varies geographically, depending on the hardness of water, as magnesium is more abundant in hard water (156, 220). The dietary magnesium intake in the EPIC-Norfolk cohort does not take account of water hardness due to the variability of mineral composition of local water. This may mean that the dietary magnesium intakes of this cohort are higher than those reported. Magnesium is present in lower levels in meat, dairy products, white flour and rice (219). In the UK however, cereals and cereal products are the main contributing sources of dietary magnesium in the adult diet. These products account for approximately 27% of dietary intakes, 13% of this is from bread alone. Additionally beverages contributed highly to magnesium intake in men, approximately 20% (women ~10%). With half of this amount coming from beer and lager in men, and a further 4% from coffee, the contribution of coffee was the same for men and women (221).

Table 3.0. The magnesium content of commonly consumed foods

Food item	Magnesium content (mg/100g)
Fruit and vegetables	
New potatoes (boiled)	12
Chickpeas (dried, boiled)	37
Swiss chard (boiled)	86
Spinach (raw)	54
Banana	34
Dried mixed fruit	29
Meat and fish and animal products	
Beef mince (extra lean, stewed)	18
Chicken (roasted, light meat)	26
Cod (baked or poached)	26
Salmon (grilled)	25
Milk (semi-skimmed)	11
Egg (boiled)	12
Cereal and cereal products	
Brown rice (cooked)	43
White easy cook rice (cooked)	11
All bran cereal	240
Nuts and seeds	
Almond	270
Brazil nut	410
Sesame seeds	370
Sunflower seeds	390
Beverages	
Coffee (infusion, average)	8
Drinking chocolate powder (made up with milk)	21
Orange juice (unsweetened)	8
Tea (black, infusion, average)	2
Alcohol	
Beer (bitter, average)	7
Cider (dry)	3
Red wine	11
White wine (medium)	8

Adapted from McCance and Widdowson (189)

Table 3.0a. Dietary sources of magnesium in UK for men and women combined, ordered by percentage contribution to mean intake.

Food item	Percentage contribution
Cereal and cereal products	27
White bread	6
Wholemeal bread	4
Breakfast cereals	7
Drinks	17
Beer and lager	7
Coffee	4
Tea	2
Meat and meat products	12
Milk and milk products	11
Potatoes and savoury snacks	10
Vegetables (excluding potatoes)	8
Fruit and nuts	7
Fish and fish dishes	3
Eggs and egg dishes	1

Adapted from NDNS (202).

The literature review of previous research in this chapter which follows the section on mechanisms of action will firstly start with evidence from animal studies, followed by epidemiological studies and then research from clinical trials. Each section (animal studies, epidemiology and clinical trials) will be divided to first cover the risk factors blood pressure and lipid profile and will subsequently review the literature surrounding stroke risk.

3.0.1 Mechanisms

Experimental studies suggest that magnesium has a role in the regulation of a number of aspects of the vascular system including; endothelial function, vascular inflammation and smooth muscle tone (167, 222) which may influence stroke risk via effects on risk factors blood pressure and serum lipid levels.

The primary mechanism of action in the reduction of BP is thought to be due to magnesium acting as a natural calcium channel blocker (159) this leads to a decrease in the intracellular calcium concentration which may be linked to the reported decrease in SBP and DBP (223). Magnesium deficiency has been associated with a number of adverse vascular properties including inflammation, endothelial dysfunction, and poor regulation of calcium. This can lead to an influx of calcium ions into vascular smooth muscle cells resulting in vasospasm and subsequent constriction (223). Magnesium however, blocks calcium channels by competitive inhibition preventing an influx of calcium ions (223). In addition to this it has been proposed that vasodilatation is partly mediated by magnesium and magnesium may also reduce oxidative stress and decrease platelet coagulation. Increased magnesium intakes have also been inversely associated with C-reactive protein concentrations, an indicator of inflammation (223, 224).

The mechanism by which increased magnesium intake improves endothelial function is potentially linked to the intracellular increase in ATP, as magnesium is a cofactor for ATP, and this in turn enhances glucose metabolism (225). Additionally low dietary magnesium intake may encourage Inflammation (226). Inflammation can also lead to changes in lipid profile, characterised by increased serum triglyceride and very low density lipoprotein (VLDL) and an enhanced susceptibility to oxidation (227).

Magnesium's association with serum lipid levels may be in relation to its role during the rate limiting step of cholesterol biosynthesis where HMG-CoA is converted to mevalonate by the enzyme HMG-CoA Reductase. Two processes which can deactivate HMG-CoA are dependent on magnesium and ATP (228). Magnesium may also have a role in the activity of Lecithin—cholesterol acyltransferase, which acts to lower LDL and triglyceride levels, whilst increasing HDL concentration (228, 229).

Magnesium's role in cardiovascular risk reduction is also thought to be potentially related to the development of diabetes. Intracellular abnormalities in magnesium levels can increase insulin resistance leading to an increased risk of developing type II diabetes (168). It should be noted that it has been previously reported that there is only a weak association between dietary and serum magnesium and so the ways in which low magnesium levels may increase stroke risk are likely to be different for serum magnesium and dietary magnesium (167).

These mechanisms are summarised in **Figure 3.0** and will be further discussed where appropriate in individual sections of the following literature review. The evidence presented in this narrative literature review was obtained through searches of MEDLINE (Ovid) and PubMed. The terms "dietary magnesium" or "magnesium" were combined with relevant key words such as "blood pressure", "systolic blood pressure", "diastolic blood pressure", "cholesterol", "LDL", "HDL", and "stroke risk". Titles and abstracts of returned search items were reviewed and the full text of relevant articles was obtained. The reference lists of full text articles were also reviewed to identify additional relevant publications.

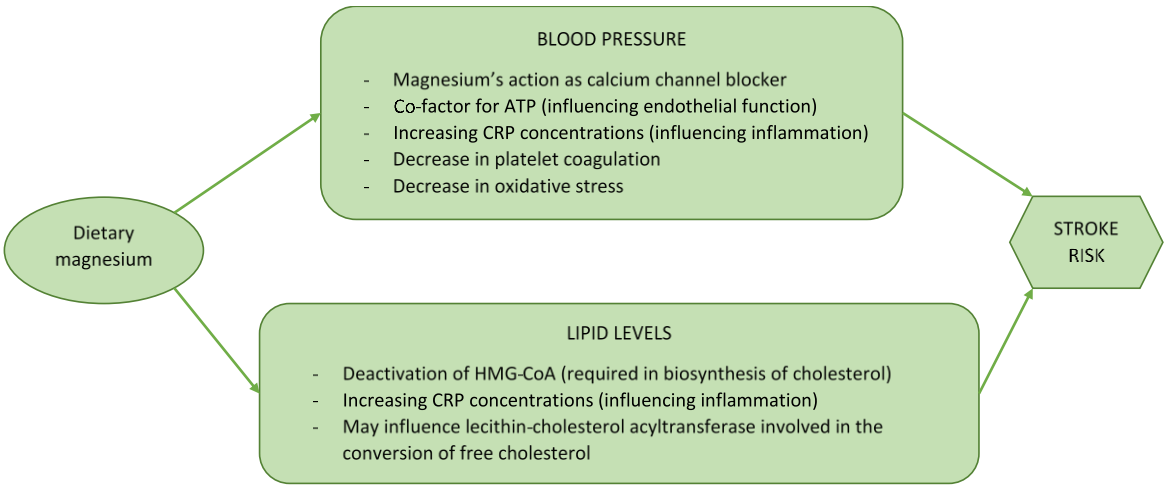


Figure 3.0 Summary of mechanisms of action of dietary magnesium on stroke risk via effects on risk factors blood pressure and lipid levels.

3.0.2 Animal models

Blood pressure

In rat models, supplementation with magnesium not only reduced blood pressure, but also exhibited beneficial effects on plasma glucose levels and circulating HDL, LDL and VLDL levels (**Table 3.1**) (230-232). However, earlier work conducted by Overlack et al (232) reported that blood pressure was not influenced by magnesium intake following 12 or 20 weeks of supplementation in normotensive or spontaneously hypertensive rats respectively. Despite this, there was a significant increase in plasma and non-significant increase in intracellular concentrations of magnesium in rats fed a higher magnesium diet (1%) compared with normal diet (0.1%). A low magnesium diet (0.01%) was associated with significantly lower plasma and intracellular magnesium compared with normal magnesium diet (0.1%) (232). The conflicting results may be attributable to differences in study methodology, for example duration, ranging from 6 to 20 weeks, the composition of experimental diet, the magnesium status of rats at the onset of the study, and presence of other disease states which affect blood pressure including diabetes (230). In this instance the studies by Mervaala (231) and Soltani (230) were conducted in rat populations at risk of low magnesium levels. The first of these was in rats treated with cyclosporin A, an immunosuppressant that is commonly associated with loss of magnesium (231). The study by Soltani et al (230) was conducted in diabetic rats, and this population have been indicated to be prone to magnesium deficiency (230, 233). Both of these studies reported beneficial effects of magnesium on blood pressure, and this effect may have resulted from these populations' increased sensitivity to magnesium that occurs when magnesium status is low.

Table 3.1. Overview of Magnesium and Blood Pressure Studies - Animal Models

Author	Model	Treatment and duration	Measurements	Results
Soltani 2007 (230)	M Wistar Rats (induced diabetes)	1. Non diabetic control no treatment, 2. Acute diabetic (10 days), 3. Chronic diabetic (8 weeks), 4. Chronic diabetic treated with 0.46 g/24h $MgSo_4$ 8 weeks	BP, blood samples: glucose, TG, TC, HDL, LDL, VLDL and Mg,	Significant lower SBP ($P<0.05$) and DBP ($P<0.05$) of Mg treated chronic diabetic compared with non-diabetic control and chronic diabetic model.
Mervaala 1997 (231)	Male SHRs	Low Na, high Na, CsA & low Na, CsA & high Na, CsA & low Na & Mg supp, CsA & high Na & Mg supp. 6 weeks	SBP, CsA conc (whole-blood, renal, myocardial, hepatic, striated muscle)	CsA & high Na and Mg sup decreased SBP by ~20 mmHg vs. CsA & high Na
Overlack 1987 (232)	Male wistar rats and SHRs	Control, low Mg - NC, High Mg - NC, 1% TC, 2% TC, low Mg & 1% TC, low Mg % 2% TC, high Mg & 1% TC or high Mg & 2% TC. High Mg water contained 17.8g Mg Ap-Hcl/litre All others distilled water 4 and 8 weeks	TC, TG, total protein, albumin, serum Mg, aorta samples-itimal and media thickness	No significant association in normotensive or hypertensive rats

Abbreviations

BP, blood pressure; CsA, cyclosporin A; DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; Mg, magnesium; Mg Ap-HCl, magnesium-aspartate HCl; Na, sodium; SBP, systolic blood pressure; TG, triglycerides; SHRs, spontaneously hypertensive rat species; supp, supplement; VLDL, very low density lipoprotein.

Lipid Profile

In some animal models, rats and rabbits, a magnesium deficiency has been associated with increased peroxidation of lipoproteins. This can subsequently lead to acceleration of atherosclerotic plaque formation, a risk factor for stroke incidence, as well as increased blood pressure (**Table 3.2**) (234, 235).

Two separate studies (233, 236), have investigated the effects of magnesium supplementation in diabetic rats. Soltani et al (233) reported that induction of diabetes led to an increase in total cholesterol, LDL, VLDL, HDL and triglyceride concentrations. A decrease in plasma magnesium was also reported. They found that supplementation with 10 g/L magnesium sulphate for 8 weeks resulted in normalisation of levels of total cholesterol, LDL, VLDL and triglycerides. Plasma HDL concentrations were significantly higher in the magnesium supplementation group compared with the non-diabetic control and chronic diabetic control.

Dou et al (237) fed rats a diet high in fat and glucose (100g lard/kg of diet and 200g glucose/kg of diet respectively) which also included 25g cholesterol/kg diet. In addition two groups were fed diets with additional magnesium; medium-magnesium diet (0.6 g/kg magnesium) and high-magnesium dose (1.2 g/kg magnesium). Compared with the control group rats in the medium-magnesium diet had significantly lower total cholesterol levels after 5 weeks of diet, but not the high-magnesium diet. HDL levels were significantly higher in the high-magnesium diet compared with the control group. There were small non-significant increases in the triglyceride and LDL levels of rats in both the medium and high-magnesium diets compared with the control group. These increases were greater in the high-magnesium diet. This study also investigated the effects of vitamin E supplementation, and when either the medium or high-magnesium diet were combined with vitamin E supplementation the triglyceride and LDL levels were higher than vitamin E alone (237) thus suggesting that perhaps the magnesium content of the diet was a contributing factor in the increases in LDL and triglyceride levels.

Two other studies looking at the effects of deficient or low magnesium diets in rats and rabbits respectively (234, 238), noted that there were significant increases in total cholesterol and triglyceride levels in rats consuming a diet deficient in magnesium (80 mg/kg) compared with the control diet (960 mg/kg) (238) and rabbits consuming low magnesium (35% of normal diet) compared with a normal magnesium diet (0.23% of diet) (234). In rabbits that were additionally fed diets with added cholesterol (either 1% or 2%) the low magnesium diet was associated with greatly increased presence of atherosclerotic lesions and intima thickening, whereas the high magnesium diet appeared to reduce the likelihood and extent of occurrence (234). The formation of atherosclerotic plaques and associated thickening of the intimal surface are associated with increased risk of stroke.

Table 3.2. Overview of Magnesium and Lipid Profile - Animal Models

Author	Model	Treatment and duration	Measurements	Results
Dou 2009 (237)	M/F Wistar Rats (diabetic)	1 week run in, randomised to 1. Control, 2. Vit E (0.5 g/kg), 3. Middle-dose Mg (0.6 g/kg), 4. High dose M (1.2 g/kg), 5. Vit E & middle-dose Mg, 6. Vit E & high dose Mg and high fat high glucose diet. 4 weeks	Fasting blood glucose, weight, MDA, serum lipid levels.	NS difference in MDA on Mg alone diets. TC sig different in mid dose Mg but other lipid levels NS. HDL sig in high dose Mg but other lipid levels NS. Mid and high dose Mg sig reduced blood viscosity.
Soltani 2007 (230)	M Wistar Rats (induced diabetes)	1. Non diabetic control no treatment, 2. Acute diabetic (10 days), 3. Chronic diabetic (8 weeks), 4. Chronic diabetic treated with 0.46 g/24h $MgSo_4$ 8 weeks	BP, blood samples: glucose, TG, TC, HDL, LDL, VLDL and Mg,	Induced diabetes lead to increased plasma glucose, TG, TC, HDL, LDL, and VLDL. Mg supp returned TC, TG, LDL and VDL levels to baseline
Laurant 1999 (238)	M Wistar Rats	Mg deficient 80 mg/kg. Control 960 mg/kg. 19 weeks	SBP, blood samples: Ca, Mg, TG TC. Diameter carotid artery.	Sig increase in TC and TG after 19 wks $P<0.005$.
Altura 1990 (234)	Male, New Zealand rabbits	Control, low Mg - NC, High Mg - NC, 1% TC, 2% TC, low Mg & 1% TC, low Mg % 2% TC, high Mg & 1% TC or high Mg & 2% TC. High Mg water contained 17.8g Mg Ap-Hcl/litre All others distilled water 4 and 8 weeks	TC, TG, total protein, albumin, serum Mg, aorta samples-itimal and media thickness	Low Mg – NC; 35% increase serum TC, 20% increase TG (4 weeks) vs control. High Mg – NC; 24% decrease serum TC, 33% decrease TG

Abbreviations

BP, blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; MDA, malondialdehyde; Mg, magnesium; Mg Ap-Hcl, magnesium-aspartate HCl; Na, sodium; NC, normal chow; SBP, systolic blood pressure; TG, triglycerides; SHRs, spontaneously hypertensive rat species; supp, supplement; Vit E, vitamin E; VLDL, very low density lipoprotein.

Stroke Risk

Few studies have been conducted looking specifically at magnesium intake and stroke risk in animal models. However, one study investigated the effects of magnesium on the infarct size of stroke (239). They identified that in magnesium deficient rats, fed a diet with 0.015% magnesium for 5-6 weeks, the infarct size was up to 45% larger at 24 hours compared with the normal (0.08%) and high (0.32%) magnesium diets. Other studies which have investigated the effects of extracellular magnesium availability indicated that acute or long-term deficiency may lead to greater endothelial damage and thus an increased susceptibility to stroke (**Table 3.3**). Touyz et al (240) investigated the effects of low magnesium on hypertension in stroke prone spontaneously hypertensive rat species (SPSHRs). They identified that although initially a low magnesium diet, <0.1% magnesium, led to a decrease in SBP, after 5 weeks of the diet SBP rose significantly. Circulating magnesium levels are tightly controlled, and this may suggest that short term diets low or deficient in magnesium may be compensated by internal regulation, but this cannot be maintained and thus at this point there is a sharp rise in SBP. The authors also noted signs of endothelial dysfunction with increased media thickness and ratio of media:lumen in the rats fed a low magnesium diet. Additionally an increased susceptibility to oxidative stress was also identified in the rats after consuming a low magnesium diet. This can lead to the initiation of inflammatory response. The inflammatory response is characterised by increased synthesis of vascular cell adhesion molecule, enhanced susceptibility to oxidative stress and subsequent changes to vascular structure including an increase in media:lumen ratio (240, 241). Magnesium deficiency was also shown to impair acetylcholine mediated vasodilation and increase intracellular calcium concentration resulting in vasoconstriction. Many of these factors have been associated with increased risk of stroke, and their combined effects may be associated with increased risk of stroke incidence (240, 241).

Table 3.3. Overview of magnesium, stroke risk and risk factors in animal models

Author	Model	Treatment and duration	Measurements	Results
Demougeot 2004 (239)	Male Wistar Rats	Low (0.015%) normal (0.08%) or high (0.32%) magnesium diet 5-6 weeks Induced infarction using; photothrombosis	SBP, Infarct volume, plasma total Mg, Ca and K, plasma antioxidant activity at 4hr and 24hr post photothrombosis	Mg deficient rats 45% higher infarct volume at 24hr
Touyz 2002 (240)	Male SPSHRs	Low (<0.1% Mg^{2+}) control (0.21% Mg^{2+}) or high (0.75% Mg^{2+}) MO diet 16 weeks	Serum Mg^{2+} TBARS, small artery samples, SBP	Low Mg diet: decrease plasma Mg and rapid increased SBP after 6 weeks diet. High Mg diet; Decreased SBP, oxidative stress and improved vascular structure
Malpuech-Brugere 2000 (242)	Male wistar rats	Mg deficient or control diet 32 or 950 g/kg MO respectively (4 or 8 days)	WBC, Plasma Mg and Zn, TNFα, IL-6,	Decrease in plasma Mg 0.14±0.02 vs 0.81±0.01 ($p < 0.01$) Increase circulating leukocytes; primarily neutrophils, IL-6, and plasma fibrinogen
Mervaala 1997 (231)	Male SHRs	Low Na, high Na, CsA & low Na, CsA & high Na, CsA & low Na & Mg supp, CsA & high Na & Mg supp. 6 weeks	SBP, CsA conc (whole-blood, renal, myocardial, hepatic, striated muscle)	CsA & high Na and Mg sup decreased SBP by ~20 mmHg vs. CsA & high Na

Altura 1990 (234)	Male, New Zealand rabbits	Control, low Mg - NC, High Mg - NC, 1% TC, 2% TC, low Mg & 1% TC, low Mg % 2% TC, high Mg & 1% TC or high Mg & 2% TC. High Mg water contained 17.8g Mg Ap-Hcl/litre All others distilled water 4 and 8 weeks	TC, TG, total protein, albumin, serum Mg, aorta samples-itimal and media thickness	Low Mg – NC; 35% increase serum TC, 20% increase TG (4 weeks) vs control. High Mg – NC; 24% decrease serum TC, 33% decrease TG
Overlack 1987 (232)	Male wistar rats and SHRs	Low 0.01%, normal 0.1% or high 1% Mg diet (Mg Asp-Hcl) 20 wks (SHRs) or 12 wks (wistar) Nifedipine 300-1000 ppm added wks 9-12 (2 wks each dose)	SBP (weekly), plasma and intracellular; Na, K, Mg, (wks 8 and 12)	Wistar; ns diff in SBP w/ low or high Mg intake SHRs; ns diff in SBP w/ low or high Mg intake

Abbreviations

Ca, calcium; K, potassium; Mg, magnesium; Mg Ap-Hcl, magnesium-aspartate HCl; Na, sodium; NC, normal chow; SBP, systolic blood pressure; TBARS, thiobarbituric acid reactive substances; TG, triglycerides; SHRs, spontaneously hypertensive rat species; supp, supplement; Vit E, vitamin E; VLDL, very low density lipoprotein; WBC, white blood cell; Zn, zinc.

3.0.3 Epidemiological studies

Blood pressure

A number of prospective studies and several cross-sectional studies have aimed to investigate the associations between dietary magnesium and blood pressure or hypertension **(Table 3.4)** with varying findings. A number of different population groups have been studied including American and Asian, but little has been conducted in European or English populations.

One of the earliest studies by Joffres et al (165) which investigated the associations between total magnesium intake and blood pressure in 615 Japanese men living in Hawaii identified a significant inverse association between SBP and DBP and magnesium intake (P 0.01 and 0.01 respectively). They also reported significant differences in the blood pressure of those in the extreme quartiles of total magnesium intake. A difference of -6.4 mmHg and -3.1 mmHg for SBP and DBP respectively was seen between those with the highest and lowest intakes. Dietary intake was assessed using 24hr recall and adjustment was made for age and BMI. In a study of 58,218 women, in the Nurse's Health Study, aged 34-59 years, a potential benefit from increased consumption of magnesium was also reported. Using dietary data collected from an FFQ, Witteman et al (166) showed a reduction in relative risk (RR 0.77 95%CI: 0.67-0.88) for developing hypertension in women with magnesium intakes greater than 300 mg/d compared with intakes less than 200 mg/d, after adjustment for age, Quetlet's score and alcohol intake.

Two prospective studies by Peacock et al (164) in men and women, and Ascherio et al (22) in women only, reported no significant associations between dietary magnesium intake and incidence of hypertension. It was however, noted that women who developed hypertension over the course of the study had lower serum magnesium levels at baseline (164). Both studies utilized FFQ to determine dietary intakes. In a separate study of 30,681 men aged 40-75 years, Ascherio et al (243) reported a significant increase in risk of developing hypertension in men consuming <250 mg/d magnesium compared to those consuming >400 mg/d (RR 1.49 95% CI 1.15-1.92) (P trend 0.003). However, after

additionally adjusting for potassium and fibre intakes the trend was attenuated and was non-significant (P trend 0.66).

Table 3.4. Overview of dietary magnesium and cross sectional and prospective epidemiological blood pressure studies

Author	Study Popn-	Sex, Age (y)	Dietary Method	Endpoints	Adjustments	Size of Effect
Song 2006 (244)	Women's Health Study (n=28,349)	W, ≥45	FFQ	Incident hypertension	Age, randomized treatment (Vit E and aspirin), family history MI<60, physical activity, alcohol intake, HRT, multivitamin, smoking, total energy intake, BMI, DM, high cholesterol, Q5: SFA, cholesterol, GL, and Na	RR 0.93 (95% CI0.86-1.02) for developing hypertension P trend 0.03
Zhao 2004 (245)	Northern & Southern Chinese INTERMAP (n=561/278)	M & W, 40-59	24h recall	N/A	Age, sex, sample, CVD disease, DM, special diet, heavy physical activity, nutrient groups: Na & K, electrolytes & Mg, electrolytes & phosphorus	Addition of Mg to model reduced differences in North/South SBP coefficient by -11.7% and -28.4% for DBP
Stamler 2003 (246)	US INTERMAP (n=2195)	M & W, 40-59	24h recall	N/A	Ethnicity, history; high BP, heart disease, MI, stroke or DM. special diet, age, sample, sex, BMI, dietary variables singly including: Mg	Adjusting for BMI and Mg intake reduced association between education and BP by -12.4% and -11.6% for SBP and DBP respectively
Peacock 1999 (164)	ARIC Study (n=7731 (3541/4190))	M & W, 45-64	FFQ	Incident hypertension	Age, sex, race, ARIC centre, BMI, WHR, DM, education, family history hypertension, leisure activity, HRT,	Mean dietary Mg lower in with hypertension vs without (239±3.1 mg/d vs 246±1.5 mg/d P=0.006). NS in M.

					baseline SBP, total energy, K, Ca, fibre, protein, ratio PUFA:SFA/protein/caffeine.	NS association between dietary Mg and incident hypertension in M/W
Ascherio 1996 (22)	Nurses' Health Study (n=41,541)	W, 38-63	FFQ	Incident hypertension	Age, BMI, alcohol, WHR, physical activity, caffeine intake, menopausal status, HRT use, dietary intakes	No significant association between Mg intake and incidence of hypertension. In normotensive inverse association between Mg and BP; w/ average BP 1.1/1.3 mmHg lower in women w/ intakes ≥0.35 g/d compared w/ intake ≤0.2 g/d.
Ascherio 1992 (243)	Health Professionals Follow-Up (n=30,681)	M,40-75	FFQ	Incident hypertension	Energy adjusted, age, weight, alcohol, and additional fibre and K	RR 1.49 for intakes <0.250 g/d compared with intakes ≥0.400 g/d (P trend 0.003) after adjustment for fibre and K became non-significant.
Witteman 1989 (166)	Nurses' Health Study (n=58,218)	W, 34-59	FFQ	Incident sr hypertension	Energy adjusted, age,	RR 0.77 (95% CI, 0.67-0.88) for intakes about 300 mg/d compared with intakes <200 mg/d
Joffres 1987 (165)	Honolulu Heart Study (n=615) Japanese popn-	M, 63-82	24h recall	N/A	Age and BMI	Total Mg strong inverse associated with SBP and DBP (P-value 0.006 and 0.008 respectively).

Abbreviations

BMI, body mass index; Ca, calcium; DBP, diastolic blood pressure; DM, diabetes; GL; glycaemic load HRT, hormone replacement therapy; K, potassium; Mg, magnesium; MI, myocardial infarction; Na, sodium; NS: non-significant; PUFA, polyunsaturated fatty acids; Q5, quintile 5; SBP, systolic blood pressure; SFA, saturated fat; sr, self-reported; supp, supplement; Vit E, vitamin E; WHR, waist-to-hip ratio.

Lipid Profile

Total serum cholesterol has been implicated as a major modifiable risk factor for stroke (74, 77, 79, 247). Over the years substantial research has been conducted investigating the influence of diet on total serum cholesterol level, and more recently on specific lipoproteins namely HDL and LDL (**Table 3.5**) (31, 32, 248-250). However, research has predominately focused on the effects of macronutrients with most emphasis on dietary fat intake (25, 138, 248, 249, 251). Other dietary constituents including those of plant origin have, to date, been less thoroughly studied (13, 252, 253).

A cross-sectional study by Huang et al (254) in 210 Taiwanese elderly patients, aged over 65 years, with type II diabetes reported that magnesium intake was positively correlated with HDL levels ($p<0.006$). Triglyceride levels were not significantly associated with magnesium intake after adjustment for a number of confounding factors including age, sex, physical activity, total energy intake and smoking. Despite this there was a non-significant inverse trend with increasing magnesium intakes for triglyceride levels. The majority of this population (88.6%) had magnesium intakes which were below the reference nutrient intake (RNI) which in Taiwan is 360 mg/d for men and 315 mg/d for women (UK RNI values are 300 mg/d for men and 270 mg/d for women). They also reported a further 37.1% of the population with deficient circulating magnesium levels (<0.75 mmol/L). In the UK the normal range for magnesium is 0.7-1.0 mmol/L, mild hypomagnesaemia is between 0.7-0.5 mmol/L and moderate to severe hypomagnesaemia is <0.5 mmol/L. Therefore the effects seen in this population may differ from those of a healthy population free from type II diabetes and frequently associated co-morbidities. In a prospective study, of 14,221 men and women aged 45-64 years, investigating the associations between dietary and serum magnesium and stroke risk Ohira et al (167) reported a significant inverse association between dietary magnesium intake and LDL cholesterol ($P<0.001$) adjusting for age, sex and race. A significant positive association was also shown with HDL and dietary magnesium intake ($P<0.001$) after adjustment for age, sex and race. However, no other adjustments were made and other factors such as BMI, smoking status, physical activity levels and dietary factors including total energy and total fat intakes amongst others may also affect and potentially attenuate the relationship.

Table 3.5. Overview of magnesium intake and lipid profile in cross sectional and prospective epidemiological studies

Author	Study Popn-	Sex, Age (y)	Dietary Method	Adjustments	Size of Effect
Huang 2012 (254)	Type II diabetics Taiwan	M/W >65	24h recall	Sex, Age, PA, total energy, CHO (% energy), protein (% energy), total fat (% energy), smoking and alcohol intake	Sig increase in HDL across quartile 5.3 mg/dL (P trend 0.006) NS change in TG levels. NS OR low HDL or high TG, but lower OR with increasing quartile.
Larsson 2008 (169)	ATBC Finland (n=26,556)	M 50-69	FFQ	Energy adjusted Mg, K, Ca, Na	NS difference in TC across quintile Mg at baseline Sig diff in HDL (P<0.01) across quintile Mg at baseline
Ohira 2009 (167)	ARIC (n=13,560)	M/W, 45-64	FFQ	Age, Sex, Race	Sig difference in LDL (P trend 0.01) and HDL (P trend 0.001) across quartile dietary Mg intake.
Song 2005 (168)	Women's Health Study (n=35,601) US popn-	W, 39-89	FFQ	Nutrients energy adjusted	NS difference in percentage with hypercholesterolemia at baseline across quintile dietary Mg intake.

Abbreviations

Ca, calcium; CHO, carbohydrate; K, potassium; HDL, high density lipoprotein; LDL, low density lipoprotein; Mg, magnesium; MI, myocardial infarction; Na, sodium; NS: non-significant; PA, physical activity; PUFA, polyunsaturated fatty acids; SBP, systolic blood pressure; SFA, saturated fat; sr, self-reported; supp, supplement; TC, total cholesterol; TG, triglyceride.

Stroke Risk

Iso et al (255) and Song et al (168) independently reported no significant association between magnesium intake (dietary and/or supplements) and stroke risk after adjustment for cardiovascular risk factors including BMI, smoking status and hypertension (**Table 3.6**). These two studies were exclusively comprised of American female health professionals. These participants were part of the Nurses' Health Study and Women's Health Study respectively and therefore were not population-based. They were also relatively healthy, reflected by their magnesium intakes. There was a relatively narrow range of magnesium intakes between the lowest and highest quintile; median of 211-318 mg magnesium and 255-433 mg respectively (168, 255). Despite these null findings in association with stroke risk, dietary magnesium intake may be linked to a reduced risk of developing hypertension and beneficial influences on lipid profile which are significant risk factors for stroke (255).

A systematic review and meta-analysis conducted by Larsson et al (161) evaluated the data from prospective studies, published between January 1997 and September 2011, on dietary magnesium intake and stroke risk. They identified seven prospective studies which met their search criteria amounting to 241,378 participants and 6,477 stroke cases in total. In summary the findings indicated that a 100 mg increase of magnesium intake per day, the equivalent to approximately 40 g serving of all bran breakfast cereal with 125 ml semi skimmed milk or 8 Brazil nuts, could lead to a decrease in total stroke risk by 8% and ischaemic stroke risk by 9%. No association was shown between magnesium intake and haemorrhagic stroke, although this may be due to relatively small sample size (n=1,015) for haemorrhagic stroke (161). It should be noted that only two out of the seven studies were conducted on specific European populations. One study was conducted in a Finnish population, which comprised of males who smoked ≥5 cigarettes/d (169). The other study conducted in Sweden was comprised exclusively of females, and only presented significant associations in those reporting a history of hypertension (256).

A more recent meta-analysis by Nie et al (163) which also included earlier literature than that conducted by Larsson et al (161), examined the period between 1966 and 2011, reported similar findings. They identified eight prospective studies, a total of 304,551 participants, this included one study, by Zhang et al (257) that was not present in the

previously mentioned meta-analysis. Zhang et al (2012) also reported a significant association between dietary magnesium and cardiovascular mortality in 58,615 Japanese men and women 40-79 years. They concluded that higher magnesium intakes (compared with lowest intakes) were associated with a reduction in risk of haemorrhagic stroke in males, HR 0.59 (0.35-0.99, P trend 0.03) after adjusting for cardiovascular risk factors including age, BMI, alcohol intake, educational attainment and menopausal status and use of HRT in women among other factors. However, this association was attenuated when additionally adjusted for sodium, calcium and potassium intakes. In women, total stroke was significantly inversely associated with magnesium intake after additional adjustment for dietary sodium HR 0.68 (HR 0.48-0.96, P=0.009). However, with the addition of calcium to the model this association was attenuated (P=0.952). Additional adjustment for potassium intake, further influenced the association, a significant positive trend was reported (P=0.015). Ischaemic stroke was significantly associated with magnesium intakes after adjustment for dietary sodium intake only (P<0.001) and was attenuated with the addition of dietary calcium and potassium intakes.

A small meta-analysis, of six prospective studies including 210,776 men and women, by Xu et al (258) on the association between dietary magnesium intake and CVD disease mortality, also included subgroup analysis on stroke risk. However, the subgroup analysis only included 1 study, Leurs et al (259), which was separated into men and women to create two studies and therefore the results which suggest no significant association, relative risk (RR) 0.85 (95% CI 0.70-1.04), between dietary magnesium intake and stroke risk could not be described as conclusive. There was also no significant association reported in association with overall CVD mortality across ten study groups. The study included in the stroke risk analysis was investigating the associations between water hardness in relation to magnesium levels and ischaemic heart disease (IHD) and stroke in a Dutch population of 4,114 men and women aged 55-69 years (259). For analyses of water hardness, soft water (≤1.50 mmol/L calcium carbonate) was used as reference category and compared with two other categories of water hardness, medium (1.60-2.00 mmol/L calcium carbonate) and hard (>2.00 mmol/L calcium carbonate). They did not report any significant association between water hardness and stroke risk in men or women. For men HR 0.90 (95% CI 66-

1.21) P trend 0.46 and for women HR 0.86 (95% CI 0.61-1.30) P trend 0.37 was reported. They also analysed associations between quintiles of magnesium concentration in water and stroke risk in men and women, with a range of 1.7-26.2 mg/L. No significant associations were reported between the concentration of magnesium in water and stroke risk in men or women HR 0.69 (95% CI 0.37-1.31) P trend 0.44 men and HR 0.77 (95% CI 0.38-1.57) P trend 0.97 women (259).

Another more recent systematic review and meta-analysis, investigating the associations between dietary and circulating magnesium on CVD risk reported no significant association between dietary magnesium intake and CVD endpoints. However, a significant inverse association was shown between circulating magnesium and CVD risk (162). Their literature range was limited to articles in submission up to May 2012. The primary aim was to establish if there was an association between dietary or circulating magnesium and CVD, IHD and mortality from IHD, rather than stroke specifically. A total of 16 studies met the inclusion criteria, 313,041 individuals, and the authors concluded that there was no significant association between dietary magnesium intake, measured in 200 g increments, and CVD (RR 0.89 95%CI 0.75-1.05) based on 11,995 CVD incidents. In additional analyses using generalised least squares with fixed effects model a RR of 0.87 (95% CI 0.80-0.95) was reported. However, this fixed-effects method is based on the premise that the effect size is constant across the studies included in the analysis. In contrast, the random effects model, which illustrated no significant trend, assumes that there are differences between the effect sizes of the different studies. Therefore the more cautious findings of the random effects model are often more appropriate.

Most recently Adebamowo et al (171) investigated the associations of intakes of magnesium, potassium and calcium on stroke risk in two Nurses' Health Study cohorts. They sought to investigate the relationship of these nutrients individually and as a combined score with stroke risk. The women in cohort I were aged 30-55 years and in cohort II 25-42 years at baseline. Follow-up was 30 years and 22 years for cohorts I and II respectively, with a total of 3,780 incident stroke cases reported. The two cohorts were pooled for analyses. After adjusting for a number of relevant confounding factors which included, age, calendar year, total energy intake, BMI, parental history of heart disease ≤60

years, alcohol intake, physical activity, smoking, HRT use, oral contraceptive, menopausal status, aspirin use, multivitamin, hypertension, hypercholesterolemia, DM baseline, and thiazide use the RR for total stroke between extreme quintiles of total magnesium was 0.87 (95% CI 0.78-0.97) P trend = 0.007. However, this was attenuated with the addition of potassium and calcium intakes to the model; RR 0.93 (95% CI 0.79-1.08) P trend = 0.69 for total stroke and total magnesium intake. A stronger association was seen between dietary magnesium and total stroke, RR 0.81 (95% CI 0.73-0.90) P trend = 0.001 before adjustment for potassium and calcium. After additional adjustment for calcium and potassium the RR of extreme quintiles of dietary magnesium intake and total stroke was 0.82 (95% CI 0.69-0.97) P trend = 0.08. They also investigated the relationship of a mineral score combining mineral intakes of magnesium, potassium and calcium. This was achieved by assigning each quintile of each mineral a point, 1 for the lowest quintile through to 5 for the highest quintile, and summing the total score. The score ranged between 3 and 15 points. Pooled analysis of the two cohorts indicated a significant trend toward lower stroke risk with higher intakes of these three minerals P trend = 0.003 across quintiles of the score. A RR of 0.81 (95% CI 0.72-0.91) was reported between the extreme quintiles after adjustment for the confounding variables previously mentioned.

Table 3.6. Overview of Magnesium and Stroke Risk Studies in prospective and cross-sectional cohort studies

Author	Study Popn-	Sex, Age (y)	Dietary Method	Endpoints	Adjustments	Size of Effect
Adebamowo 2015 (171)	Nurse's Health Study I & II (n=86,149 & 94,715)	W, 60-85 & 47-64	FFQ	stroke	Age, calendar year, energy, BMI, parental history heart disease ≤60 years, alcohol, PA, smoking, HRT, oral contraceptive, menopausal status, aspirin use, multivitamin, hypertension, hypercholesterolemia, DM baseline, thiazide use, (model 2; dietary potassium and calcium)	Attenuated with addition of K and Ca to model Total stroke dietary magnesium Q5 vs. Q1: RR 0.82 (95% CI 0.69-0.97) P trend = 0.08 Ischemic stroke: RR 0.91 (95% CI 0.71-1.15) P trend = 0.71 Heamorrhagic stroke: RR 0.87 (95% CI 0.59-1.29) P trend = 0.69
Guasch-Ferré 2014 (260)	PREDIMED (n=7216)	M/W, 55-80/60-80	FFQ	CVD events CVD, cancer & all-cause mortality	Stratified by recruitment centre. Sex, age, intervention group, BMI, smoking status, leisure time PA, education, hypertension, DM, hypercholesterolemia, family history CHD, aspirin use, oral diabetic medication, antihypertensive, hypocholesterolemia medication, alcohol, (dietary fibre, Ca – not included in stroke risk model).	Stroke: RR 1.10 (95% CI 0.70-1.74) P trend = 0.64

Del Gobbo 2013 (162)	Meta-analysis 16 prospective studies (n=313,041)	M/W	-	CVD	-	Dietary Mg 200 mg increment not associated with CVD risk (RR 0.89 95% CI 0.75-1.05) Circulating Mg 0.2 mmol/L increment decrease CVD risk (RR 0.70 95% CI 0.56-0.88)
Zhang 2012 (257)	The Japan Collaborative Cohort Study (n=58,615)	M/W, 40-79	FFQ	Death from CVD incl stroke, (IS and HS), CHD and heart failure	Age-adjusted. Covariates; BMI, smoking, alcohol, history; hypertension, diabetes. Physical activity, walking time, education, perceived mental stress, menopausal status, HRT. Dietary intake; sodium, calcium, potassium.	Men; HR (lowest vs highest mg quintile) 0.49 (0.26-0.95), P trend 0.074 for HS for sodium (inverse non-sig when potassium and calcium included) Women; HR 0.68 (0.48-0.96) P trend 0.010 and 0.47 (0.29-0.77) P trend <0.001 for total stroke and IS respectively, with sodium (weakened when potassium and calcium included)
Larsson 2012 (161)	Meta-analysis 7 prospective studies (n= 241,378)	M/W,	-	stroke	-	100 mg/d Mg associated 8% decrease total stroke risk (RR 0.92 95% CI 0.88-0.97) Mg intake inversely associated with IS risk (RR 0.91 95% CI 0.87-0.96)
Larsson 2011 (256)	Swedish Mammography	W, 49-83	FFQ	stroke	Age, energy, smoking (incl pack years) education, BMI, self-reported	Mg intake was strongly positively correlated with potassium

	Cohort (n=34,670)				hypertension, aspirin, familial MI history, alcohol, protein, cholesterol, fibre and folate.	(r=0.81) No association with stroke risk reduction and Mg
Leurs 2010 (259)	Netherlands Cohort Study (n=4,114)	M/W, 55-69	FFQ	stroke, IHD	Sex stratified. Age, smoking, hypertension, BMI, leisure time PA, education, total energy, alcohol, energy adjusted SFA/MUFA/PUFA, fruit & vegetable (g/d), use of diuretics, multivitamin use, Ca supplementation, energy adjusted dietary Ca/Mg, volume tap water (for Mg analysis tap water Ca concentration)	No significant association water hardness and stroke risk HR 0.90 (95% CI 66-1.21) P trend 0.46 men and HR 0.86 (95% CI 0.61-1.30) P trend 0.37 women No significant association concentration of Mg in water and stroke risk HR 0.69 (95% CI 0.37-1.31) P trend 0.44 men and HR 0.77 (95% CI 0.38-1.57) P trend 0.97 women.
Larsson 2008 (169)	ATBC trial Finnish Cohort (n=26,556)	M, 50-69	FFQ	First stroke	Age, energy, supplement group, alcohol, smoking, BMI, S/DBP, serum and HDL levels, history diabetes or CHD, PA, folate, vitamin C,E, fat, CHO, protein and fibre	RR 0.85 (95% CI 0.76-0.97) P trend 0.004 increasing Mg RR 0.76 (95%CI, 0.64-0.89) for men <60 and 1.02 (95% CI 0.84-1.23) for men ≥60 highest vs lowest Mg quintile for cerebral infarction
Ohira 2009 (167)	ARIC (n=13,560)	M/W, 45-64	FFQ	stroke events	age, sex, race, smoking, BMI, LDL, HDL, cholesterol, fibrinogen, von Willebran factor, educational, energy, SBP,	after adjustment rate ratios of ischaemic stroke incidence were

					antihypertensive medication , diabetes status.	non-significantly associated with dietary Mg
Weng 2008 (261)	CardioVascular Disease risk FACtor Two-township Study - Taiwan (n=1772)	M/W, >40	FFQ	Ischaemic stroke	Age, energy, sex, age-sex interaction, urea?, alcohol, smoking, sex-smoking habit, hypertension, diabetes, hypertensive medication, BMI, sr CHD, central obesity, PA, hypertriglyceridemia, hypercholesterolemia, plasminogen, apolipoprotein B and fibrinogen	Mg was weakly inversely associated with risk of IS with a borderline significant P-value 0.059
Song 2005 (168)	Women's Health Study (n=35,601) US popn-	W, 39-89	FFQ	CVD incl stroke, non-fatal MI, fatal CVD.	Age, energy, randomised assignments (aspirin and vitamin E), smoking, exercise, alcohol, HRT, BMI, multivitamin, hypertension, high cholesterol, diabetes and parental MI <60.	No significant trend between mg and total stroke. RR Q1-Q5 0.80 for total stroke, 0.84 for IS and 0.87 for HS.
Ascherio 1998 (262)	The Health Professionals Follow-Up Study (n=43,738)	M, 40-75	FFQ	fatal or nonfatal stroke	Time (2 year intervals), energy, smoking, alcohol, history hypertension, hypercholesterolemia, parental history MI <65, profession, quintiles BMI and PA.	Multivariable + K and fibre RR 0.92 (95% CI, 0.58-1.46)
Iso 1999 (255)	Nurse's Health Study (n=85,764)	W, 34-59	FFQ	fatal or nonfatal stroke	Age, energy, smoking, time interval, hypertension, BMI, alcohol, menopausal status, HRT, vigorous exercise, aspirin use, multivitamin use, vit E, w=3 fatty acid, diabetes, high cholesterol.	Mg strongly positively correlated with potassium (r=0.83) all stroke RR 0.80 (95% Ci, 0.63-1.01) age and smoking adjusted.

		Ischaemic stroke 0.84 (95% CI 0.60-1.19) after adjustment, 1.04 (95% CI 0.71-1.52) when calcium added Mg not correlated with subarachnoid or intraparenchymal haemorrhage after adjustment,

Abbreviations

Ca, calcium; CHO, carbohydrate; CHD, coronary heart disease; CVD, cardiovascular; K, potassium; HDL, high density lipoprotein; HRT, hormone replacement therapy; HS, haemorrhagic stroke; IHD, ischaemic heart disease; IS, ischaemic stroke; LDL, low density lipoprotein; Mg, magnesium; MI, myocardial infarction; MUFA, monounsaturated fatty acids; Na, sodium; NS: non-significant; PA, physical activity; PUFA, polyunsaturated fatty acids; SBP, systolic blood pressure; SFA, saturated fat; sr, self-reported; TC, total cholesterol; TG, triglyceride.

3.0.4 Supplementation trials

Blood pressure

Clinical studies investigating the effect of oral magnesium supplementation indicate a potential benefit on BP (**Table 3.7**). To date the majority of these trials have investigated the effects of supplementation rather than increased dietary intakes (263-265). Direct comparisons between the studies are difficult due to differences in the source of magnesium supplemented and in addition to this the varying concentrations and durations of supplementation. This may partially explain the variation in reported findings as Walker et al (266) illustrated significant differences in the bioavailability of different magnesium supplements. Bioavailability of magnesium is largely dependent on physiological requirements and absorption from the gastrointestinal tract, with the primary site of absorption being the ileum. Approximately 30-40% of magnesium consumed will be absorbed, however this can range from 20-80% in saturated and deficient individuals respectively (267-269). The bioavailability of oral supplements has not been extensively researched. However, evidence indicates that organic magnesium supplements, including, magnesium citrate have a higher bioavailability than inorganic formulations such as magnesium oxide whereby bioavailability may be as little as 4% (266, 268). Therefore, increased dietary magnesium intakes may produce differing results to those exhibited with oral supplementation. RCTs investigating the effects of dietary magnesium intake compared with oral supplements and their effect on blood pressure may help to elucidate these potential differences.

Table 3.7. Overview of human studies investigating the effects of magnesium supplementation on blood pressure

Author	Study Popn	Sex, Age (y)	Design	Intervention	Blinding	Duration	Results
Lee 2009 (264)	Overweight Korean (n=141)	M/W, 30-60	RCT	300 mg Mg/d (Mg oxide) or placebo	DB	12 weeks	Sig change in SBP from baseline to 12 weeks for Mg and placebo (P<0.001) and DBP for Mg (P=0.006)
Walker 2002 (263)	Mild hypertensives (n=36)	M/W, middle aged	Randomised Parallel	1. Placebo – cellulose, 2. 600 mg Mg/d (Mg aa chelate), 3. 500 mg/d dried extract hawthorn leaves and berries, 4. Combination of 2&3	DB	10 weeks	NS difference in SBP or DBP with Mg, hawthorn or combination supplement.
Sacks 1998 (265)	Nurses' Health Study II (n=290)	W, mean 39	Randomised Parallel	4 wks placebo, then; 4 mmol/d potassium chloride, 1200 mg/d calcium carbonate, 336 mg/d Mg lactate, combination of these or placebo-matching calcium carbonate	DB	16 weeks	Average change vs placebo Mg SBP -0.9 (P=0.29) DBP -0.7 (P0.32) Combined SBP -1.3 (P=0.13) DBP-0.9 (P-0.17)
Kawano 1998 (270)	Japanese mild-mod hypertensives (n=60)	M/W (34/26), 35-74	Randomised crossover	20 mmol/d Mg oxide control period no placebo given	No	8 weeks	Office: 144.9 ±1.7/88.3 ±0.9 (P0.01/0.05) Home: 134.4 ±1.4/85.4 ±0.8 (P=0.01)

							ABPM day: 135.2 ±1.3/79.6 ±0.8 (P=0.01) ABPM night: 123.4 ±1.5/73.6 ±0.9 (NS)
Borrello 1996 (271)	Italian, untreated mild hypertensives (n=83)	M/W Mg group 51 ±7 placebo 49 ±5	Parallel	4 wk placebo, then either placebo (n=41) or 200 Mg oxide (n=42)	DB	12 weeks	Supine: 148.5 ±7.1/87.5 6.3 (P0.01/NS) ABPM NS
Sacks 1995 (272)	American, Untreated mild-mod hypertensives (n=125)	M/W 21-70	Randomised parallel	4-6 wk placebo, then; Ca (25 mmol/d) and Mg (15 mmol Mg diglycine chelate), Ca and K(60 mmol), K and Mg or placebo	DB	6 months	Difference: Ca & Mg: -0.6 ±1.9/-0.4 ±2.0 (NS) K & Mg: -3.9 ±1.2/-2.7 ±1.2(P<0.05) NS from placebo
Witteman 1994 (273)	Dutch, mild-mod untreated hypertensives (n=91)	W, 35-77	RCT	2 wk placebo, then either 20 mmol Mg/d (magnesium aspartate HCl) or placebo	DB	6 months	143.8 ±14/86.1 ±7.0 (P=0.18/0.003)
Cappuccio 1985 (274)	Mild-mod untreated hypertensives (n=17)	M/W, 33-66	Randomised crossover	15 mmol/d Mg aspartate HCl and then placebo	DB	1 month	Supine: 154 ±3.4/98 ±2.2 (NS) Standing: 157 ±4.3 105 ±1.9 (NS)

Dyckner 1983 (275)	Patients on long term diuretics for hypertension or congestive heart failure (n=39)	M/W 62.2 ±4.2 SD	Randomised	Continued diuretic treatment and potassium supplementation. Additionally supplemented 15 mmol/d Mg aspartate HCl (n=20) (as 365 mg magnesium or 3689 mg Mg aspartate HCl/d) or control (n=19)	DB	6 months	Supine: 140 ±15/85 ±7 (P=0.001) Standing: 139 ±18/87 ±10 (P=0.05)

Abbreviations

ABPM, ambulatory blood pressure monitor; Ca, calcium; DBP, diastolic blood pressure; K, potassium; Mg, magnesium; NS, non-significant; PA, physical activity; PUFA, polyunsaturated fatty acids; RCT, randomised control trial; SBP, systolic blood pressure; SFA, saturated fat; sr, self-reported; TC, total cholesterol; TG, triglyceride.

Lipid Profile

A number of studies have investigated the effects of oral magnesium supplementation on the symptoms of the metabolic syndrome, which includes dyslipidaemia and hypertension, in diabetic patients (**Table 3.8**). The findings have been equivocal with several studies reporting no effect of magnesium on serum lipid levels (276, 277). However, others illustrated positive associations between magnesium and HDL, LDL, TC and TG levels (233, 254, 278). This may in part be explained by the variation in oral magnesium supplement and also duration of the study with interventions ranging in length from 1 meal to 24 weeks, with many of the shorter studies (3 months duration) reporting no significant differences in serum lipid levels with magnesium supplementation (229, 276-278). However, even in the study by Djurhuss et al (233) where 10 patients with type I diabetes, and 5 controls, were given 1000 mg/d magnesium oxide there were differences in the effects on serum lipids. A significant decrease in total cholesterol and LDL ($P<0.02$ and $P<0.05$ respectively) were reported but no significant effect was shown on triglyceride, HDL or ratio of LDL:HDL levels (233).

Fewer studies have involved healthy participants, but in one study where healthy participants received high doses of oral magnesium supplements, 411-585 mg/d, an increase in circulating HDL was reported. An improvement in TC:HDL was also noted (229). Thus indicating that the effects of magnesium supplementation may also be relevant to more general populations. Kishimoto et al (279) investigated the effects of magnesium supplementation on postprandial lipid profile. Sixteen healthy men, with a mean age of 41.7 (±2.6) took part in the randomised crossover study. Their findings suggest that supplementation with the equivalent of 500 mg magnesium in the form of $MgCl_2$ may lead to improvement in postprandial lipid profile compared with a meal without supplementation.

Itoh et al. (1997) conducted a trial in 33 healthy subjects and illustrated a benefit of supplementation with high doses of oral magnesium, 411 mg/d for women and 584 mg/d for men. The effect was reported for both LDL and HDL levels. Although, this benefit was not reported in relation to TC or triglyceride levels. A second small study on 16 healthy middle aged men by Kishimoto et al (279) indicated a potential improvement in

postprandial lipid levels with magnesium supplementation. Before the start of the study the participants underwent a 12 hour fast, after which a venous blood sample was taken. Participants were then given a standardised meal of a bread roll and 30 g butter. The intervention meal also included 5 ml bittern, which contained 500 mg magnesium in the form of $MgCl_2$. Venous blood samples were taken postprandially at 2, 3, 4 and 6 hours. Samples were analysed for serum TG, chylomicron TG, non-esterified fatty acids (NEFA), remnant-like particle cholesterol and apo-B48. After a one week washout period the participants were given the other meal combination. Serum TG was significantly reduced compared with the fat only meal at 2 and 3 hours, whilst chylomicron TG was additionally significantly reduced at 4 hours. However, at 6 hours, postprandial levels of both serum TG and chylomicron TG were non-significantly higher than the fat only meal. Although levels did not spike in the same way as was seen with the fat only meal. The authors hypothesised that magnesium supplementation, which significantly increased serum magnesium levels but not calcium, reduced absorption of fat by binding with fatty acids to form insoluble structures (279). Evidence suggests that postprandial elevated lipid levels may have atherogenic effects, particularly the presence of TG-remnants, which in high levels may decrease HDL concentrations whilst also increasing LDL concentration (279).

These findings, although inconclusive, suggest this is a relationship which requires further investigation to elucidate potential associations. The identification of dietary factors that may have favourable associations with lipid profile is of importance because even relatively small differences in lipid levels can substantially reduce stroke risk. For example a 1.0 mmol/l reduction in LDL may reduce stroke risk by approximately 10% in the general population (280).

Table 3.8. Overview of human intervention studies investigating the effects of magnesium supplementation on lipid profile

Author	Study Popn	Sex, Age y	Design	Intervention	Blinding	Duration	Measurements	Results
Kishimoto 2010 (279)	Healthy n=16	M, 41.7 (mean)	Randomised crossover	Bread roll and 30 g butter, with 5 ml bittern (500 mg Mg) during intervention	-	1 meal	Fasting blood sample. Postprandial blood samples 2, 3, 4 & 6h after meal. Serum TG, NEFA, lipoproteins, apoB48, serum Mg & Ca	Mg supplementation may delay postprandial serum and chylomicron TG, up to 4h. And RLP-cholesterol and NEFA at 2h post meal.
Farvid 2004 (278)	Type II diabetic n=69	M/W 30-69	RCT	1. 200 mg Mg oxide & 30 mg Zn sulfate, 2. 200 mg vit C & 150 mg Vit E, 3. Mg, Zn, Vit C & Vit E, 4. placebo	DB	3 months	Fasted blood for: ascorbic acid serum α-tocopherol, Zn, Mg, TC, TG, HDL, LDL, apo AI & B. spot urine: Mg, Zn, creatinine 24h recall,	NS difference in serum lipid levels with Mg and Zn supp. Ns difference in serum or urine Mg with supp.
Djurhuus 2001 (233)	Type I diabetic n=14	M/W 25-53	Case control	IV 30 mmol $MgSO_4$ in 500 ml 0.9% NaCl over 12h	No	24 weeks	blood sample for: serum insulin, free insulin, HbA_{1c}	IV $MgSO_4$ sig decreased TC (P <0.001) and TG (P<0.005)

				Then type I patients: 1000 mg/d MgO			fructosamine, lipid, Mg. 24h urine for: Mg, K. muscle biopsy muscle Mg, dietary intake (7d)	24 wks supp sig decreased TC P<0.02), LDL (P<0.05) NS TG, HDL, LDL/HDL ratio.
De Valk 1998 (277)	Type II diabetic n=50	M/W mean age intv 63	Placebo controlled	15 mmol/d Mg aspartate HCl or placebo	DB	3 months	Fasted blood sample before and after supp; plasma Mg(+ erythrocyte), Ca, glucose, TC, HDL, TG, creatinine, HbA_{1c}. 24h urine for: Mg, Ca, creatinine.	NS difference in TC, HDL or TG levels between supp and control
Itoh 1997 (229)	Healthy Japanese n=33	M/W mean age intv 64	Placebo controlled	548 mg/d $Mg(OH)_2$ M, or 411 mg Mg/d W	DB	1 wk run in 4 weeks	Dietary intake (food samples), BP, heart rate, weight, fasted blood samples: serum TC, HDL, TG, apo – AI, AII, B, CII, CIII, and E lecithin-cholesterol acyltransferase, total protein, glucose and insulin. Plasma renin activity. 24h urine: Na, K, Ca, Mg, creatinine, aldosterone,	NS change in TC, HDL, LDL or TG with Mg sup in M or W.

							noradrenaline, and adrenaline.	
Eibl 1995 (276)	Diabetic with hypo-magnesemia n=68	M/W mean age intv 63	RCT	30 mmol/d Mg citrate or placebo	DB	4 wks run in 3 months	**Baseline and end:** oral glucose tolerance, dietary questionnaire. **Monthly:** fasting blood for; plasma Mg, blood glucose, lipids, HbA_{1c}. 24h Urine for Mg, plasma insulin,	NS change in lipid profile with Mg. Plasma Mg lower in diabetic patients than controls at baseline. No change at 2 mo. Increase at 3 mo. Return to baseline 6mo after study

Abbreviations

ABPM, ambulatory blood pressure monitor; Ca, calcium; DBP, diastolic blood pressure; HDL, high density lipoprotein; IV, intravenous; K, potassium; LDL, low density lipoprotein; Mg, magnesium; NEFA, non-esterified fatty acid; NS, non-significant; PA, physical activity; PUFA, polyunsaturated fatty acids; RCT, randomised control trial; RLP-cholesterol, remnant like particle cholesterol; SBP, systolic blood pressure; SFA, saturated fat; sr, self-reported; TC, total cholesterol; TG, triglyceride; Vit C, vitamin C; Vit E, vitamin E; Zn, zinc.

In summary many of the previously undertaken studies have been conducted in single sex populations. Therefore direct sex comparisons within the same population group are limited. Many studies were also recruited solely from specific populations with certain synonymous characteristics such as occupation, or social class, as opposed to the general population. For associations with serum lipid levels, previous studies have often comprised of a particular at risk group such as diabetics and therefore are not necessarily representative of the effects in the general healthy population. In addition only a handful of studies have been conducted on European populations to date (169, 256) and, to the best of my knowledge no other study has simultaneously investigated the associations of dietary magnesium intake blood pressure, serum lipid levels and stroke risk in one single population of men and women. EPIC-Norfolk is a mixed sex cohort that is representative of the general UK population. It also utilises data from 7DDs which may provide a more accurate representation of dietary intake compared with other commonly used dietary assessment methods (167-169, 255, 256, 261, 262).

3.1 Aims and hypotheses

This chapter aims to address research question 1, outlined in the introductory Chapter, Chapter One.

"What is the relationship between dietary magnesium intake and stroke risk factors, blood pressure and serum lipid levels and the risk of stroke in middle and older aged men and women?"

They hypothesis was that higher intakes of dietary magnesium intake would be associated with lower blood pressure, more favourable lipid profile and overall lower risk of stroke.

The relationship between dietary magnesium intakes with blood pressure, lipid profile and stroke risk are understudied, particular with reference to the UK general population. In addition the proposed study allows for the concurrent analysis of associations between dietary magnesium intake with stroke risk factors and risk of stroke in one population of men and women. The findings of the present study may be relevant for the development of future interventions which where appropriate may inform public health policies aiming to reduce the risk of stroke in the UK population.

3.2 Methods

Four thousand four hundred and forty three participants were included in the main analyses for blood pressure and stroke risk. Participants were excluded from analyses if they reported stroke at baseline or had missing data for any of the variables included in the multivariable models (n=477). Additional sub-group analyses were conducted on 4,268 men and women to assess associations between dietary magnesium intake and serum cholesterol. In these analyses participants were excluded if they had missing data for serum total cholesterol, triglyceride, HDL or LDL levels (n=175). This sub-group analysis differs from that presented in the paper 'The relationship between dietary magnesium intake, stroke and its major risk factors, blood pressure and cholesterol in the EPIC-Norfolk cohort' whereby only associations between dietary magnesium intake and total cholesterol were investigated in 4,443 participants (281) .

Missing data were recoded to the 'no' category where there was no response for use of aspirin (n=813) and dietary supplements (n=2). However, with regard to smoking status, to reduce the risk of bias from under reporting individuals with missing data for cigarette smoking were coded as current smokers (n=37).

Dietary mis-reporting

Dietary mis-reporting was evaluated by calculating the ratio of reported energy intake, determined from 7 day food diary, with expected energy requirement (EER). EER was estimated using the equation and physical activity coefficients supplied by Otten et al (282). Physical activity coefficients used were 1.00 (both men and women) 'sedentary' representing typical daily living activities e.g. household chores; 1.11 and 1.12 (men and women respectively) 'low active' representing typical daily living activities and 30-60 mins of daily moderate activity; and 1.25 and 1.27 (men and women respectively) 'active' representing typical daily living activities and at least 60 mins of moderate activity. The coefficient for 'very active' representing typical daily living activities, at least 60mins of moderate activity and 60 mins of vigorous activity or 120 mins of moderate activity, was not used as the majority of the general population do not meet the physical activity

requirements of this level. The physical activity categories determined from the HLQ for EPIC-Norfolk participants included four classifications. These classifications were given the following physical activity coefficients: 'inactive' 1.00 (men and women); 'moderately inactive' and 'moderately active' 1.11 and 1.12 (for men and women respectively); 'active' 1.25 and 1.27 (for men and women respectively).

The following formulae were used to calculate the EER of men and women:

Men

EER = 662 - (9.53 * age[y]) + physical activity x ((15.91*weight [kg]) = (539.6 * height [m]))

Women

EER = 354 - (6.91 * age [y]) + physical activity x ((9.36*weight [kg]) = (726 * height [m]))

Analysis

The main risk factors analysed were blood pressure and serum lipid levels. Hypertension (blood pressure of >140/90 mmHg) is the most modifiable risk factor for stroke and is reported to have a contributory role in up to 70% of all strokes (41, 283). Abnormal lipid levels can negatively affect stroke risk. For example a 0.55 mmol/l increase in serum LDL increases stroke risk by ~11% (284). A similar increase in triglyceride levels, 0.55 mmol/l may increase stroke risk by 5.5% although it is not clear if accumulative increases in both LDL and triglycerides would lead to a further increase in stroke risk (77).

Multiple regression analysis with multivariable adjustment, for relevant confounding variables, was used to identify potential associations between quintiles of dietary magnesium intake in relation to blood pressure (SBP and DBP) and lipid profile (TC, LDL, HDL and TG). A modified Prentice weighted cox regression analysis was used to determine risk of stroke across quintiles of dietary magnesium intake and data-derived groups of intake. Quintile 1, lowest dietary intakes, was used as the reference category. For data derived categories of intake the Group 1, containing those with the lowest 10% of dietary magnesium intakes, was used as the reference category. The three subsequent groups each contained a third of the remaining dietary magnesium intakes. All analyses were sex stratified.

Full study methods and further detail of the covariates included in each model, including justification, can be found in Chapter Two – Subjects and methods. Additionally the statistical models used are stated below each table of results.

3.3 Results EPIC

Baseline characteristics

In the 4,443 participants included in the main analyses 45.0% were male. The mean age was 61 and 60 years for men and women respectively **(Table 3.9)**. Mean BP was 140/85 and 136/82 mmHg for men and women respectively which was significantly different (P<0.001 for both SBP and DBP). TC was 6.07 (±1.10) and 6.36 (±1.22) in men and women respectively. A significantly higher percentage of men reported taking aspirin continuously for 3 months or more 13.6% compared with 8.1% of women (P<0.001), but there was no significant difference in the use of antihypertensive medication. There was also no significant difference in the percentage of men and women reporting a family history of stroke, MI or diabetes. Women were however, more likely than men to have never smoked, but also had a slightly higher percentage of current smokers 12.9% than men 11.7%. Physical activity levels between men and women were significantly different (P<0.001). Similar percentages of men and women reported being inactive 32.2% and 32.8% respectively. However, more women than men reported being moderately inactive 32.3% compared with 23.8% of men, whilst men were more likely to be active (the highest of four categories) 22.0% compared with 13.9% of women. Men were most likely to be educated to A-Level or equivalent 44.4% (women 33.7%) whilst women were most likely to have no qualifications 44.5% (men (33.4%).

Dietary intakes were significantly different (P<0.001) between men and women for total energy and a number of macronutrients including magnesium, sodium and potassium. However, there was no significant difference in the dietary ratio of calcium to magnesium intakes of men and women. Alcohol intakes were significantly higher in men than women (P<0.001). Women were significantly more likely to take calcium or magnesium supplements than men (P<0.001 and P<0.01 respectively).

Dietary mis-reporting

Dietary mis-reporting was evaluated by calculating the ratio of reported energy intake, determined from 7 day food diary, with EER and assessed across quintiles of dietary magnesium intake (data not shown). The mean ratios of energy intake to EER across

quintiles of dietary magnesium intake indicated that the majority of men and women under-reported dietary intakes as illustrated by ratio less than 1.00. The highest level of under-reporting was in quintile 1 for both men and women. The mean ratios of energy intake to EER were 0.72 for men and 0.73 for women in quintile 1 of dietary magnesium intake. Whereas in quintile 5 (the highest dietary magnesium intake) there were mean ratios of 1.02 for both men and women indicating a potential small amount of over-reporting. The level of mis-reporting was similar between men and women across quintiles of magnesium intake.

Table 3.9 Baseline characteristics by sex in 4443 men and women, aged 39-80 years in EPIC-Norfolk cohort (1993-1997)

	Men n=2000	**Women n=2443**	**P-value[1]**
Age (years)	61.1 (±9.53)	60.4 (±9.71)	0.02
BMI (kg/m$^{2)}$	26.5 (±3.18)	26.2 (±4.24)	<0.01
Family History Stroke (%)	465 (23.3%)	601 (24.6%)	0.29
Family History MI (%)	720 (36.0%)	934 (38.2%)	0.13
Family History DM (%)	222 (11.1%)	305 (12.5%)	0.16
Blood pressure mmHg			
SBP	140 (±18.5)	136 (±19.5)	<0.001
DBP	85.3 (±11.5)	81.8 (±11.4)	<0.001
Pulse pressure	54.2 (±11.2)	54.0 (±11.4)	0.66
Antihypertensive Use (%)	417 (20.9%)	516 (21.1%)	0.83
Blood lipids mmol/l			
Total Cholesterol	6.07 (±1.10)	6.36 (±1.22)	<0.001
Aspirin Use (%)	271 (13.6%)	197 (8.06%)	<0.001
Smoking (%)			
Current	234 (11.7%)	314 (12.9%)	<0.001
Former	1114 (55.7%)	774 (31.7%)	
Never	652 (32.6%)	1355 (55.5%)	
Physical activity (%)			
Inactive	644 (32.2%)	800 (32.8%)	<0.001
Moderately Inactive	476 (23.8%)	790 (32.3%)	
Moderately Active	440 (22.0%)	514 (21.0%)	
Active	440 (22.0%)	339 (13.9%)	
Education level (%)			
0 – No Qualifications	667 (33.4%)	1086 (44.5%)	<0.001
1 – O-Level or Equivalent	165 (8.3%)	249 (10.2%)	
2 – A-Level or Equivalent	887 (44.4%)	822 (33.7%)	
3 – Degree or Equivalent	281 (14.1%)	286 (11.7%)	
Dietary factors			
Total Energy (kcal/d)	2218 (±505)	1685 (±384)	<0.001
Magnesium (mg/d)	318 (±92.0)	265 (±73.2)	<0.001
Ca:Mg Ratio	2.93	2.93	0.96
Potassium (mg/d)	3423 (±819)	2962 (±683)	<0.001
Alcohol (g/d)	15.9 (±20.8)	7.70 (±11.7)	<0.001
Sodium (mg/d)	3150 (±864)	2405 (±660)	<0.001
Calcium Supplement Use (%)	34 (1.70%)	160 (6.55%)	<0.001
Magnesium supplement use (%)	22 (1.10%)	53 (2.17%)	<0.01

[1]P-value difference between males and females.

Values are mean and standard deviations where continuous and number and percentage where categorical.

Table 3.10 Characteristics of 2000 men aged 39-80 years stratified by quintiles of magnesium intake. Values expressed as mean and standard deviation or frequency and percentage

	Q1 85-242 mg 206 mg n=400	**Q2 243-284 mg 266 mg n=400**	**Q3 285-328 mg 307 mg n=400**	**Q4 329-385 mg 355 mg n=400**	**Q5 386-829 456 mg n=400**	**P trend**
Age (years)	64.2 (±8.94)	62.3 (±9.17)	61.1 (±9.43)	59.6 (±9.47)	58.1 (±9.46)	<0.001
BMI (kg/m^2)	26.8 (±3.34)	26.9 (±3.27)	26.5 (±3.16)	26.3 (±3.08)	26.0 (±2.97	<0.001
Waist/Hip ratio (cm)	0.95 (±0.06)	0.94 (±0.06)	0.93 (±0.06)	0.92 (±0.06)	0.93 (±0.06)	<0.001
Current smoker (%)	68 (17.0%)	63 (15.6%)	40 (10.0%)	33 (8.25%)	30 (7.50%)	<0.001
Inactive (%)	152 (38.0%)	161 (40.3%)	125 (31.3%)	116 (29.0%)	90 (22.5%)	0.02
Educated to degree or equivalent (%)	38 (9.50%)	36 (9.00%)	50 (12.5%)	74 (18.5%)	83 (20.8%)	0.01
Aspirin use >3months (%)	57 (14.3%)	58 (14.5%)	54 (13.5%)	53 (13.3%)	49 (12.3%)	0.33
Antihypertensive medication use (%)	104 (26.0%)	99 (24.8%)	78 (19.5%)	80 (20.0%)	56 (14.0%)	<0.001
Family history of stroke (%)	97 (24.3%)	90 (22.5%)	90 (22.5%)	101 (25.3%)	87 (21.8%)	0.74
Dietary intake						
Total energy (kcal/d)	1727 (±376)	2082 (±374)	2269 (±382)	2392 (±392)	2618 (±496)	<0.001
Fruit (g/d)	91.2 (±95.2)	112 (±96.0)	140 (±118)	158 (±119)	213 (±173)	<0.001
Vegetables (g/d)	72.9 (±59.5)	81.3 (±63.2)	89.4 (±62.9)	97.9 (±70.8)	109 (±81.8)	<0.001
Nuts and seeds (g/d)	0.82 (±2.92)	1.70 (±7.35)	2.16 (±5.97)	3.18 (±7.25)	5.28 (±13.10)	<0.001
Legumes (g/d)	26.0 (±26.2)	28.8 (±29.0)	33.0 (±30.1)	34.3 (±32.1)	38.4 (±37.9)	<0.001
Bread and cereals (g/d)	205 (±90.0)	248 (±94.9)	277 (±109)	307 (±108)	350 (±137)	<0.001
Dairy (g/d)	193 (±124)	250 (±139)	297 (±158)	311 (±169)	378 (±198)	<0.001
Meat (g/d)	82.8 (±73.0)	80.9 (±54.3)	97.1 (±69.3)	98.7 (±72.9)	95.3 (±74.7)	<0.001
Alcohol (g/d)	10.6 (±17.1)	13.5 (±19.2)	15.7 (±17.7)	17.9 (±20.7)	21.7 (±26.1)	<0.001

Potassium (mg)	2496 (±465)	3058 (±361)	3422 (±424)	3731 (±440)	4404 (±766)	<0.001
Ca:Mg ratio	3.23 (±0.94)	3.10 (±0.80)	3.03 (±0.80)	2.78 (±0.69)	2.52 (±0.70)	<0.001
Vitamin C (mg)	57.9 (±32.5)	74.5 (±41.9)	85.8 (±45.3)	91.8 (±44.4)	111.5 (±61.7)	<0.001
Sodium (mg)	2510 (±678)	3000 (±710)	3208 (±778)	3403 (±757)	3631 (±930)	<0.001
Magnesium supplement use (%)	1 (0.25%)	2 (0.50%)	3 (0.75%)	8 (2.00%)	8 (2.00%)	<0.01
Calcium supplement use (%)	5 (1.25%)	4 (1.00%)	5 (1.25%)	12 (3.00%)	8 (2.00%)	0.09

Table 3.11 Characteristics of 2443 women aged 39-80 years stratified by quintiles of dietary magnesium intake. Values expressed as mean and standard deviation or frequency and percentage

	Q1 48-204 mg 176 mg n=489	**Q2 205-240 mg 223 mg n=489**	**Q3 241-274 mg 258 mg n=489**	**Q4 275-319 mg 295 mg n=489**	**Q5 320-692 mg 374 mg n=488**	**P trend**
Age (years)	63.3 (±9.62)	60.7 (±9.59)	59.8 (±9.76)	59.6 (±9.48)	58.6 (±9.49)	<0.001
BMI (kg/m^2)	27.0 (±4.59)	26.3 (±4.36)	26.1 (±4.14)	25.9 (±3.91)	25.5 (±4.05)	<0.001
Waist/Hip ratio (cm)	0.81 (±0.07)	0.79 (±0.06)	0.80 (±0.07)	0.79 (±0.06)	0.78 (±0.06)	<0.001
Current smoker (%)	85 (17.4%)	73 (14.9%)	56 (11.5%)	48 (9.82%)	52 (10.7%)	0.001
Inactive (%)	223 (45.6%)	176 (36.0%)	147 (30.1%)	135 (27.6%)	119 (24.4%)	<0.001
Educated to degree or equivalent (%)	25 (5.11%)	39 (7.98%)	65 (13.3%)	67 (13.7%)	90 (18.4)	<0.001
Aspirin use >3months (%)	48 (9.82%)	33 (6.75%)	43 (8.81%)	37 (7.57%)	36 (7.38%)	0.30
Antihypertensive medication use (%)	141 (28.8%)	98 (20.0%)	100 (20.5%)	87 (17.8)	90 (18.4%)	<0.001
Family history of stroke (%)	125 (25.6%)	114 (23.3%)	119 (24.4%)	124 (25.4%)	119 (24.4%)	0.96
Dietary intakes						
Total energy (kcal/d)	1340 (±284)	1571 (±301)	1706 (±305)	1812 (±308)	1999 (±365)	<0.001
Fruit (g/d)	109 (±97.3)	138 (±101)	172 (±118)	194 (±121)	253 (±157)	<0.001
Vegetables (g/d)	69.2 (±51.3)	91.4 (±58.9)	95.0 (±66.5)	107 (±67.9)	117 (±73.2)	<0.001
Nuts and seeds (g/d)	0.52 (±2.43)	1.07 (±3.01)	1.50 (±4.15)	2.73 (±6.15)	4.44 (±9.46)	<0.001
Legumes (g/d)	19.2 (±23.3)	21.2 (±21.0)	23.0 (±23.1)	24.2 (±24.0)	26.6 (±27.5)	<0.001
Bread and cereals (g/d)	154 (±69.8)	188 (±69.5)	213 (±85.3)	226 (±86.9)	264 (±105)	<0.001
Dairy (g/d)	176 (±107)	233 (±130)	263 (±140)	298 (±149)	359 (±191)	<0.001
Meat (g/d)	64.7 (±54.0)	72.2 (±53.9)	76.2 (±59.0)	75.9 (±58.7)	82.8 (±70.0)	<0.001
Alcohol (g/d)	5.09 (±9.70)	6.76 (±10.56)	7.98 (±11.9)	9.14 (±12.7)	9.54 (±12.9)	<0.001

Potassium (mg)	2171 (±367)	2648 (±310)	2949 (±353)	3254 (±366)	3788 (±591)	<0.001
Ca:Mg ratio (mg)	3.16 (±0.89)	3.08 (±0.80)	2.97 (±0.81)	2.84 (±0.71)	2.60 (±0.72)	<0.001
Vitamin C (mg)	63.2 (±37.0)	78.2 (±40.8)	88.6 (±50.8)	97.7 (±46.2)	118 (±58.9)	<0.001
Vitamin K (mg)	64.9 (±40.5)	80.9 (±50.8)	87.3 (±45.1)	95.4 (±52.5)	107 (±56.8)	<0.001
Sodium (mg)	1932 (±525)	2236 (±506)	2463 (±578)	2552 (±580)	2843 (±714)	<0.001
Magnesium supplement use (%)	1 (0.20%)	10 (2.04%)	14 (2.87%)	11 (2.25%)	17 (3.48%)	0.001
Calcium supplement use (%)	14 (2.86%)	31 (6.34%)	30 (6.15%)	37 (7.57%)	48 (9.84%)	<0.001

Table 3.12 Association of quintiles of dietary magnesium intake (range and mean quintile intake) and blood pressure (means and SE) in 2000 men, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary Magnesium Intake					
		Q1 85-242 mg 206 mg n=400	**Q2 243-284 mg 266 mg n=400**	**Q3 285-328 mg 307 mg n=400**	**Q4 329-385 mg 355 mg n=400**	**Q5 386-829 456 mg n=400**	**P trend**
SBP	Unadjusted	143 (±0.98)	140 (±0.97)	140 (±0.88)	139 (±0.90)	136 (±0.87)	<0.001
	Model 1[1]	140 (±0.87)	139 (±0.86)	140 (±0.86)	140 (±0.86)	139 (±0.87)	0.64
	Model 2[2]	143 (±1.16)	140 (±0.90)*[3]	140 (±0.85)*	138 (±0.89)**	136 (±1.18)***	0.002
DBP	Unadjusted	86.1 (±0.58)	85.9 (±0.60)	85.4 (±0.57)	85.2 (±0.56)	84.1 (±0.56)	0.008
	Model 1[1]	85.4 (±0.57)	85.4 (±0.56)	85.3 (±0.56)	85.5 (±0.56)	85.0 (±0.57)	0.68
	Model 2[2]	87.2 (±0.76)	86.1 (±0.59)	85.1 (±0.55)*	84.9 (±0.58)*	83.4 (±0.77)**	0.01

[1]Model 1: age, BMI, smoking status, physical activity, education level, antihypertensive medication use

[2]Model 2: model 1 + baseline MI or diabetes, family history stroke, family history MI, alcohol intake, dietary sodium, potassium, ratio Ca:Mg, total energy and calcium supplement use (including contribution from medication)

[3]P value for significance compared with Q1: *= P value ≤0.05, ** = P value ≤0.01, *** = P value ≤0.001

Table 3.13 Association of quintiles of dietary magnesium intake (range and mean quintile intake) and blood pressure (means and SE) in 2443 women, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary Magnesium Intake					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		48-204 mg 176 mg n=489	**205-240 mg 223 mg n=489**	**241-274 mg 258 mg n=489**	**275-319 mg 295 mg n=489**	**320-692 mg 374 mg n=488**	**P trend**
SBP	Unadjusted	140 (±0.90)	135 (±0.85)	137 (±0.93)	135 (±0.89)	133 (±0.81)	<0.001
	Model 1[1]	136 (±0.79)	135 (±0.77)	137 (±0.77)	136 (±0.77)	135 (±0.78)	0.85
	Model 2[2]	137 (±1.07)	135 (±0.82)	137 (±0.77)	135 (±0.81)	135 (±1.09)	0.45
DBP	Unadjusted	83.5 (±0.51)	81.5 (±0.50)	82.4 (±0.55)	80.9 (±0.53)	80.7 (±0.48)	<0.001
	Model 1	82.0 (±0.49)	81.3 (±0.48)	82.6 (±0.48)	81.4 (±0.48)	81.7 (±0.49)	0.71
	Model 2	82.5 (±0.67)	81.6 (±0.51)	82.5 (±0.48)	81.1 (±0.51)	81.2 (±0.68)	0.26

[1]Model 1: age, BMI, smoking status, physical activity, education level, antihypertensive medication use

[2]Model 2: model 1 + baseline MI or diabetes, family history stroke, family history MI, alcohol intake, dietary sodium, potassium, ratio Ca:Mg, total energy and calcium supplement use (including contribution from medication)

Blood Pressure

In men a significant inverse association was identified between dietary magnesium intake and SBP and DBP ($P<0.01$ and $P=0.01$ respectively) after full adjustment for confounding variables **(Table 3.12)**. A significant difference in extreme quintiles was also seen. This difference was 7 mmHg for SBP ($P\leq0.001$) and 3.8 mmHg for DBP ($P=0.01$). In women no significant association was seen with either SBP or DBP after adjustment for relevant confounding variables including age, smoking status, physical activity and dietary factors such as total energy, sodium, potassium and the ratio of dietary Ca:Mg intakes **(Table 3.13)**.

Table 3.14 Association of quintiles of dietary magnesium intake (range and mean quintile intake) and blood lipids (means and SE) in 1888 men, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary magnesium intake					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		85.1-243 mg 207 mg n=378	**244-286 mg 266 mg n=378**	**287-329 mg 308 mg n=377**	**330-386 mg 357 mg n=378**	**387-829 mg 458 mg n=377**	**P trend**
Total Cholesterol	Unadjusted	6.17 (±0.06)	6.07 (±0.06)	5.96 (±0.06)	6.02 (±0.06)	5.86 (±0.05)	<0.001
	Model 1[1]	6.14 (±0.06)	6.06 (±0.06)	5.97 (±0.06)* [3]	6.03 (±0.06)	5.88 (±0.06)**	0.003
	Model 2[2]	6.14 (±0.07)	6.06 (±0.06)	5.97 (±0.05)*	6.02 (±0.06)	5.89 (±0.07)*	0.04
Triglycerides	Unadjusted	1.89 (±0.05)	2.02 (±0.05)	1.86 (±0.05)	1.85 (±0.04)	1.83 (±0.05)	0.06
	Model 1	1.85 (±0.05)	1.98 (±0.05)*	1.87 (±0.05)	1.87 (±0.05)	1.88 (±0.05)	0.77
	Model 2	1.92 (±0.06)	2.00 (±0.05)	1.86 (±0.05)	1.85 (±0.05)	1.82 (±0.06)	0.06
HDL	Unadjusted	1.21 (±0.02)	1.19 (±0.02)	1.21 (±0.02)	1.27 (±0.02)	1.26 (±0.02)	<0.001
	Model 1	1.21 (±0.02)	1.20 (±0.02)	1.21 (±0.02)	1.26 (±0.02)*	1.25 (±0.02)	0.02
	Model 2	1.23 (±0.02)	1.21 (±0.02)	1.21 (±0.02)	1.26 (±0.02)	1.24 (±0.02)	0.23
LDL	Unadjusted	4.11 (±0.05)	3.97 (±0.05)	3.91 (±0.05)	3.91 (±0.05)	3.77 (±0.05)	<0.001
	Model 1	4.09 (±0.05)	3.97 (±0.05)	3.92 (±0.05)*	3.92 (±0.05)*	3.77 (±0.05)***	<0.001
	Model 2	4.04 (±0.06)	3.95 (±0.05)	3.92 (±0.05)	3.93 (±0.05)	3.83 (±0.06)*	0.06
HDL:LDL	Unadjusted	0.31 (±0.01)	0.32 (±0.01)	0.33 (±0.01)	0.35 (±0.01)	0.36 (±0.01)	<0.001
	Model 1	0.32 (±0.01)	0.33 (±0.01)	0.33 (±0.01)	0.34 (±0.01)**	0.36 (±0.01)***	<0.001
	Model 2	0.32 (±0.01)	0.33 (±0.01)	0.33 (±0.01)	0.34 (±0.01)	0.35 (±0.01)*	0.02

[1]Model 1: age, BMI, smoking status, physical activity, education level, baseline MI or diabetes, family history stroke, family history MI, statin medication use

[2]Model 2: model 1 + alcohol, dietary total fat intake, ratio Ca:Mg, total energy and calcium supplement use (including contribution from medication).

[3]P value for significance compared with Q1: *= P value ≤0.05, **= P value ≤0.01, *** = P value ≤0.001

Table 3.15 Association of quintiles of dietary magnesium intake (range and mean quintile intake) and blood lipids (means and SE) in 2380 women, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary magnesium intake					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		48.3-204 mg 176 mg n=476	**205-240 mg 223 mg n=476**	**241-274 mg 258 mg n=476**	**275-318 mg 295 mg n=476**	**319-692 mg 375 mg n=476**	**P trend**
Total Cholesterol	Unadjusted	6.60 (±0.06)	6.33 (±0.06)	6.21 (±0.05)	6.29 (±0.05)	6.13 (±0.05)	<0.001
	Model 1[1]	6.45 (±0.05)	6.32 (±0.05)* [3]	6.24 (±0.05)**	6.33 (±0.05)	6.22 (±0.05)**	0.01
	Model 2[2]	6.45 (±0.06)	6.32 (±0.05)	6.24 (±0.05)**	6.34 (±0.05)	6.22 (±0.06)*	0.09
Triglycerides	Unadjusted	1.76 (±0.04)	1.56 (±0.03)	1.56 (±0.03)	1.57 (±0.04)	1.46 (±0.03)	<0.001
	Model 1[1]	1.64 (±0.03)	1.54 (±0.03)*	1.58 (±0.03)	1.60 (±0.03)	1.53 (±0.03)*	0.14
	Model 2[2]	1.73 (±0.04)	1.57 (±0.03)***	1.58 (±0.03)**	1.57 (±0.03)**	1.45 (±0.04)***	<0.001
HDL	Unadjusted	1.49 (±0.02)	1.57 (±0.02)	1.57 (±0.02)	1.60 (±0.02)	1.59 (±0.02)	<0.001
	Model 1[1]	1.51 (±0.02)	1.58 (±0.02)**	1.56 (±0.02)	1.59 (±0.02)**	1.57 (±0.02)*	0.03
	Model 2[2]	1.49 (±0.02)	1.57 (±0.02)**	1.56 (±0.02)**	1.60 (±0.02)***	1.60 (±0.02)**	0.01
LDL	Unadjusted	4.32 (±0.05)	4.06 (±0.05)	3.93 (±0.05)	3.99 (±0.05)	3.88 (±0.05)	<0.001
	Model 1[1]	4.20 (±0.05)	4.04 (±0.05)*	3.96 (±0.05)***	4.02 (±0.05)**	3.96 (±0.05)***	<0.01
	Model 2[2]	4.18 (±0.06)	4.04 (±0.05)*	3.97 (±0.05)**	4.03 (±0.05)	3.97 (±0.06)*	0.08
HDL:LDL	Unadjusted	0.37 (±0.01)	0.43 (±0.01)	0.44 (±0.01)	0.45 (±0.02)	0.45 (±0.01)	<0.001
	Model 1[1]	0.39 (±0.01)	0.44 (±0.01)**	0.43 (±0.01)*	0.45 (±0.01)***	0.43 (±0.01)*	0.01
	Model 2[2]	0.39 (±0.01)	0.44 (±0.01)**	0.43 (±0.01)*	0.45 (±0.01)**	0.44 (±0.01)*	0.05

[1]Model 1: age, BMI, smoking status, physical activity, education level, baseline MI or diabetes, family history stroke, family history MI, statin medication use

[2]Model 2: model 1 + alcohol, dietary total fat intake, ratio Ca:Mg, total energy and calcium supplement use (including contribution from medication).

[3]P value for significance compared with Q1: *= P value ≤0.05, ** = P value ≤0.01, *** = P value ≤0.001

Lipid Profile

Analyses of associations of dietary magnesium intake and serum lipid levels were conducted on a sub-group of the cohort, additionally excluding participants with missing data for serum lipid levels (n=175). This approach differs to that presented in our paper 'The relationship between dietary magnesium intake, stroke and its major risk factors, blood pressure and cholesterol in the EPIC-Norfolk cohort' (281) where only associations between dietary magnesium intake and total cholesterol were assessed and thus those with missing data for LDL, HDL, and triglycerides (n=175) were not excluded as they have been in these sub-group analyses. As such there are slight differences in the presented results, most notably in the significance of the association between dietary magnesium intake and total cholesterol levels in women. In the article, we report a significant inverse association between dietary magnesium intake and total cholesterol in women. However, after exclusion of those with missing data for other lipid fractions the association is attenuated and becomes non-significant in the sub-group analyses presented in this chapter.

In the 1888 men, a significant inverse association was identified in relation to dietary magnesium intake and total cholesterol (P=0.04) after full adjustment for confounding variables including age, use of statin medication, and dietary intakes of alcohol, fat and total energy amongst other factors **(Table 3.14)**. No significant association was identified in relation to triglyceride or LDL levels; however, there was a non-significant inverse trend in both triglyceride and LDL levels and additionally a non-significant difference of 0.10 mmol/L between the extreme quintiles in the fully adjusted model for triglycerides. The difference in LDL levels of the extreme quintiles reached significance (P≤0.05) the difference was 0.21 mmol/L. There was no significant association between dietary magnesium intake and HDL levels in men, however, a significant positive association was identified in relation to the ratio of HDL:LDL (P=0.02). Additionally a significant difference of 0.03 mmol/L was seen between the extreme quintiles of the fully adjusted model. There were some contrasting results in associations of dietary magnesium intake and serum lipid levels in women **(Table 3.15)**. A significant inverse association was identified for total cholesterol (P=0.01) in the first model of adjustment for factors including age, smoking

status, and use of statin medication. However, in the fully adjusted model, with the addition of dietary factors such as total energy, total fat and alcohol intakes, the association was attenuated (P=0.09). There was still a significant difference of 0.05 mmol/L between the serum cholesterol levels of the extreme quintiles in the fully adjusted model (P≤0.05). There was a strongly significant inverse association between magnesium intakes and triglyceride levels (P<0.001), in the fully adjusted model, and a significant difference of 0.28 mmol/L was also identified (P≤0.001). A significant positive association was identified in relation to HDL levels and dietary magnesium intake after adjustment for model 1 (P0.03). This association was strengthened with the addition of dietary factors such as total energy, ratio of dietary Ca:Mg intake, total fat and alcohol intakes to the model (P=0.01). There was also a significant difference of 0.11 mmol/L between the extreme quintiles in the fully adjusted model (P≤0.01). For LDL cholesterol a significant inverse association was seen after adjustment for model 1 which included age, BMI, use of statin medication and smoking status amongst other factors (P<0.01), however, this association was attenuated with the addition of dietary factors in model 2 (P=0.08). There was however, still a significant difference of 0.21 mmol/L between the mean LDL levels of the extreme quintiles (P≤0.05). Finally a significant positive association was identified between the ratio of HDL:LDL and dietary magnesium intake. After adjustment for factors such as age and use of statin medication in model 1 the significance was P=0.01, this association was attenuated but remained significant with the addition of dietary factors in model 2 (P=0.05). Additionally there was a significant difference of 0.05 mmol/L between the extreme quintiles in the fully adjusted model (P≤0.05).

Table 3.16 Quintiles of dietary magnesium intake (range and mean quintile intake) at baseline (1993-1997) and stroke risk (HR and 95%CI), follow-up March 2008, in 2000 men, aged 40-75 in EPIC-Norfolk cohort.

	Dietary magnesium intake					
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	
	85-242 mg 206 mg n=400	**243-284 mg 266 mg n=400**	**285-328 mg 307 mg n=400**	**329-385 mg 355 mg n=400**	**386-829 456 mg n=400**	**P trend**
Stroke Events	126 (30.6%)	111 (26.9%)	93 (22.6%)	85 (20.6%)	75 (18.3%)	
Model 1[1]	1.0 (reference)	0.85 (0.60-1.20)	0.70 (0.49-1.00)	0.86 (0.60-1.24)	0.80 (0.55-1.16)	0.22
Model 2[2]	1.0 (reference)	0.86 (0.60-1.20)	0.68 (0.47-0.99)	0.81 (0.56-1.17)	0.74 (0.50-1.09)	0.11
Model 3[3]	1.0 (reference)	0.87 (0.61-1.25)	0.73 (0.50-1.06)	0.80 (0.55-1.17)	0.81 (0.53-1.22)	0.21

[1]Model 1: age, BMI, education status, physical activity, smoking status, alcohol intake

[2]Model 2: model 1 + serum total cholesterol, baseline MI or diabetes, family history stroke, or MI

[3]Model 3: model 2 + SBP, DBP, aspirin use >3 months, antihypertensive medication, ratio Ca:Mg and magnesium and calcium supplement use (including contribution from medication)

Table 3.17 Quintiles of dietary magnesium intake (range and mean quintile intake) at baseline (1993-1997) and stroke risk (HR and 95%CI), follow-up March 2008, in 2443 women, aged 40-75 in EPIC-Norfolk cohort.

	Dietary magnesium intake					
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	
	48-204 mg	**205-240 mg**	**241-274 mg**	**275-319 mg**	**320-692 mg**	**P trend**
	176 mg	**223 mg**	**258 mg**	**295 mg**	**374 mg**	
	n=489	**n=489**	**n=489**	**n=489**	**n=488**	
Stroke Events	152 (30.5%)	102 (20.5%)	87 (17.5%)	82 (16.5%)	88 (17.7%)	
Model 1[1]	1.0 (reference)	0.74 (0.53-1.05)	0.74 (0.51-1.06)	0.84 (0.59-1.20)	0.83 (0.57-1.20)	0.39
Model 2[2]	1.0 (reference)	0.71 (0.50-1.01)	0.71 (0.49-1.03)	0.82 (0.57-1.17)	0.76 (0.52-1.11)	0.23
Model 3[3]	1.0 (reference)	0.72 (0.50-1.04)	0.73 (0.50-1.08)	0.86 (0.59-1.26)	0.82 (0.54-1.24)	0.45

[1]Model 1: age, BMI, education status, physical activity, smoking status, alcohol intake

[2]Model 2: model 1 + serum total cholesterol, baseline MI or diabetes, family history stroke, or MI

[3]Model 3: model 2 + SBP, DBP, aspirin use >3 months, antihypertensive medication, ratio Ca:Mg and magnesium and calcium supplement use (including contribution from medication)

Table 3.18 Stroke risk (HR 95% CI) by magnesium groups (range and mean intake), bottom 10% (Group 1 reference category) and 3 groups of 30% intakes each, in 2000 men, aged 40-75 in EPIC-Norfolk cohort.

	Dietary magnesium intake				
	Group 1	**Group 2**	**Group 3**	**Group 4**	
	85-214 mg	**215-285 mg**	**286-353 mg**	**354-828mg**	**P trend**
	181 mg	**254 mg**	**318 mg**	**427 mg**	
	n=199	**n=605**	**n=591**	**n=605**	
Stroke Events	65 (32.7%)	157 (26.0%)	123 (20.8%)	104 (17.2%)	
Model 1[1]	1.00	0.73 (0.50-1.07)	0.63 (0.43-0.94)*[4]	0.67 (0.44-1.01) *	0.07
Model 2[2]	1.00	0.72 (0.48-1.07)	0.61 (0.41-0.92) *	0.61 (0.40-0.94) *	0.03
Model 3[3]	1.00	0.67 (0.45-1.01)*	0.60 (0.40-0.90) *	0.59 (0.38-0.93) *	0.04

[1]Model 1: age, BMI, education status, physical activity, smoking status, alcohol intake

[2]Model 2: model 1 + serum total cholesterol, baseline MI or diabetes, family history stroke, or MI

[3]Model 3: model 2 + SBP, DBP, aspirin use >3 months, antihypertensive medication, ratio Ca:Mg and magnesium and calcium supplement use (including contribution from medication)

[4]P value for significance compared with reference (Group 1): *= P value ≤0.05

Table 3.19 Stroke risk (HR 95% CI) by magnesium groups (range and mean intake), bottom 10% (Group 1 reference category) and 3 groups of 30% intakes each, in 2443 women, aged 40-75 in EPIC-Norfolk cohort.

	Dietary magnesium intake				
	Group 1	**Group 2**	**Group 3**	**Group 4**	
	48-180 mg	**181-240 mg**	**241-294 mg**	**295-691 mg**	**P trend**
	156 mg	**213 mg**	**267 mg**	**352 mg**	
	n=232	**n=745**	**n=740**	**n=726**	
Stroke Events	73 (31.5%)	165 (22.2%)	126 (17.0%)	115 (15.8%)	
Model 1[1]	1.00	0.73 (0.49-1.08)	0.70 (0.46-1.06)	0.74 (0.48-1.12)	0.27
Model 2[2]	1.00	0.67 (0.45-1.00) *[4]	0.65 (0.43-0.98) *	0.66 (0.43-1.02)	0.14
Model 3[3]	1.00	0.65 (0.43-0.99) *	0.65 (0.42-1.01)	0.69 (0.44-1.09)	0.27

[1]Model 1: age, BMI, education status, physical activity, smoking status, alcohol intake

[2]Model 2: model 1 + serum total cholesterol, baseline MI or diabetes, family history stroke, or MI

[3]Model 3: model 2 + SBP, DBP, aspirin use >3 months, antihypertensive medication, ratio Ca:Mg and magnesium and calcium supplement use (including contribution from medication)

[4]P value for significance compared with reference (Group 1): *= P value ≤0.05

Stroke risk

A modified Prentice weighted cox proportional hazards regression was used to determine hazard ratios of risk of stroke in association with quintiles and groups of dietary magnesium intake. There was no significant association between dietary magnesium intake, by quintiles of intake, and stroke risk in men **(Table 3.16)**. There was however, a non-significant trend towards lower risk with increasing intakes of dietary magnesium. To assess whether those with the very lowest magnesium intakes (lowest 10% of magnesium intakes) had the greatest risk of stroke we also stratified analyses by data-derived groups of magnesium intake. These groups were the lowest 10% of magnesium intake and 3 further groups (the lowest intake was used as the reference category). A significant association of reduction in risk with increasing magnesium intakes was identified in men after full adjustment for factors including age, BMI, smoking status, serum total cholesterol levels, family history of stroke, use of aspirin or antihypertensive medication and dietary factors including alcohol intake the ratio of Ca:Mg intake amongst other factors (P=0.04) **(Table 3.18)**. There was also a significant difference in the HR between those in the lowest 10% of dietary magnesium intakes and those in in the category with the highest intakes. HR for men consuming the highest magnesium intakes was 0.59 (95% CI 0.38-0.93). In women there was no significant association between dietary magnesium intake by quintiles and stroke risk **(Table 3.17).** Although non-significant the HR in Q5 indicated a potential lower risk of stroke with increasing magnesium intakes. For analyses by groups of magnesium intake, group 1 comprising of lowest 10% of dietary magnesium intakes was the reference category, there was a no significant association across the groups of magnesium intake **(Table 3.19)**. However, there was a significant difference in the HR of those in group 2, magnesium intakes 181-240 mg/d, compared with the lowest 10% of intakes, 48-180 mg/d (P≤0.05) HR 0.65 (95% CI 0.43-0.99). A decreased risk was also seen in those with the highest magnesium intakes, 295-691 mg/d, however this was non-significant, HR 0.69 (95% CI 0.44-1.09).

3.4 Discussion

The main findings from this chapter, summarised in **Table 3.0**, suggest that after adjustment for several important confounding factors including age, smoking status, history of MI or DM, medication use and total energy intake and other dietary variables there was a significant association between dietary magnesium intake and SBP and DBP in males. However, in females there was no significant association between dietary magnesium intake and blood pressure after adjustment for confounding variables. Total cholesterol was inversely associated with magnesium intake in men and positively associated with ratio of HDL:LDL in the fully adjusted model. For women a significant inverse association was identified in relation to triglyceride levels, whilst a significant positive association was shown in relation to HDL and the ratio of HDL:LDL after full adjustment. Dietary magnesium intake, stratified by quintiles, was not significantly associated with stroke risk in either sex. However, when investigating the effects of very low intakes (lowest 10% of dietary magnesium intakes) compared to higher intakes a significant inverse association was identified for men only.

Table 3.20 Summary of results investigating the relationship between dietary magnesium intake and blood pressure, serum lipid levels and stroke risk in men and women aged 39-80 years.

	Men	**Women**
Blood Pressure		
SBP	↓	↔
DBP	↓	↔
Cholesterol		
Total Cholesterol	↓	↔
Triglycerides	↔	↓
HDL	↔	↑
LDL	↔	↔
HDL:LDL	↑	↑
Stroke Risk		
Quintiles of magnesium intake	↔	↔
Groups magnesium intake	↓	↔

↔ - no significant association
↓ - significant inverse association
↑ - significant positive association

The strongest associations with blood pressure were found in males after adjusting for total energy, age, BMI, smoking status, physical activity, education level, baseline reported stroke, MI or DM, family history of stroke or MI, use of antihypertensive or aspirin medication, dietary intakes of sodium, potassium, total fat and alcohol, for SBP and DBP (P trend <0.001 and P=0.01 respectively) (Table 3.12). This difference in effect of dietary intakes on men and women may be due to in their consumption of other nutrients such as sodium and potassium which have been identified to have an influence on blood pressure. For example men had significantly higher sodium intakes compared with women (P<0.001) (Table 3.9).

Increased dietary magnesium intake was inversely associated with LDL and positively associated with HDL levels in men after adjustment for multivariable model 1. However, these associations were attenuated when adjusting for dietary covariates; total fat and alcohol intakes, total energy, ratio of dietary Ca:Mg intake and use of calcium supplements (Table 3.14). For total cholesterol and the ratio of HDL:LDL cholesterol the association remained significant (P trend = 0.04 and 0.02 respectively) after full adjustment. Significant differences were also identified between the extreme quintiles of dietary magnesium intake for serum total cholesterol, LDL and ratio of HDL:LDL cholesterol levels, small differences of 0.25 mmol/L, 0.21 mmol/L and 0.03 mmol/L for TC, LDL and ratio HDL:LDL respectively was also observed in men.

In women a significant inverse trend was identified between dietary magnesium intake and total cholesterol and LDL levels after adjustment for model 1 including age, BMI, smoking status, use of aspirin medication and educational status amongst other factors, but was attenuated with the addition of dietary variables (P trend 0.09 and P trend 0.08 for total cholesterol and LDL respectively in the fully adjusted model) (Table 3.15). However, significant differences of the TC and LDL levels of the extreme quintiles were also observed as was seen in men. The difference was 0.23 mmol/L for TC and 0.21 mmol/L for LDL between those with the highest and lowest dietary magnesium intakes. Despite being relatively small reductions, these differences may still be relevant for reducing stroke risk. Previously a decrease of 1 mmol/l in serum LDL concentration has been associated to decrease RR of total stroke by approximately 10% (280). A significant positive association

was found for HDL cholesterol level and dietary magnesium after adjustment in women, with a difference of 0.11 mmol/L between those with the highest and lowest dietary magnesium intakes. In men a significant positive trend was seen after adjustment for model 1 but was attenuated and became non-significant with the addition of dietary factors in model 2. This would suggest that for men dietary factors such as alcohol intake, total energy and total fat intakes have a greater influence on HDL levels than dietary magnesium intakes. Additionally a significant positive association was identified between dietary magnesium intake and the ratio of HDL:LDL cholesterol in women, which was maintained with full adjustment (P trend = 0.05). No significant association was observed between serum triglyceride levels and dietary magnesium intake for men. However, in women a strongly significant inverse association was identified after full adjustment for confounding factors (P trend <0.001).

The participants mean dietary intakes (Table 3.09) were mainly in line with the dietary reference value (DRV) set out for adult men and women in UK (216). Percentage energy intake from carbohydrates, total and saturated fat was slightly greater than the recommendations for both men and women. For both sexes percentage energy from MUFA and PUFA were in accordance with recommendations and despite protein intake being greater than the DRV, participant's intakes were in line with the average consumption of the general population (285) and percentage energy from protein was also similar to that of the general population.

Blood pressure and dietary magnesium Intake

The present findings agree with those of Joffres et al (165) who found a significant inverse association between dietary magnesium intake and SBP and DBP. Their cohort was relatively small (n=615) and exclusively comprised of elderly males, aged between 63-82 years, and conducted on a Japanese population and is therefore not necessarily directly comparable to this cohort. In addition they used a 24hr recall to assess dietary intakes which have previously been indicated to provide different intakes of a number of nutrients, particularly micronutrients when compared with 7DD (286). To my knowledge, to date, there has not been a directly comparable study conducted on a UK population. Joffres et al (165) reported the greatest reduction in blood pressure between the first and second

quartile, with a decrease of 4.6 mmHg and 2.8 mmHg for SBP and DBP. In the current analyses a more substantial difference in blood pressure was observed between those consuming lowest and highest dietary magnesium intakes with a difference of 7 mmHg (P<0.001) and 3.8 mmHg (P<0.01) for SBP and DBP respectively in males in the fully adjusted model. There were also, linear decreases in SBP, DBP across the quintiles in males (P trend 0.001 for SBP and 0.01 for DBP) (Table 3.12).

Witteman et al (166) also showed a potential benefit from increased consumption of magnesium, in women only. They reported almost a quarter of reduction in relative risk (RR 0.77 (95%CI: 12%-33%)) for developing hypertension, with magnesium intakes greater than 300 mg/d compared with intakes less than 200 mg/d. More recently, Song et al (244) indicated a potential inverse association between dietary magnesium intake and the development of hypertension. They reported a RR 0.91 (95% CI 0.83-0.99) for women in the highest quintile, with median dietary magnesium intakes of 400 mg/d, compared to those in the lowest quintile, with median intakes of 253 mg/d. The cohort was however, exclusively female. In the present analyses no significant association was identified between dietary magnesium intake in women (Table 3.13). These contrasting findings may in part be due to differences in the confounding variables included during adjustment. For example Witteman et al (166) did not adjust for several factors, such as smoking status and physical activity levels, which have been shown to influence blood pressure.

The majority of intervention trials investigating the effects of magnesium on blood pressure have used oral magnesium supplements. Many studies have reported significant reductions in blood pressure ranging from 2.0 – 12.0 and 2.7 – 8.0 mmHg for SBP and DBP respectively (270-273, 275). This range in effect size is likely due to variations in study methodology implemented, including the dose and type of magnesium supplementation used. The largest effects for both SBP and DBP were seen in the study by Dyckner and Wester (275) which involved hypertensive participants who were being treated with long term diuretics. Therefore it cannot be ruled out that the participants medication use may have influenced those findings, as the same dose of magnesium supplementation was used by Cappuccio et al (274) which showed no effect. Diuretics may have a negative effect on magnesium status. Therefore it is possible that participants in Dyckner and Wester's (275)

study were depleted in magnesium. It has been reported that depletion can lead to an increase in the bioavailability of dietary magnesium consumed. Another possibility may be in part attributable to differences in the length of supplementation. In Dyckner and Wester's (275) trial, supplementation was for 6 months compared to only 1 month in the study by Cappuccio et al (274). Therefore it may be that in order to obtain a beneficial effect from magnesium supplementation it may need to be taken on a more long-term basis. Future clinical trials and dietary intervention studies could aim to address this, in order to establish the long-term effects of increased magnesium intake.

A limited number of studies to date have assessed and investigated the effects of increasing dietary magnesium intakes on blood pressure in relation to stroke risk in UK general population. The present study is one of the few to have involved participants of both sexes, allowing for direct comparison of effects of sex on a given population.

Lipid profile and dietary magnesium intake

An abnormal lipid profile is an established risk factor for stroke, and the influence of certain aspects of the diet, specifically fat intakes, has been extensively studied. However, less research has, to date, investigated the effect of other dietary components including those of plant origins on the lipid profile.

A number of studies have investigated the effects of nut consumption on serum lipid levels, as nuts contain a number of potentially beneficial macro and micronutrients including unsaturated fats. They are also a good source of magnesium and the benefit associated with nut consumption may not solely be due to their unsaturated fat content (287, 288). Therefore it is possible that positive effects on lipid profile may at least in part be due to the magnesium content of nuts (Table 3.0). Although nut consumption was relatively low in this cohort there was a significant positive trend for higher nut and seed consumption with increasing dietary magnesium intakes (Tables 3.10 and 3.11). In addition to this, one of the primary contributing food groups to magnesium intake in the average UK diet (8), cereal and cereal products, also significantly increased across quintiles of magnesium intake in both men and women ($P<0.001$) (Table 3.10 and 3.11).

Several studies have investigated the effects of oral magnesium supplementation on serum lipid levels in diabetic patients with varied findings. Djurhuus et al (233) reported similar decreases in LDL levels after the supplementation period, of 24 weeks, (0.29 mmol/l) as were reported between the quintiles of dietary magnesium in the current analyses (0.21 and 0.21 mmol/l for men and women respectively). However, Eibl (276) did not report any significant effects on TC, HDL, LDL or triglyceride levels after 3 months supplementation with 30 mmol/l magnesium citrate (equivalent to 365 mg magnesium). Although these studies are not directly applicable to the general population or dietary magnesium, due to the potential differences in absorption and bioavailability of dietary and supplemental magnesium, they suggest magnesium could have a positive influence on serum lipid profiles.

Itoh et al (229) conducted a trial in healthy subjects and illustrated a benefit of supplementation with high doses of oral magnesium, 411 mg/d for women and 584 mg/d for men. The effect was reported for both LDL and HDL levels. Although, this benefit was not reported in relation to TC or triglyceride levels. A second small study on 16 healthy middle aged men by Kishimoto et al (279) indicated a potential improvement in postprandial lipid levels with magnesium supplementation. The authors hypothesised that magnesium supplementation, which significantly increased serum magnesium levels but not calcium, reduced absorption of fat by binding with fatty acids to form insoluble structures (279). Evidence suggests that postprandial elevated lipid levels may have atherogenic effects, particularly the presence of TG-remnants, which in high levels may decrease HDL concentrations whilst also increasing LDL concentration (279).

However, these supplementation trials are not necessarily comparable with observational studies of the general population, as the lowest supplementation doses were high ranging from 400-580 mg/d. The RNI is 300 mg/d and 270 mg/d men and women respectively. In addition to this currently, the average magnesium intake of the UK general population is below the RNI (285). There is also evidence to indicate that magnesium intakes, particular from supplements, of this level may result in adverse side effects including diarrhoea and nausea. Although these were not reported in participants during the course of either of these studies (229, 279).

Several studies have previously investigated stroke risk and dietary magnesium intakes, however, to our knowledge none have included general populations of both men and women simultaneously or concurrently analysed major risk factors as well as stroke risk (167-169, 171, 255-257, 261, 262, 289, 290). A number of these studies in large populations of American, Taiwanese and Northern European cohorts have reported no association between dietary magnesium intake and stroke risk (167, 168, 255, 256, 289, 290). However, several studies have reported significant associations in men (169), women (171), and men and women (261). Additionally, a large meta-analysis by Larsson et al (161), in 241,378 people, reported an inverse association between dietary magnesium intake, recorded by FFQ, and risk of stroke. The findings of this thesis indicated a non-significant trend across quintiles of dietary magnesium intake and stroke risk in men and women. However, a significant inverse trend (P=0.04) was identified when we compared men with the lowest 10% of magnesium intake with the remainder of the cohort. This finding would suggest that it is the very lowest magnesium intakes that may infer the greatest risk of stroke incidence in men.

3.4.1 Strengths and Limitations

The strengths of the present analyses include; the size of the cohort and prospective design, which reduces the susceptibility of the study to selection bias. Additionally adjustment for a number of potential confounding factors allows for the identification of dietary associations independent of known risk factors.

As with all epidemiological studies involving self-reported dietary intakes, there is the potential for response bias. However, in comparison with the government guidelines and the average intakes of the UK population this EPIC-Norfolk sub-cohort was representative of the general population. A number of participants had one or more missing values for variables of interest and were excluded from analyses. In the case of smoking status and the use of aspirin medication the observations with missing values were recoded and categorised into the current smoker and no category for aspirin medication use respectively. In this way it may be possible that individual observations have been misclassified. In addition to this, by excluding those participants with missing values for

some variables including; history at baseline of stroke or MI, BP measurements or lipid profile variables bias may have been introduced. Selection bias is also possible although the whole EPIC-Norfolk cohort was representative of the UK population. Additionally, truncation of the sample distribution would likely only attenuate the observed associations and therefore associations may actually be stronger than are presented.

The possibility of mis-reporting of dietary intakes also needs to be considered. When comparing the reported energy intake with EER there was evidence of under-reporting for the majority of the population across quintiles of dietary magnesium intake. With the exception of highest magnesium intakes, quintile 5, where the mean ratio of energy intake to EER suggested slight over-reporting by participants. However, analyses assessing the associations between dietary magnesium intake and risk factors for stroke, blood pressure and lipid levels, were adjusted for total energy intake and BMI and therefore I do not believe that the mis-reporting identified would have a substantial influence on the findings of theses analyses.

Despite adjusting for a number of relevant covariates it is still possible for residual confounding to occur, although the likelihood is reduced due to previous validation of dietary methods and results of EPIC-Norfolk cohort.

Finally due to the nature of this investigation analyses are restricted to one time point (baseline), and therefore do not take into account changes in dietary habits or lifestyle variables over the course of the follow-up.

3.5 Summary

In summary, from the review of the literature it was identified that the current research in relation to dietary magnesium intake and stroke risk, and risk factors; blood pressure and lipid profile, indicated a potential benefit of increased consumption of dietary magnesium. Gaps in the literature, of areas which to date have been understudied were also highlighted. This includes the requirement for observational studies involving both sexes to allow direct comparisons of differences in risk and risk factors in the sexes. Additionally there are limited studies to date involving cohorts that are representative of the general population, particularly within European countries and more specifically to the best of my knowledge no other study has been conducted in the UK.

Therefore further research was required to identify if dietary magnesium intake beneficially influences BP and lipid profile in this specific population independent of a number of confounding factors and therefore potentially also contribute to a reduction in overall stroke risk.

The results of the current analyses indicate the potential of increased dietary magnesium intakes to impact positively on blood pressure and lipid profile in men and women. It has been reported that a reduction of SBP and DBP by 1-3 mmHg and 4 mmHg respectively may reduce stroke risk by up to 30% and 23% for SBP and DBP respectively. In this cohort the effects on BP were stronger in males than females with differences in males of -7 mmHg and -4.3 mmHg, for SBP and DBP, between those with the highest and lowest dietary magnesium intakes. In females a difference of -1.4 mmHg was reported in relation to DBP only prior to adjustment for specific dietary confounders. Additionally in males a linear trend across the quintiles was also identified with all blood pressure variables which indicates that modest increases dietary magnesium intakes may be of benefit to the male population.

Significant inverse associations with TC in men and triglyceride levels in women were seen in relation to dietary magnesium intakes. Significant positive associations were seen in relation to HDL levels in women only and between the ratio of HDL:LDL in both men and

women and dietary magnesium intake. Despite differences in LDL levels between highest and lowest magnesium intakes being relatively small (0.21 mmol/L and 0.21 mmol/L for men and women respectively) they are still relevant in terms of reduction of stroke risk, as a 1 mmol/L decrease in LDL is associated with a 10% reduction in total stroke risk (280). This would indicate a potential benefit to the UK general public of increased dietary magnesium intake on lipid levels in relation to a reduction in stroke risk. Therefore dietary magnesium intakes may be important in reducing stroke risk in terms of beneficial effects on risk factors including blood pressure and lipid levels.

A significant decrease in stroke risk was identified in men for higher magnesium intakes compared with the lowest 10% of dietary magnesium intake (HR 0.59 (95% CI 0.38-0.93) P trend = 0.04). There was no significant association in women, and also no significant association in either sex when analysed by quintiles of dietary magnesium intake. Despite this, non-significant trends towards a lower risk of stroke were shown in both sexes across the quintiles and also in the data derived categorised analysis for women. In this instance the HR was 0.69 (95% CI 0.44-1.09) P trend = 0.27. These findings may suggest that it may be those with the very lowest dietary magnesium intakes that are most at risk and as a higher dietary magnesium intake may also influence stroke risk factors beneficially it may be an important addition to the diet to reduce the overall risk of stroke.

Chapter Four

DIETARY PROTEIN INTAKE AND CONTRIBUTION OF FOOD SOURCES

4.0 Introduction

In the early nineteenth century animal studies showed that diets free from nitrogen (thus containing no protein) lead to muscle and tissue wasting and subsequently premature death (291, 292) thereby illustrating that protein is an integral part of human requirements and dietary needs. Protein is a key nutrient for growth and development in early life and ongoing repair throughout the life-cycle. Protein has a number of roles in sustaining good physical health, including maintaining muscle condition and the prevention of sarcopenia (293, 294) and CHD (295). Although total protein is important, the subtypes of protein (animal and vegetable) may also be important for health. There is also evidence that total protein and intake of foods higher in protein such as soy (296), processed meats (175) and fish (297) may influence stroke risk and risk factors including blood pressure and lipid levels (140, 174, 175). There is also emerging evidence of potential effects of overall plant or animal intakes, although this is an understudied area (124, 174).

It is likely that dietary sources of protein differ between countries, within 10 European countries there was substantial variation in the reported meat intakes alone (298). In addition dietary patterns and nutrient intakes have also been shown to differ between European countries and therefore it is likely differences would also exist with other populations across the world (299, 300). To date there is limited data investigating the main contributing sources to total protein and subtypes in the UK diet. As illustrated above the composition of food sources contributing to total protein and subtypes likely differs between countries, this chapter therefore aims to address this gap in knowledge of the UK diet.

Proteins are synthesised from combinations of 20 individual amino acids and protein is the greatest source of nitrogen both in the human body and the diet (219, 292). All proteins, with the exception of proline, are comprised of the same basic structure, shown in **Figure 4.0,** of an amine ($-NH_3^+$) and carboxylic acid (-COOH) group, whilst the additional R group allows for specificity of function (219, 292).

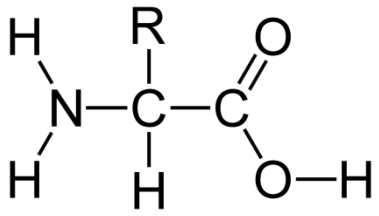


Figure 4.0 Basic Amino Acid Structure adapted from (301).

Amino acids are sub classified into one of three groups; essential, non-essential and conditionally essential. Essential amino acids are those that, under normal conditions, cannot be produced within the body at a rate required for normal growth and development. Therefore they must be obtained from dietary sources such as meat, eggs and dairy. Essential amino acids include leucine, methionine and phenylalanine amongst others (219). Non-essential amino acids are those which can be synthesised within the body such as alanine and aspartate and are present in higher quantities in plant foods (219). Conditionally essential amino acids are those which may in limited quantities be synthesised in the body, often only in specific tissues, and not necessarily at a rate applicable to growth or function. Conditionally essential amino acids also generally rely on the presence of another amino acid to act as the donor of a carbon or accessory group. This includes arginine, glycine and proline (302). Sources of protein (animal and plant) are composed of differing amino acids. Animal protein, such as meat and eggs, contain all the essential amino acids. Plant-based protein foods, such as whole grains, legumes, fruit and vegetables, contain lower levels of some amino acids such as lysine, threonine and tryptophan which may be present in rate limiting levels if not consumed with other sources as their synthesis can require additional amino acids, for example, citrulline for arginine synthesis (219).

The protein content of plant and animal foods differs substantially as is illustrated in **Table 4.0**. From this table it is evident that animal sources have higher protein content per serving than plant based sources. Therefore in order for plant sources to contribute highly to daily protein intake, they are required to be consumed more frequently or in higher quantities.

Table 4.0 The protein content in an average portion of a selection of animal and plant foods.

Food Item	Protein content per average portion (g)
Animal based foods	
Minced beef	34.6
Roast chicken	27.3
Semi-skimmed milk (glass)	7.00
Cheddar cheese	10.2
Plain whole milk yogurt	7.13
Boiled egg	6.25
Cod	25.7
Plant based foods	
Easy cook white basmati rice	4.68
White spaghetti	7.92
Brown bread (1 slice)	2.84
Weetabix (2 biscuits)	4.48
New potatoes	2.45
Baked beans	7.02
Red lentils (1 tbsp)	3.04
Peas	4.20
Carrots	0.36
Apple	0.44
Peach (canned in juice)	1.08

Based on calculations from McCance and Widdowson's 6th edition (189, 303).

This chapter will give an overview of the current knowledge of the contributing sources to dietary total protein and types of protein (plant or animal) intakes in adult populations. The subsequent chapter 'Chapter Five – Dietary protein intake and stroke risk factors and risk of stroke' is concerned with the influence of dietary total protein and types of protein on stroke risk factors, blood pressure and serum lipids, and risk of stroke in the EPIC-Norfolk sub-cohort. The evidence presented in this narrative literature review was obtained through searches of MEDLINE (Ovid) and PubMed and published reports from relevant organisations including Department of Health and the European Food Safety Authority. The search term dietary was combined with; protein, total protein, plant protein, animal protein and relevant key words such as intake* or source*. Titles and abstracts of returned search items were reviewed and the full text of relevant articles was obtained. The reference lists of full text articles were subsequently reviewed to identify additional relevant publications.

Dietary recommendations for protein intake

The European Food Safety Authority indicates a European Population Reference Intake of 0.83 g/kg of body weight (304), this value was deemed applicable to both high quality protein intakes and protein from mixed diets. The average requirements of European population were estimated to be 0.66 g/kg body weight (304). More specifically for the UK population the RNI for protein is 55.5 g/d for men aged 19-50 years and 53.3 g/d for men over 50. The RNI for women in the UK is 45.0 g/d and 46.5 g/d for ages 19-50 and over 50 years respectively (216). Although it is worth noting that these reference intakes for the UK population were last updated in 1991. In addition it is recommended that protein contributes to approximately 15% of total energy intake (305). In the UK National Diet and Nutrition Survey (NDNS) 2008-09 rolling programme, the current average percentage of energy from protein is 16.8% and 17.1% for adults aged 19-64 years in men and women respectively (306). There are currently no recommendations on the type of protein to consume. However, in older populations for healthier ageing intakes of 0.75 g/kg per day have been suggested (294). Additionally in relation to the development of sarcopenia specifically, age-related muscle loss, or in those with established sarcopenia, dietary

protein intake is suggested to be 1.0-1.2 g/kg per day (293) but the exact recommendation remains to be determined.

Difficulties in comparing discreet studies often arise due to differences in the classification and aggregation/disaggregation of food items. For example in the subsequent paragraphs some studies such as NDNS (221) and MONICA project (307) used aggregated meat and meat products as a variable whereas others such as Cade and Margetts (178) and NHANES (145) used disaggregated variables. Composite food items such as meat pie can either remain as aggregated foods and are categorised based on their predominant component, often meat, whereas disaggregated foods take account of each individual component of the food item. For example a meat pie could be crudely broken down into meat, sauce, vegetables and pastry respectively. This can lead to differences in the overall estimation of nutrient intakes, the rank order of contributing foods to protein intake, and also presents difficulties in making direct comparisons between studies. Some studies have also investigated protein intake and sources of protein for the whole population, whilst others have conducted sex-stratified analyses.

4.0.1 Sources of protein in the UK diet

The quality of protein may be dependent on the amino acid content of the food items but is also related to the bioavailability of amino acids. In developed countries the primary source of protein is meat and meat products, whilst in the developing world the major source is from cereals (308). In the NDNS (221) the mean daily protein intake for men and women was 88.2 g and 63.7 g respectively and the main sources of protein were meat and meat products (36% of intake), cereal and cereal products (23% of intake) and milk and milk products (16% of intake) (221). When comparing the meat consumption of European countries as part of the EPIC study, the adjusted mean total meat intake of the UK general population (72.3 g/d ±3.3) (EPIC-Norfolk cohort) was lower than the majority of other 26 EPIC centres, with the exception of Greece, Granada (Spain), Naples (Italy) and Oxford (UK) (298). However, it should be noted that the EPIC-Oxford cohort was comprised of health conscious individuals, and a large proportion were vegetarian, and was thus not representative of the general population (298).

Cade and Margetts (178) studied the dietary protein intakes of participants in three English towns, Ipswich, Stoke and Wakefield. Dietary intakes in the study were based on a 1-day record using household measures, where this was not completed a 24h recall was taken; ~20% of participants had recalls. The authors reported that the nutrient intakes of those providing a recall tended to be lower than those completing the 1-day record. Their study highlighted the primary contributing source to protein intake was white bread and flour (10.5% of total intake) in a cohort of 2,402 middle aged men and women aged 35-54 years. However, the primary food source of protein would have been different if a combined total meat intake was used, as was the approach in NDNS (178). Whole milk was the second highest contributing food to protein intake, accounting for 9.8% of protein intake. A total of 30 different food items contributed to 90% of protein intake in this population, illustrating that protein is obtained from a variety of sources. The majority of these (18 out of 30) were animal food items (including meat, fish and animal products). In terms of vegetable sources potatoes contributed 4.3% (7th highest item) and peas 1.0% of total protein intake (26th item). No fruit featured in the list. Cereal based products including wholemeal bread, cakes, puddings and biscuits contributed 2.3%, 1.9%, 1.9% and 1.3% of protein intake respectively.

A study on the food sources of protein intake in European countries included small sub-samples of the EPIC-Norfolk (general population), n=972, and EPIC-Oxford (health conscious), n=311, cohorts (309). Dietary intakes were recorded using 24h recall. Analyses were sex stratified and adjusted for age and weighted for day of recall and seasonality. The mean intakes of total protein were 91.2 (±1.9) and 70.8 (±1.1) for men and women in EPIC-Norfolk and 72.1 (±3.5) and 59.8 (±1.9) in EPIC-Oxford respectively. They also investigated the percentage contribution of subtypes of protein intake (animal, plant and unknown) to total protein intake. In men and women from the general population animal protein intake contributed similarly to total protein intake 58% and 57% respectively. However, in the health conscious EPIC-Oxford group the percentage intake of animal protein was higher in women 29% compared with 23% in men, in addition the percentage from unclassified protein sources was the same for both men and women. In the EPIC-Norfolk general population the percentage intake from plant protein was the same for men and women

34% of total protein intake (309). Halkjær et al (309) additionally ascertained the percentage contribution of food sources to animal and plant protein intake. Analyses were also sex stratified, age adjusted and weighted for seasonality and day of recall. They reported that for both men and women in the EPIC-Norfolk general population cohort the total meat intake contributed most highly to animal protein intake (55.3% and 50.4% for men and women respectively). Subgroup analysis of meat sources indicated that men had higher intakes of red meat (22.1% vs. 18.8%) and processed meat (15.1% and 8.3%) than women. Whereas women had higher intakes of poultry (19.2% vs. 17.3%), fish (12.6% vs. 10.3%), and eggs (4.3% vs. 3.0). In the EPIC-Oxford cohort, including a large number of lacto-ovo vegetarians and vegans, considerably lower mean intakes of meat, but higher intakes of dairy and eggs than the general population were reported (309).

4.0.2 Sources of protein in the European diet

Belgium

A Belgian study aimed to determine the association of plant and animal protein with overweight and obesity in 3,083 men and women aged 15 years and older (310). Dietary intake was ascertained using two non-consecutive 24hr recalls. The recalls were completed 2-8 weeks apart (with the median time being 3 weeks). In addition the dietary recall period of the whole study was spread throughout the year to account for variation in season across the population (310).

Lin et al (310) reported a mean animal protein intake of 47 g/d compared with plant protein which contributed 25 g/d to the total protein intake of 72 g/d. Total protein intake accounted for 15.4% of energy intake, whilst animal protein intake contributed to 64% of total protein intake. Men had significantly higher intakes of total, animal and plant protein than women ($P<0.001$ for all). Meat and meat products were the primary sources contributing 53% of animal protein, and cereal and cereal products contributed most highly to plant protein 54% (310).

They also noted that total protein intake in g/d tended to decrease with increasing age. However, as a percentage of energy contribution in adult life (19 years and older) there

was no significant difference between the age groups (19-59, 60-74 and 75 years and older). This was the same for men and women (310).

In terms of specific food contributions, dairy products contributed similarly to animal protein intake in men and women. However, in the age groups 60-74 and 75 years and older the percentage contribution to animal protein intake was significantly lower than for younger age groups (16-18 and 19-59 years). Men had significantly higher intakes of meat and meat products (g/d) than women for all age groups ($P<0.001$) and also for fresh meat, which included red meat and pork, $P<0.001$ for 15-18, 19-59, and 60-74, and $P=0.029$ for age group 75 years and older. There was no significant difference in intake of poultry between men and women at any age group. Women had significantly lower intakes of processed meat compared with men at all ages ($P<0.001$ for all). Fish and shellfish intake was only significantly different between men and women aged 60-74 years, at other ages women tended to have slightly lower intakes than men (but not significantly). A similar pattern was seen with egg consumption whereby there was no significant difference between intake of younger men and women, but in those aged 75 and older, men consumed significantly more animal protein from eggs than women ($P=0.009$) 1.4 g/d for men vs 0.75 g/d for women, although in real terms this intake is likely to be negligible. The percentage contributions to total energy intake across the sexes were relatively similar (310).

The majority of plant protein was derived from cereal and cereal products for both men and women, in all age groups. Cereal and cereal products accounted for 18-20% (13-18 g/d) of plant protein intake in adult men and 16-20% (9-12.5 g/d) of plant protein intake in adult women. This was followed by potatoes and other tubers contributing 3.4-4.8% (2.8-3.5 g/d) in adult men and 2.8-4.4% (1.7-2.5 g/d) in adult women and vegetables contributing 2.5-2.9 % (2.1-2.4 g/d) in adult men and 3.2-3.7% (1.9-2.3 g/d) in adult women. The difference in intake of cereal and cereal products and potatoes and tubers was significant between men and women ($P<0.001$ for all ages). There was no significant difference in the vegetable intake of men and women at any age (310). Women did however, tend to have a higher contribution to plant protein from combined fruit and vegetables than men. Legumes, soya products, and disaggregated fresh fruit and nuts and

seeds categories contributed only minimally to plant protein intake in this cohort of Belgian men and women (310).

France

In 1,912 French adults, dietary intake was assessed using a 7-day open ended food record and the nutritional adequacy of the diet was determined using PANDiet (311). PANDiet assessed the probability of nutritional adequacy of the diet on a scale, with a range of 0-100. A score of 100 indicates the highest nutritional adequacy and 0 indicates lowest nutritional adequacy. The study aimed to determine associations between the adequacy of nutrient intakes and protein intake from plant and animal sources (311).

In this cohort men had an energy adjusted mean total protein intake of 87.7 g/d (17.1% of energy) and women had an energy adjusted mean intake of 84.4 g/d (16.5% of total energy) ($P<0.0001$ for both g/d and %en). The difference in intake in both g/d and as a percentage of total energy was statistically significant between men and women. Men also had significantly higher intakes of animal protein in g/d than women, 62.1 g/d compared with 59.0 g/d ($P<0.0001$). However, there was no significant difference between animal protein intake as a percentage of total energy 12.0% for men and 11.7% for women or as a percentage of total protein intake 69.8% for men and 69.3% for women. Mean plant protein intake was 25.7 g/d for men and 25.4 g/d for women, which was not significantly different. However, men had a significantly higher percentage of energy from plant protein 5.1% than women 4.8% ($P<0.0001$). There was no significant difference in plant protein intake as a percentage of total protein 30.2% for men and 30.7% for women.

In terms of intake of specific animal protein foods in g/d, men had significantly higher intakes of red meat ($P<0.0001$), poultry ($P<0.05$) and processed meat ($P<0.01$) than women. Whereas women had significantly higher intakes of fish ($P<0.05$), and dairy products ($P<0.05$) including disaggregated milk ($P<0.05$), yogurt ($P<0.0001$) and other dairy products ($P<0.001$) but not cheese. There was no significant difference in egg intake. For intake of plant foods in g/d men had significantly higher cereals intake ($P<0.01$) and women had significantly higher intakes of nuts and seeds ($P<0.05$), and fruit ($P<0.001$) but there was no significant difference in intake of potatoes, vegetables or legumes.

There were also some reported changes in consumption pattern with age. For example in men in older age groups lower amounts of red meat were consumed, but higher amounts of offal and fish were eaten, perhaps due to offal being a more common constituent of the older generation's diets than the younger population. In women there was an overall increase in animal protein intake with age, with individual increases in offal, fish and yogurt consumption. In both sexes milk intake was lower in older individuals (aged 55-79) compared with younger age groups (18-34 years). There was higher fruit and vegetable intake in older age groups of both men and women, and potato intake in women only compared with younger age group. In women nuts and seeds intake was lowest in the oldest group (55-79 years). There was no significant change in poultry or processed meat, although intakes tended to be lower in older age groups. Egg and cereals intake was largely similar across the age groups for both men and women.

Total protein intake in this cohort was largely driven by animal protein intake for both men and women. Analysis of protein intakes from specific foods across quartiles of protein intake showed significant increases in a number of animal protein foods including muscle red meat, poultry and fish ($P<0.0001$ for all) and dairy proteins ($P<0.0001$) for both men and women. Fruit intake in men decreased significantly ($P<0.001$) across quartiles and vegetable intake in both men and women showed a significant positive trend with increasing total protein intake ($P<0.01$ for men and $P<0.0001$ for women). Whereas there was little change in intake of other plant protein food sources across quartiles of total protein intake for men and women.

Germany

In a German population of men and women aged 45-64, two 7-day weighed records completed as part of the MONICA Project were used to assess trends in sources of dietary nutrients (307). Survey two was carried out in 1994/95 close to the time the EPIC-Norfolk food diaries were completed. Meat and meat products were the highest source of protein and accounted for 44.0% of protein intake. In an earlier survey, completed in 1984-85, the contribution of meat was higher and accounted for 47.5% of protein intake (307). Milk products, including cheese, and bread and cereal products also contributed highly to protein intake 13.3% and ~20% respectively. Vegetables contributed more highly, 8.0%,

than fruit, 1.4%. Fish and eggs were also relatively small contributors to protein intake 4.4% and 5.1% respectively in this German cohort.

Spain

A population study of 4,701 Spanish men and women aged 10-75 years assessed dietary intake using two repeated 24hr recalls between 1992 and 93 (312). This study assessed protein intake for the whole population and also across a range of age groups. For the purpose of this thesis only age groups which correspond with the age range of the EPIC-Norfolk cohort (39-80 years) will be included. In addition the protein requirements of children and adolescents differs from that of the adult population due to increased demand to support growth and development. The total protein intake of adults in the Spanish cohort was 110.5, 96.4 and 90.2 g/d for men aged 25-44, 45-64 and 65-75 years respectively (312). Total protein intake was lower in women 85.8, 84.7 and 79.4 g/d for women aged 25-44, 45-64 and 65-75 years respectively. In both men and women animal protein intake contributed most highly to total protein intake in all age groups, 82.2, 70.9 and 64.8 g/d for men aged 25-44, 45-64 and 65-75 years respectively and 64.0, 65.3 and 56.7 g/d for women. Vegetable protein intake was higher in men 27.8, 24.5 and 24.2 g/d for men and aged 25-44, 45-64 and 65-75 years respectively than women 19.3, 19.0 and 18.6 g/d for women aged 25-44, 45-64 and 65-75 respectively (312).

The three main contributing sources to total protein intake as a percentage energy for the whole cohort (ages 10-75 years) were meats, accounting for 18% of intake, fish contributing to 14.7% of intake and poultry contributing 14.0% to total protein (312). These also formed the three highest contributing sources to animal protein intake. There was also a relatively high contribution from dairy products, 16.7%, and sausages, 9.9%. The majority of plant protein was from cereals, 51.4%, and this contributed 13% to total protein intake. Vegetables, pulses and fruits also contributed highly to plant protein intake 9.2%, 8.3% and 7.8% respectively but a lesser extent to total protein intake 2.3%, 2.1% and 2.0% for vegetables, pulses and fruits respectively (312).

Comparisons across European populations

In the previously mentioned study by Halkjær et al (309), there is further evidence of differences to protein intake and the contributing food sources across countries (309). In their study, which included 27 cohorts across 10 European countries, the highest total protein intakes were seen in Spanish populations. The San Sebastian cohort had the highest total protein intakes for both men and women, 144.0 and 102.2 g/d respectively. This was also reflected in the animal protein consumption where the San Sebastian cohort also had the highest intake. Animal protein contributed to 73% and 71% of total protein intake of men and women in the Spanish cohort. The lowest protein intakes, other than the health conscious group in the UK were from the Greek cohort with mean intakes of 88.5 and 62.2 g/d for men and women. Men and women in the Potsdam, Germany, cohort also had relatively low protein intakes, compared with other cohorts, 89.7 and 62.7 g/d for men and women (309). There was also wide variation in the mean intakes of animal and plant protein between the different cohorts. Animal protein intakes ranged from 105.2 g/d in the San Sebastian cohort to 16.5 g/d in the UK health conscious cohort in men and 66.8 g/d and 17.1 g/d for women in the same cohorts. There was also variation in the percentage contribution to total protein intake, 73% in Asturias, Spain, and 23 % in the health conscious UK group for men. In women the highest contribution to total protein was 71% in the San Sebastian cohort, Spain, and lowest was 29% in the health conscious UK cohort.

The contributing food sources to protein subtypes also tended to differ between countries. For example in the San Sebastian cohort where total protein and animal protein intakes were highest, red meat contributed 30.2% and 22.2% of intake for men and women. Whereas in Umeå, Sweden, it was only 21.9% and 18.7% respectively (note intakes were lower in health conscious UK cohort). There was also wide variation in the consumption of processed meat, the lowest contributions were seen in the Greek cohort where processed meat contributed 3.5% and 3.3% of animal protein intake for men and women respectively. This was actually lower than the health conscious UK cohort. The highest percentage contribution from processed meat was 29.7% and 20.9% for men and women in the Potsdam, Germany, cohort (309).

There was also a difference in the percentage contribution of food sources to plant protein intake. Cereals were the highest contributing source to plant protein intake in all cohorts. In men the highest contribution of 69.1 % in the Ragusa, Italy, cohort and in women it was 60.7% in the female only Naples, Italy, cohort. Vegetable intake tended to be the second highest source of plant protein for both men and women, followed by fruit. There was more variation in the intake of legumes, with higher intake tending to be in the more southern European countries for example 15.6% in Asturias, Spain and 0.1% in Aarhus, Denmark for men. The contribution of cakes to plant protein intake ranged from 1.4% in Ragusa, Italy, to 6.3% in Umeå, Sweden, in men. In women the range was 3.1% in Florence, Italy and Copenhagen, Denmark, to 8.1% in Umeå, Sweden. In some countries, Germany and Denmark, there was also relatively high percentage contribution of non-alcoholic drinks to plant protein intake. The highest was 13.1% and 11.0% for men and women respectively in Heidelberg, Germany, compared with lowest of 0.7% in men in San Sebastian and 1.4% for women in Murcia, both Spain (309).

Halkjaer et al (309) also highlighted the difference in protein intakes of men and women, and contribution of animal and plant protein to total protein intake. In addition there is evidence to suggest that the contributing sources to animal and plant protein intake also differ between sexes and it is therefore important to conduct sex-stratified analyses.

These differences in the overall intake of protein and also variation in the contributing sources to protein intakes illustrate the requirement to investigate the effects of dietary protein intakes and health outcomes in individual countries.

4.0.3 Sources of protein in the American Diet

In the US as part of the National Health and Nutrition Examination Survey (NHANES) the dietary sources of a number of nutrients, including protein, were determined. Data from 24h recalls completed between 1989-1991 highlighted the main contributing source to protein intake in American adults was beef (313) which contributed to 17.7% of energy from protein for adults. Poultry accounted for a further 13.7%. In an update of the NHANES study 2003-2006 the two highest contributing sources of protein in adults diets remained

beef and poultry, however poultry contributed slightly more to total protein intake than beef.

Using dietary data from NHANES 1988-1991 Smit et al (145) sought to establish intakes of animal and plant protein in US population. They reported a mean intake of 97 (±1.5) g/d total protein for men and 65 (±0.7) g/d for women. This was equivalent to 15% of energy intake for both men and women. Total animal protein intakes were 67.5 (±0.4) g/d and 66.1 (±0.4) g/d for men and women. This difference was statistically significant (P≤0.01). The highest individual source was beef 18.4% protein for men and 13.6% for women (P≤0.01). Total plant protein intakes were significantly higher in women, 33.9% of protein compared with men 32.5% (P≤0.01). Plant protein intake was largely made up of grain consumption accounting for 18.0% and 18.1% of protein intake for men and women respectively. Women had significantly higher fruit and vegetable intake than men 10.5% compared with 9.1% (P≤0.01). There was no significant difference in percentage contribution of legumes or nuts and seeds. In addition Smit et al (145) investigated at the ratio of animal:plant protein intake, and noted that men had a significantly higher ratio of animal:plant protein intake than women, 2.9 compared with 2.7 (P≤0.05).

4.0.4 Sources of protein in other countries

Japan

In a small study of 59 men and 60 women conducted in a rural Japanese population, the main contributing source of protein intake was found to be rice, accounting for 13.0% of protein intake (314). Intakes were recorded using four 3 day food diaries repeated over the period of a year to account for potential effects of different seasons on dietary intakes (314). Miso soup was the second highest contributing source to protein intake (8.7%) and intakes of fish also contributed highly (7.5 and 6.2% for roast and raw fish respectively). Two of these foods, Miso soup and roast fish, were the two primary sources of sodium in the diet accounting for a cumulative total of 24.5% of total sodium intake. In terms of stroke risk this is significant, as high salt intakes are a risk factor for stroke directly and also for blood pressure, which is a contributing factor to stroke risk.

Iran

In a cohort of 2,537 Iranian men and women aged 19-70 years, part of the Tehran Lipid and Glucose Study, total protein intake was significantly associated with higher animal protein intake ($P<0.05$) but not plant protein intake in men and women (173). There was also a significant trend towards a higher ratio of animal:plant protein intake with increase total protein intake in both men and women ($P<0.05$). Several differences in the association of total protein intake and ratio of animal:plant protein intake were noted between men and women. For example a higher total protein and ratio of animal:plant protein intake was associated with significantly lower waist circumference in women but not men. And a higher ratio of animal:plant protein intake was associated with significant lower fasting serum glucose in women, however a higher total protein intake appeared to have the converse affect (173). This suggests that it is perhaps the ratio of consumption of animal and plant protein that is of importance to reduce fasting glucose, and potentially other risk factors, rather than overall protein intake.

In summary this evidence demonstrates differences in the contributing sources to protein intake in different countries. In the UK Cade and Margetts (178) reported white bread and flour as the primary source of dietary protein. However, this was largely due to the methodology employed to disaggregate food items in this study, for example choosing to look at individual meat components e.g. beef as opposed to total meat intake which is more commonly used approach in other studies. More recently, the NDNS identified meat and meat products as the highest contributors to total and animal protein intake (221). This is similar to a study reporting on dietary intakes of a German population in 1994/95 where meat and meat products contributed to 44% of protein intake and in the US NHANES study beef was identified as the highest single contributing source to protein in %en accounting for 17.7 %en. In contrast to Western populations in a rural Japanese population rice was the primary source of protein, although fish was the second highest source of protein (314).

The majority of previous research has been focussed on total dietary protein intake and consumption of specific high protein foods such as red meat and fish. There has, to date, been less emphasis on the origin of protein being plant or animal based and how contributing sources differ across a range of intakes of animal and plant-based protein %en

and ratio of plant:animal protein (178, 307, 309-315). However, it is clear that the contributing sources to protein intake differ between the UK and other countries, and there has been limited research on the contributing sources to protein intake in the UK diet. The quality of dietary protein intake is an emerging field in terms of health outcomes, therefore it is important to establish the pattern of protein consumption in the UK diet so that the findings, where appropriate, may inform future dietary interventions and public health policy. To the best of my knowledge no other study has investigated how contributions of foods differ depending on the type of protein (animal, plant and ratio plant:animal). It is important to understand the contributions to protein intake and how they differ between subtypes of protein and sex. This knowledge may help to elucidate mechanisms of action of potential associations between dietary protein intake (including subtypes of protein) and stroke risk and risk factors.

4.1 Aims and hypotheses

This chapter aims to address research question 2, outlined in the introductory chapter, Chapter One.

2. “What are the main contributing sources of dietary protein intake, and how do the subtypes of protein (animal, plant and ratio of plant:animal) and food group sources of protein differ between men and women?”

2a. How do different groups of animal and plant-based protein intakes (animal-land, -marine, -derived and plant protein) contribute to intakes of total protein, total animal, total plant protein and the ratio of plant:animal protein. The hypothesis was that higher total protein intake would be associated with higher intakes of animal protein and lower intakes of plant-based protein.

2b. To investigate if there was a difference in the main contributing sources of protein for men and women. The hypothesis was that protein intake would come from different sources for men and women.

2c. To investigate whether there were differences in the main contributions for the different sources of protein (animal, plant and ratio of plant:animal). The hypothesis was that the contribution of protein (animal-land, -marine, -derived and plant protein) would differ across quintiles of subtypes of protein (animal protein, plant protein and ratio of plant:animal protein). This also included investigating how the contribution of individual food items of plant and animal origin, such as red meat, eggs, milk, bread, cereals and fruit and vegetables, contribute to the ratio of plant:animal protein intake specifically, as it was hypothesised this approach would be less prone to potential bias associated with total energy intake. The hypothesis was that the consumption of plant-based foods such as bread, cereal, fruits and vegetables would contribute more highly to protein intake with an increasing ratio of plant:animal protein intake and that consumption of animal-based foods would decrease.

4.2 Methods

This chapter is based on the dietary intakes of 4,443 men and women aged 39-80 years. These participants are a representative sub-sample of the larger (n>25,000) EPIC-Norfolk cohort. Full study methods including further detail on the study population are presented in Chapter Two – Subjects and Methods.

Dietary protein intake was measured using 7DD. Myself and another PhD candidate in nutritional epidemiology independently classified the protein composition of approximately 11,000 food items from the previously entered EPIC-Norfolk food diary database. Food items were classified to contain one or more of the following types of protein; animal-land, animal-marine, animal-derived and plant protein, more detail on the criteria for inclusion into each category is detailed in the subsequent paragraph. After independently classifying food items, we compared results and discussed discrepancies. For food items where we were unsure of their classification we further discussed classification with collaborators at EPIC-Norfolk. In some instances foods were not able to be classified according to source of protein and these were incorporated into an unclassified group.

The types of food and food items included in each of these categories were as follows; animal-land encompassed items which included meat from land based animals (such as beef, poultry, and pork). Animal-marine was comprised of items containing fish or seafood. Animal-derived included items where animal products, such as milk, eggs and lard formed part or all-of the items. Plant-protein was food items from any plant based source including grains, pulses, fruits and vegetables. In addition there was a small 'unclassified' category which included products such as honey. These products were deemed to not substantially influence overall protein intake and therefore their exclusion from other groups is unlikely to influence findings and conclusions. It was possible that items could contain more than one source of protein. For example 'fish in white sauce' would be categorised to include animal-marine, animal-derived and plant-protein for the fish, milk and plain flour components respectively. Collaborators at EPIC-Norfolk then assigned the amount of

protein from each subtype (animal-land, -marine, -derived, plant protein) during the creation of new variables.

Statistical analysis

Sex stratified analyses were conducted due to the differences in dietary requirements of men and women and to reflect the stratification by sex throughout the thesis. Dietary intakes were stratified by quintiles of type of protein and included quintiles of protein as %en, animal protein %en intake, plant protein %en intake and the ratio of plant to animal protein intake. These categories were chosen to identify if there were differences in patterns of intake in those with higher and lower intakes of plant and animal based protein intakes. Additionally quintiles of ratio of plant:animal protein intake were used to determine if there were differences in the main contributing food items and the intake of nutrients and foods with established associations with stroke risk in those with a higher ratio of plant:animal protein (i.e. high plant-protein intake) than those with lower ratio (i.e. higher animal-protein intake). The mean and SD of each type of protein (animal-land, animal-marine, animal-derived and plant protein) were calculated for each quintile and expressed as g/d.

Variables of protein intake as a percentage of total energy were created by multiplying the protein intake in g/d by 4 (the number of kcal in one gram of protein), divided by total energy intake and multiplying by 100:

$$(\text{protein}*4/\text{total energy})*100$$

The ratio of plant:animal protein intake was calculated by dividing plant protein intake by animal protein intake.

Regression analysis was used to determine trends across quintiles of protein intake (including subtypes) as %en and significant differences in intakes compared with the lowest quintile. A P value was considered significant if ≤0.05.

4.3 Results

Baseline characteristics are as described in full in the previous chapter, Chapter Three – Influence of dietary magnesium intake on stroke risk and risk factors, Table 3.9. Briefly the cohort was comprised of 4,443 men and women, with a mean age of 61 and 60 years respectively. Forty five percent of the cohort were male. Mean BP was 140/85 and 136/82 mmHg for men and women respectively which was significantly different (P<0.001 for both SBP and DBP). TC was 6.07 (±1.10) and 6.36 (±1.22) in men and women respectively. Significantly more men than women reported taking aspirin, 13.6% compared with 8.1% (P<0.001), there was no significant difference in the use of antihypertensive medication. There was also no significant difference in the percentage of men and women reporting a family history of stroke, MI or diabetes. Women were more likely to have never smoked, but be less physically active than men. Men were most likely to be educated to A-Level or equivalent 44.4% (women 33.7%) whilst women were most likely to have no qualifications 44.5% (men (33.4%).

Dietary protein intakes were mostly significantly different between men and women **(Table 4.0)** with the exception of animal-land %en (P=0.11) and the ratio of plant:animal protein intake (P=0.82). Women had a slightly higher percentage of energy from total protein, 15.8% vs 15.0% (P<0.001), total animal protein, 67.8% vs 67.2% (P<0.001), animal-marine 1.54% vs 1.36% (P<0.001), animal-derived, 4.29% vs 3.69% (P<0.001) and plant protein 4.73% vs 4.64 (P=0.01).

Table 4.0 Mean intakes of dietary total protein and types of protein in grams/d, as a percentage of energy intake and as a percentage of protein intake for men and women (mean and standard deviation).

	Men n=2000	**Women n=2443**	**P value**
Total Energy kcal/d	2218 (±505)	1685 (±384)	<0.001
Total protein			
Total protein g/d	81.5 (±17.8)	65.1 (±14.1)	<0.001
Total protein %en	15.0 (±2.58)	15.8 (±2.87)	<0.001
Animal protein			
Animal protein g/d	55.0 (±14.8)	44.5 (±12.4)	<0.001
Animal protein % en	10.1 (±2.49)	10.8 (±2.81)	<0.001
Animal protein %protein	67.2 (±8.33)	67.8 (±8.77)	0.01
Animal-land g/d	27.4 (±12.7)	20.3 (±10.3)	<0.001
Animal-land %en	5.08 (±2.37)	4.96 (±2.60)	0.11
Animal-land %protein	33.3 (±12.6)	30.6 (±13.2)	<0.001
Animal-marine g/d	7.16 (±6.56)	6.23 (±5.24)	<0.001
Animal-marine %en	1.36 (±1.33)	1.54 (±1.38)	<0.001
Animal-marine %protein	8.79 (±7.88)	9.56 (±8.04)	0.001
Animal-derived g/d	20.4 (±8.93)	18.0 (±7.85)	<0.001
Animal-derived %en	3.69 (±1.40)	4.29 (±1.65)	<0.001
Animal-derived %protein	25.1 (±9.45)	27.6 (±10.1)	<0.001
Plant protein			
Plant protein g/d	25.5 (±7.94)	19.6 (±5.87)	<0.001
Plant protein %en	4.64 (±1.17)	4.73 (±1.21)	0.01
Plant protein %protein	31.5 (±8.20)	30.7 (±8.65)	0.001
Ratio plant:animal protein			
Ratio plant:animal protein g/d	0.50 (±0.23)	0.49 (±0.54)	0.82
Ratio plant:animal protein	0.50 (±0.23)	0.49 (±0.54)	0.82
Ratio plant:animal protein %protein	0.50 (0.23)	0.49 (±0.43)	0.82

Table 4.1. The contribution of four sources of protein (g/d), animal land, animal marine, animal derived and plant protein, stratified by quintiles of total protein intake as a percentage of total energy intake in 4443 men and women aged 39-80 years

	Total protein %en					
Men	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
	7.18-12.9%	**13.0-14.1%**	**14.2-15.2%**	**15.3-16.9%**	**17.0-32.2%**	**P trend**
	n=400	**n=400**	**n=400**	**n=400**	**n=400**	
Animal land (g/d)	20.9 (±10.5)	25.5 (±10.5)	27.6 (±11.1)	29.5 (±11.5)	33.5 (±15.8)	<0.001
Animal marine (g/d)	4.92 (±5.05)	6.33 (±5.67)	6.68 (±5.57)	8.05 (±6.29)	9.80 (±8.59)	<0.001
Animal derived (g/d)	20.6 (±9.35)	21.0 (±8.00)	20.9 (±8.51)	20.7 (±9.34)	19.0 (±9.25)	0.01
Plant (g/d)	26.3 (±8.30)	26.0 (±7.58)	25.5 (±7.12)	25.6 (±8.71)	24.0 (±7.74)	<0.001
Women	**8.13-13.2%**	**13.3-14.7%**	**14.8-16.1%**	**16.2-17.8%**	**17.9-30.8%**	
	n=489	**n=489**	**n=488**	**n=489**	**n=488**	
Animal land (g/d)	13.9 (±8.34)	18.7 (±8.36)	20.1 (±8.85)	22.6 (±9.63)	26.0 (±11.9)	<0.001
Animal marine (g/d)	4.48 (±4.73)	5.83 (±4.73)	6.48 (±4.93)	6.78 (±4.95)	7.56 (±6.19)	<0.001
Animal derived (g/d)	17.4 (±7.12)	19.1 (±7.43)	18.3 (±7.97)	17.8 (±7.12)	18.0 (±7.85)	0.40
Plant (g/d)	20.4 (±6.33)	20.4 (±6.04)	20.1 (±5.87)	19.2 (±5.22)	18.1 (±5.51)	<0.001

Animal-land includes all land-based meat and meat products such as beef, pork, sausages; animal-marine includes all fish and seafood; animal-derived includes all animal products such as milk, eggs and lard; plant is from any plant source including grains, fruit, vegetables nuts and legumes.

Total protein as percentage of total energy

Table 4.1 shows the contributions of different sources of protein across quintiles of total protein intake, expressed as a percentage of energy intake. As total protein intake increases there is a marked and significant increase in protein intakes from animal-land and animal-marine based protein sources (P trend <0.001 for animal-land and animal-marine for both men and women). These categories include items such as meat and meat products, fish and seafood, and also mixed food dishes, where the main contributing source to protein intake was deemed to be through the animal-land or animal-marine source. For example a fish or meat in a dairy based sauce that could not be disaggregated at this time into individual components. In this instance the main protein source was deemed to be from the meat or fish. In men there was a small but significant decrease in protein from animal-derived sources such as milk, eggs and cheese as total protein intakes increased (P trend 0.01). For both men and women there was a modest but significant decrease, of approximately 2 g/d, in protein from plant sources between extreme quintiles of total protein intake (P trend <0.001 for men and women respectively).

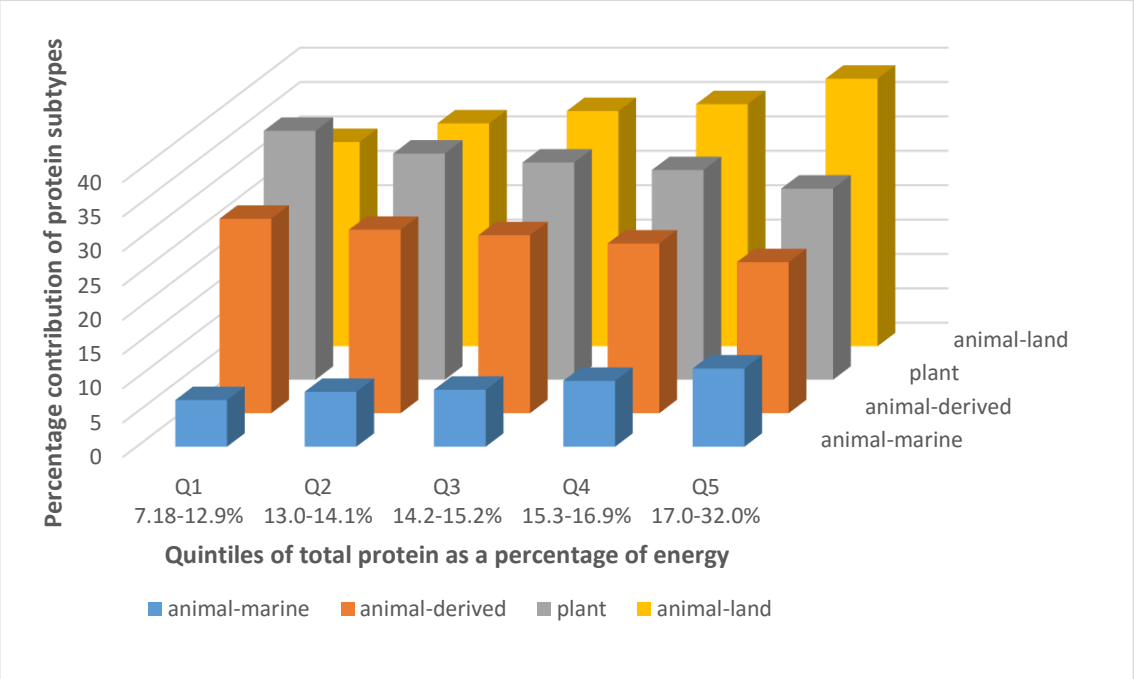


Figure 4.1. The percentage contribution of different types of protein intake (animal-land, -marine, –derived, and plant) to total protein intake %en in 2,000 men aged 39-80 years.

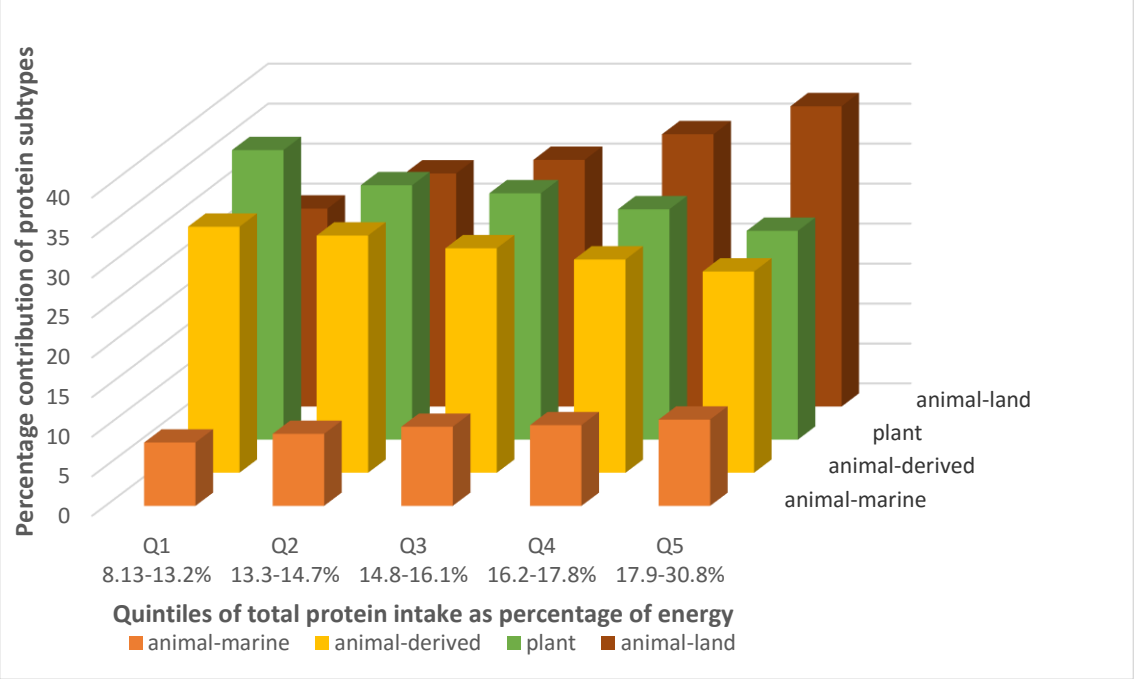


Figure 4.2. The percentage contribution of different types of protein intake (animal-land, -marine, -derived, and plant protein) to total protein %en in 2,443 women aged 39-80 years.

Figures 4.1 and 4.2 illustrate the percentage contribution of different types of protein (animal-land, -marine, -derived, and plant protein) across quintiles of total protein as %en intake for men and women respectively. In both men and women, the percentage contribution of animal-land increased sequentially with each quintile. In addition there was a steady decrease in the percentage contribution of plant protein intake with increasing total protein as %en intake. In men (figure 4.1) there was a greater increase in contribution of animal-marine across the quintiles than for women which was coupled by a slightly larger decrease in the proportion of consumption of animal-derived sources.

Table 4.2 The contribution of four sources of protein (g/d), animal land, animal marine, animal derived and plant protein, stratified by quintiles of total animal protein intake as a percentage of total energy intake in 4443 men and women aged 39-80 years

	Animal protein %en					
Men	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
	2.71-8.23%	**8.24-9.41%**	**9.42-10.4%**	**10.5-11.8%**	**11.9-27.4%**	**P trend**
	n=400	**n=400**	**n=400**	**n=400**	**n=400**	
Animal land (g/d)	18.5 (±9.50)	24.4 (±9.55)	28.1 (±10.6)	30.2 (±10.9)	35.8 (±15.2)	<0.001
Animal marine (g/d)	4.83 (±5.15)	6.02 (±5.02)	7.46 (±6.19)	7.73 (±6.34)	9.75 (±8.47)	<0.001
Animal derived (g/d)	18.9 (±8.72)	21.1 (±8.17)	21.1 (±8.46)	21.3 (±9.53)	19.8 (±9.51)	0.17
Plant (g/d)	30.5 (±9.23)	26.4 (±6.76)	25.7 (±7.12)	23.4 (±6.46)	21.4 (±6.77)	<0.001
Women	**0.43-8.58%**	**8.59-9.99%**	**10.0-11.2%**	**11.3-12.8%**	**12.9-26.0%**	
	n=489	**n=489**	**n=488**	**n=489**	**n=488**	
Animal land (g/d)	11.9 (±7.74)	18.4 (±7.48)	20.3 (±8.06)	23.5 (±9.11)	27.3 (±11.6)	<0.001
Animal marine (g/d)	4.27 (±4.25)	5.92 (±4.93)	6.40 (±4.73)	6.75 (±5.21)	7.79 (±6.22)	<0.001
Animal derived (g/d)	16.4 (±7.10)	17.9 (±6.84)	18.8 (±7.31)	18.5 (±8.01)	18.4 (±9.51)	<0.001
Plant (g/d)	23.5 (±7.11)	20.4 (±5.17)	19.5 (±4.79)	18.4 (±4.84)	16.5 (±4.63)	<0.001

Animal-land includes all land-based meat and meat products such as beef, pork, sausages; animal-marine includes all fish and seafood; animal-derived includes all animal products such as milk, eggs and lard; plant is from any plant source including grains, fruit, vegetables nuts and legumes.

Animal protein as percentage of total energy

As expected higher animal protein intakes were associated with increasing intakes of protein from animal-land and animal-marine sources **(Table 4.2)**. For men there was a difference of ~17 g/d and women ~15 g/d between the extreme quintiles of protein from animal-land sources (P trend <0.001). In men protein from animal-marine sources approximately doubled from the lowest to the highest quintile of animal protein (4.83 g/d to 9.75 g/d) (P trend <0.001). There was also a difference of protein from animal-marine sources in women of approximately 3 g/d between the extreme quintiles (P trend <0.001). The mean intakes of protein from animal-derived sources remained fairly consistent across the quintiles with small variation of 1-2 g/d for both men and women. There was no significant association between protein from animal-derived sources and intakes of animal protein in men (P trend 0.17). However in women there was a trend towards an increase in protein from animal-derived sources across the quintiles (P trend <0.001).

Table 4.3 The contribution of four sources of protein (g/d), animal land, animal marine, animal derived and plant protein, stratified by quintiles of plant protein intake as a percentage of total energy intake in 4443 men and women aged 39-80 years

	Plant protein %en					
Men	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
	1.44-3.67%	**3.68-4.23%**	**4.24-4.75%**	**4.76-5.51%**	**5.52-11.0%**	**P trend**
	n=400	**n=400**	**n=400**	**n=400**	**n=400**	
Animal land (g/d)	30.2 (±13.2)	29.8 (±11.9)	27.9 (±11.6)	26.4 (±13.2)	22.7 (±12.3)	<0.01
Animal marine (g/d)	7.24 (±7.24)	7.00 (±5.95)	6.94 (±6.17)	7.47 (±6.45)	7.14 (±6.94)	0.79
Animal derived (g/d)	22.4 (±9.98)	21.4 (±9.14)	21.1 (±8.59)	19.1 (±8.11)	18.3 (±8.08)	<0.001
Plant (g/d)	18.5 (±5.03)	22.8 (±4.88)	25.2 (±5.48)	27.5 (±6.20)	33.4 (±8.70)	<0.001
Women	**0.00-3.75%**	**3.76-4.34%**	**4.35-4.91%**	**4.92-5.58%**	**5.59-13.2%**	
	n=489	**n=489**	**n=488**	**n=489**	**n=488**	
Animal land (g/d)	23.0 (±11.4)	21.3 (±9.72)	21.1 (±9.21)	19.8 (±9.70)	16.1 (±10.1)	<0.001
Animal marine (g/d)	6.44 (±5.76)	6.42 (±5.07)	5.95 (±4.74)	6.30 (±5.43)	6.03 (±5.12)	0.21
Animal derived (g/d)	20.8 (±9.26)	18.7 (±8.05)	18.3 (±7.33)	16.9 (±6.38)	15.4 (±6.85)	<0.001
Plant (g/d)	14.6 (±3.91)	17.7 (±3.95)	19.8 (±3.98)	21.5 (±4.51)	24.7 (±6.90)	<0.001

Animal-land includes all land-based meat and meat products such as beef, pork, sausages; animal-marine includes all fish and seafood; animal-derived includes all animal products such as milk, eggs and lard; plant is from any plant source including grains, fruit, vegetables nuts and legumes.

Plant protein as a percentage of total energy

There were marked decreases in the intake of protein from animal-land sources across quintiles of plant protein in both men and women (P trend <0.001 for men and women) **(Table 4.3)**. However, protein from animal-marine sources remained relatively constant and was reflected in no significant trend (P trend = 0.79 and 0.21 for men and women respectively). There was a small, but significant, trend of lower intakes of protein from animal-derived sources with increasing plant protein %en in both men and women (P trend <0.001 for both). A difference of approximately 4 g/d and 5 g/d was identified for men and women respectively between the extreme quintiles. Protein from plant sources significantly increased, as expected, across the quintiles for both men and women (P trend <0.001 for both).

Table 4.4 The contribution of four sources of protein (g/d), animal land, animal marine, animal derived and plant protein, stratified by quintiles of the ratio of plant:animal protein intake in 4443 men and women aged 39-80 years

	Ratio plant:animal protein					
Men	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
	0.07-0.33%	**0.34-0.40%**	**0.41-0.48%**	**0.49-0.59%**	**0.60-2.71%**	**P trend**
	n=400	**n=400**	**n=400**	**n=400**	**n=400**	
Animal land (g/d)	34.4 (±14.2)	30.8 (±12.5)	27.9 (±10.6)	25.8 (±9.13)	18.1 (±10.4)	<0.001
Animal marine (g/d)	8.77 (±7.96)	7.49 (±6.48)	7.22 (±6.29)	6.36 (±5.24)	5.94 (±6.21)	<0.001
Animal derived (g/d)	22.0 (±10.2)	21.1 (±8.73)	20.8 (±8.68)	19.7 (±7.86)	18.7 (±8.75)	<0.001
Plant (g/d)	18.0 (±4.69)	22.5 (±4.74)	25.2 (±5.40)	28.0 (±5.62)	33.7 (±8.53)	<0.001
Women	**0.00-0.32%**	**0.33-0.38%**	**0.39-0.46%**	**0.47-0.57%**	**0.58-22.4%**	
	n=489	**n=489**	**n=488**	**n=489**	**n=488**	
Animal land (g/d)	26.3 (±11.7)	22.7 (±9.06)	21.4 (±8.33)	18.8 (±8.22)	12.1 (±7.94)	<0.001
Animal marine (g/d)	7.43 (±6.20)	6.94 (±5.35)	5.92 (±4.82)	614 (±4.81)	4.70 (±4.40)	<0.001
Animal derived (g/d)	20.8 (±9.65)	18.5 (±7.97)	18.2 (±7.15)	16.6 (±6.40)	15.9 (±6.76)	<0.001
Plant (g/d)	14.3 (±3.82)	17.4 (±3.54)	19.6 (±3.74)	21.5 (±4.43)	25.4 (±6.40)	<0.001

Animal-land includes all land-based meat and meat products such as beef, pork, sausages; animal-marine includes all fish and seafood; animal-derived includes all animal products such as milk, eggs and lard; plant is from any plant source including grains, fruit, vegetables nuts and legumes.

Ratio of plant:animal protein

As the ratio of protein from plant to animal sources increased there was a significant trend towards lower intakes of protein from animal-land, animal-marine and animal-derived sources in both men and women (P trend <0.001 for all) **(Table 4.4)**. In addition there was significant trend towards higher intakes of protein from plant sources across quintiles (P trend <0.001 for men and women). In men the protein from animal-land sources of the highest quintile of ratio plant:animal protein was approximately half that of the lowest quintile. There were also differences of approximately 3 and 4 g/d for protein from animal-marine and animal-derived sources between the extreme quintiles for men. Similarly in women the intake of protein from animal-land sources in the lowest quintile of ratio plant:animal was more than double that of the highest quintile and there were differences of 3 and 5 g/d for protein from animal-marine and animal-derived sources respectively.

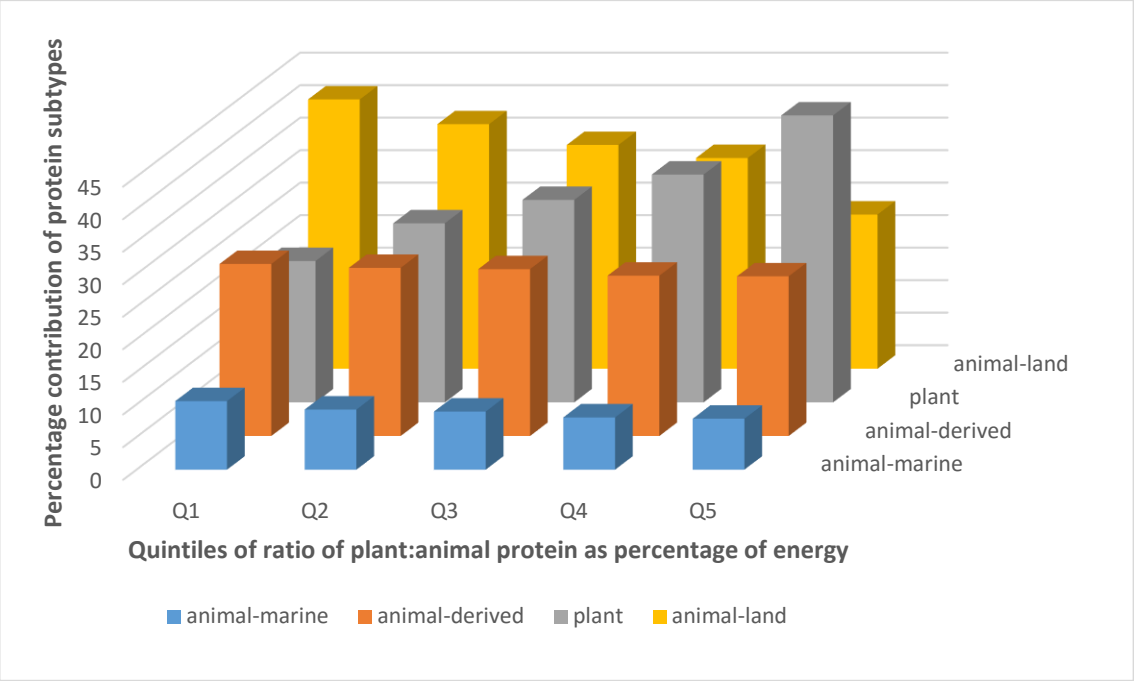


Figure 4.3 The percentage contribution of different types of protein intake (animal-land, -marine, -derived, and plant protein) across quintiles of ratio plant:animal protein in 2,000 men aged 39-80 years.

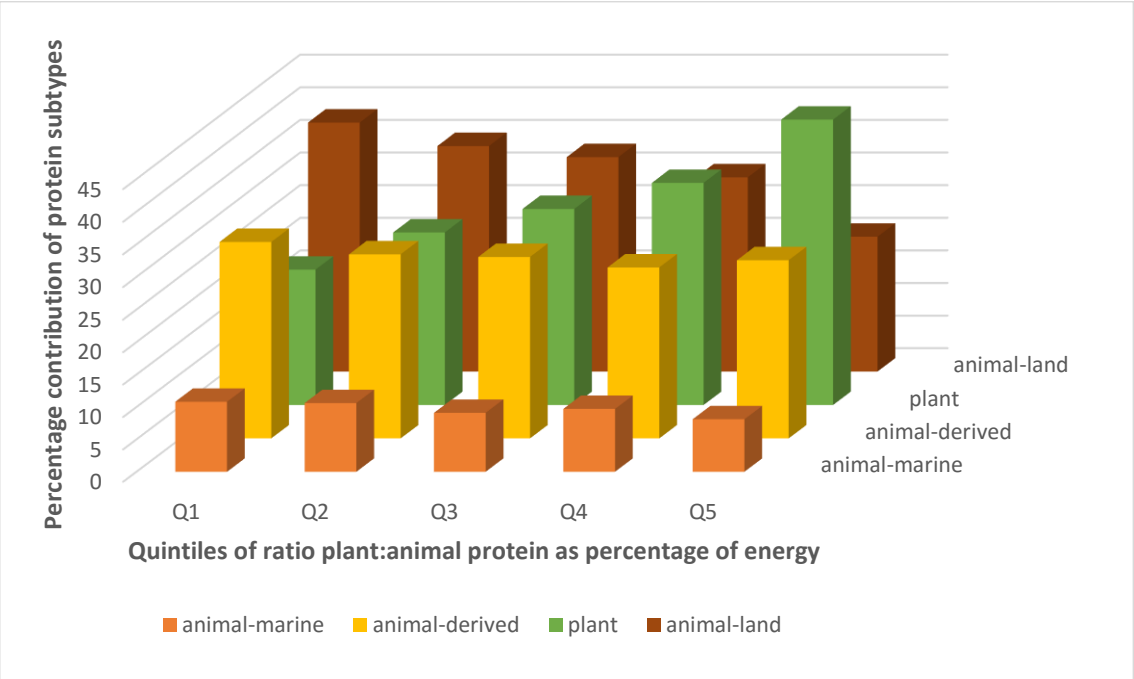


Figure 4.4 The percentage contribution of different types of protein intake (animal-land, -marine, -derived, and plant protein) across quintiles of ratio plant:animal protein in 2,443 women aged 39-80 years.

Figures 4.3 and 4.4 illustrate the differences in contributing sources of protein across quintiles of the ratio of plant:animal protein for men and women respectively representing data shown in Table 4.3. There was a similar percentage contribution of plant protein to total intakes for men and women. Differences in percentage contributing sources were mainly in relation to the proportion of animal-land and animal-derived consumption. In men animal-land contributed more highly to total intake than in women, whereas women had a higher percentage contribution from animal-derived sources. The percentage contribution of animal-marine was relatively similar for both men and women across quintiles of ratio plant:animal protein.

Table 4.5 The contribution of food items (g/d) stratified by ratio of plant:animal protein intake in 4,443 men and women age 39-80 years

	Ratio plant:animal protein					
Men	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
	0.07-0.33	**0.34-0.40**	**0.41-0.48**	**0.49-0.59**	**0.60-2.71**	**P trend**
	n=400	**n=400**	**n=400**	**n=400**	**n=400**	
Red meat (g/d)	25.7 (±33.7)	23.3 (±24.4)	21.0 (±25.4)	17.9 (±20.6)	11.0 (±17.9)	<0.001
Poultry (g/d)	18.5 (±24.0)	19.1 (±26.3)	17.1 (±20.6)	16.2 (±21.4)	11.8 (±19.7)	<0.001
Meat other (g/d)	122 (±96.1)	98.7 (±67.3)	93.4 (±66.7)	86.8 (±56.7)	64.3 (±59.4)	<0.001
Fish (g/d)	50.6 (±48.3)	42.5 (±38.8)	41.2 (±37.3)	36.6 (±30.7)	34.4 (±36.9)	<0.001
Eggs (g/d)	18.5 (±22.2)	17.2 (±19.3)	15.1 (±15.5)	15.2 (±19.9)	13.8 (±18.7)	<0.001
Milk (g/d)	234 (±186)	224 (±147)	218 (±148)	200 (±139)	196 (±137)	<0.001
Dairy other (g/d)	74.5 (±72.8)	73.2 (±66.9)	69.1 (±64.0)	72.5 (±60.0)	66.6 (±66.2)	0.12
Bread (g/d)	75.8 (±37.1)	101 (±45.8)	113 (±47.4)	125 (±51.9)	146 (±68.8)	<0.001
Cereal (g/d)	157 (±119)	161 (±107)	166 (±97.5)	162 (±91.1)	180 (±117)	0.01
Fruit and vegetables (g/d)	219 (±137)	237 (±137)	240 (±1430	262 (±143)	318 (±233)	<0.001
Nuts, seeds and legumes (g/d)	26.5 (±28.2)	30.5 (±26.3)	35.4 (±29.9)	38.2 (±33.1)	43.1 (±40.1)	<0.001
Women	**0.00-0.32**	**0.33-0.38**	**0.39-0.46**	**0.47-0.57**	**0.58-22.4**	
	n=489	**n=489**	**n=488**	**n=489**	**n=488**	
Red meat (g/d)	17.6 (±23.2)	16.2 (±18.2)	13.4 (±15.5)	13.0 (±17.3)	8.29 (±12.0)	<0.001
Poultry (g/d)	19.3 (±26.4)	16.9 (±21.0)	16.5 (±21.5)	14.5 (±17.6)	9.51 (±16.3)	<0.001
Meat other (g/d)	97.3 (±26.4)	75.0 (±51.8)	76.1 (±53.0)	63.9 (±48.6)	39.1 (±40.8)	<0.001
Fish (g/d)	41.8 (±38.4)	40.0 (±32.9)	32.8 (±27.3)	34.8 (±29.0)	27.4 (±28.7)	<0.001
Eggs (g/d)	12.9 (±14.8)	12.9 (±12.6)	12.3 (±13.6)	11.0 (±12.7)	10.8 (±16.4)	<0.01
Milk (g/d)	219 (±175)	195 (±147)	190 (±144)	171 (±117)	151 (±109)	<0.001
Dairy other (g/d)	92.3 (±95.2)	80.5 (±67.6)	77.1 (±62.2)	76.0 (±66.7)	75.3 (±67.7)	<0.001

Bread (g/d)	57.5 (±29.2)	69.1 (±28.9)	81.3 (±30.4)	86.7 (±36.0)	95.1 (±43.5)	<0.001
Cereal (g/d)	134 (±95.6)	128 (±81.5)	125 (±85.2)	128 (±78.4)	140 (±93.0)	0.35
Fruit and vegetables (g/d)	243 (±151)	276 (±144)	286 (±160)	312 (±167)	345 (±196)	<0.001
Nuts, seeds and legumes (g/d)	18.4 (±20.9)	22.5 (±20.3)	24.5 (±22.3)	25.7 (±22.8)	33.5 (±32.7)	<0.001

Meat other includes meat incorporated in dishes (poultry, red meat, offal and game) offal, game and meat products; dairy other includes all dairy except milk (e.g. cheese, yogurt, butter, cream); cereal includes breakfast cereals and other cereal and grains (e.g. rice, crispbreads); nuts, seeds and legumes includes all nuts, seeds and legumes (e.g. beans, lentils, peas).

In both men and women there was a significant trend towards lower intakes of red meat, poultry, other meat, fish, eggs and milk across quintiles of ratio of plant:animal protein intake **(Table 4.5)**. Indicating that as the proportion of plant protein in the diet increased there was a trend towards lower intakes of these food items (P trend <0.001 for all for men and women except eggs in women P trend 0.01). In men there was no significant trend with consumption of other dairy products (excluding milk) (P trend 0.12). However, in women there was a significant trend towards lower intakes of dairy with higher ratio of plant:animal protein intake (P trend <0.001). In both men and women there was a significant trend towards higher intakes of fruit and vegetables, and nuts, seeds and legumes across quintiles of ratio plant:animal protein intake (P trend <0.001 for both for men and women).

Table 4.6 Dietary intakes of selected nutrients and foods (g/d unless otherwise specified) associated with stroke risk and risk factors stratified by quintiles of ratio of plant:animal protein intake in 4,443 men and women aged 39-80 years.

	Ratio plant:animal protein					
Men	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
	1.44-3.67	**3.68-4.23**	**4.24-4.75**	**4.76-5.51**	**5.52-11.0**	**P trend**
	n=400	**n=400**	**n=400**	**n=400**	**n=400**	
Total fat	85.5 (±26.6)	86.0 (±24.2)	86.3 (±28.1)	85.7 (±24.8)	81.8 (±25.4)	0.06
MUFA	29.9 (±9.17)	30.0 (±8.67)	30.0 (±9.71)	29.9 (±8.92)	28.7 (±9.37)	0.09
PUFA	14.8 (±5.68)	15.6 (±5.32)	16.3 (±6.57)	16.4 (±6.01)	17.2 (±6.46)	<0.001
Saturated fat	33.5 (±12.4)	33.2 (±10.9)	32.7 (±12.1)	32.3 (±11.0)	29.2 (±10.9)	<0.001
Sodium (mg)	2986 (±903)	3149 (±853)	3178 (±840)	3205 (±794)	3234 (±907)	<0.001
Fruit and vegetable	219 (±137)	237 (±137)	240 (±143)	262 (±156)	318 (±233)	<0.001
Nut, seeds and legumes	26.5 (±28.2)	30.5 (±26.3)	35.4 (±29.9)	38.2 (±33.1)	43.1 (±40.1)	<0.001
Alcohol	16.2 (±19.1)	16.4 (±20.7)	16.1 (±22.6)	15.9 (±19.9)	14.9 (±21.4)	0.38
Women	**0.00-3.75**	**3.76-4.34**	**4.35-4.91**	**4.92-5.58**	**5.59-13.2**	
	n=489	**n=489**	**n=488**	**n=489**	**n=488**	
Total fat	66.4 (±22.3)	64.5 (±21.2)	65.6 (±19.4)	63.4 (±19.2)	62.9 (±20.5)	0.01
MUFA	22.9 (±7.85)	22.3 (±7.38)	22.6 (±6.81)	22.0 (±6.80)	21.7 (±7.55)	0.01
PUFA	11.6 (±4.87)	11.8 (±4.29)	12.1 (±4.15)	12.5 (±4.30)	13.2 (±5.09)	<0.001
Saturated fat	26.3 (±10.2)	25.0 (±9.68)	25.4 (±8.81)	23.6 (±8.80)	22.8 (±8.80)	<0.001
Sodium (mg)	2387 (±741)	2339 (±621)	2436 (±656)	2446 (±613)	2416 (±658)	0.08
Fruit and vegetable	243 (±151)	276 (±144)	286 (±160)	312 (±167)	345 (±196)	<0.001
Nut, seeds and legumes	18.4 (±20.9)	22.5 (±20.3)	24.5 (±22.3)	25.7 (±22.8)	33.5 (±32.7)	<0.001
Alcohol	8.13 (±12.7)	8.09 (±12.0)	7.99 (±11.4)	6.99 (±10.6)	7.30 (±11.7)	0.10

The difference in intakes of selected nutrients which can influence cardiovascular risk by quintiles of ratio plant:animal protein intake is shown in **Table 4.5**. In men there was no significant trend between intakes of total fat and MUFA (P trend 0.06 and 0.09 respectively). However, in women there was a small, but significant trend towards lower intakes of these nutrients with increasing ratio of plant:animal protein intake (P trend 0.01 for both). There were also contrasting associations between sexes for sodium intakes, in men there was a trend towards higher sodium intakes (P trend <0.001) whereas there was no significant trend in women (P trend 0.08). There was no significant association between alcohol intakes and the ratio of plant:animal protein intake in either men or women (P trend 0.38 and 0.10 for men and women respectively).

By distributing intakes of animal and plant protein as %en intake in sex-stratified tertiles it was possible to assess the relationship between the two types of protein. It tended to be that men in the lowest tertile of plant protein intake as percentage of energy were in the highest tertile of animal protein intake as %en (37.7% of population) **(Fig 4.5)**. And vice versa for the highest tertile of plant %en and lowest tertile of animal protein as %en (43.0% of population). This is also illustrated in relation to total protein intake as %en and animal protein % en **(Fig 4.6)** and plant protein as %en **(Fig 4.7)** in men. For animal protein (Fig 4.6) the majority of the population are in either the lowest tertiles of both total protein and animal protein as %en or the highest tertiles. There was a less stark contrast for plant protein as %en (Fig 4.7). The same was also true for women **(Fig 4.8, 4.9 and 4.10).**

Men

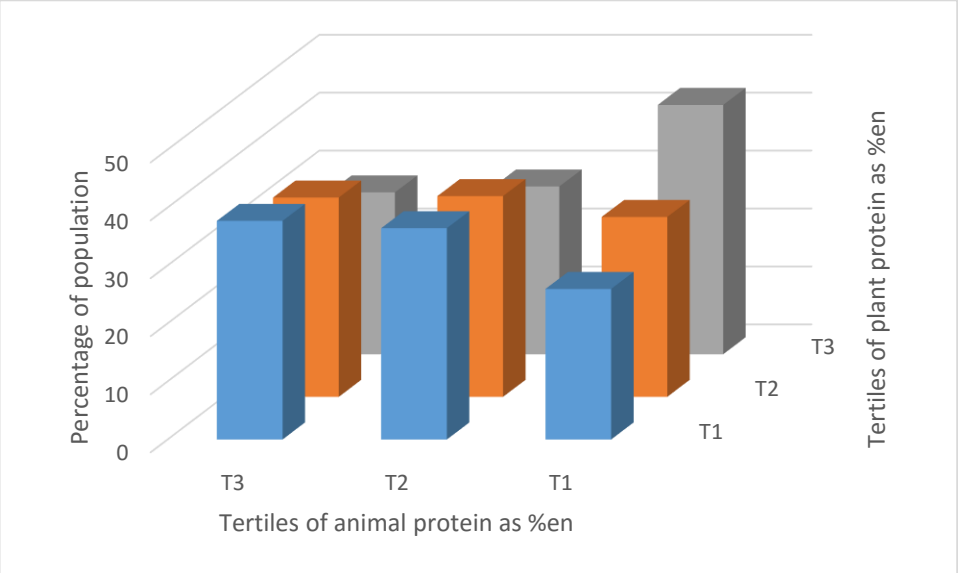


Figure 4.5 Percentage of the population in each of the tertiles of **plant and animal protein** as a percentage of total energy intake for 2,000 men.

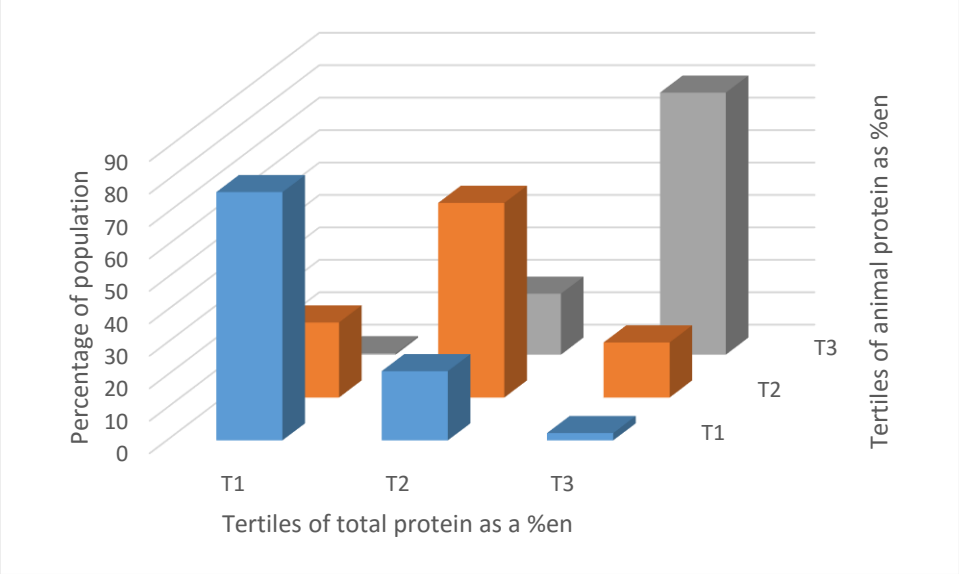


Figure 4.6 Percentage of population in each of the tertiles of **total protein and animal protein** as a percentage of total energy intake for 2,000 men.

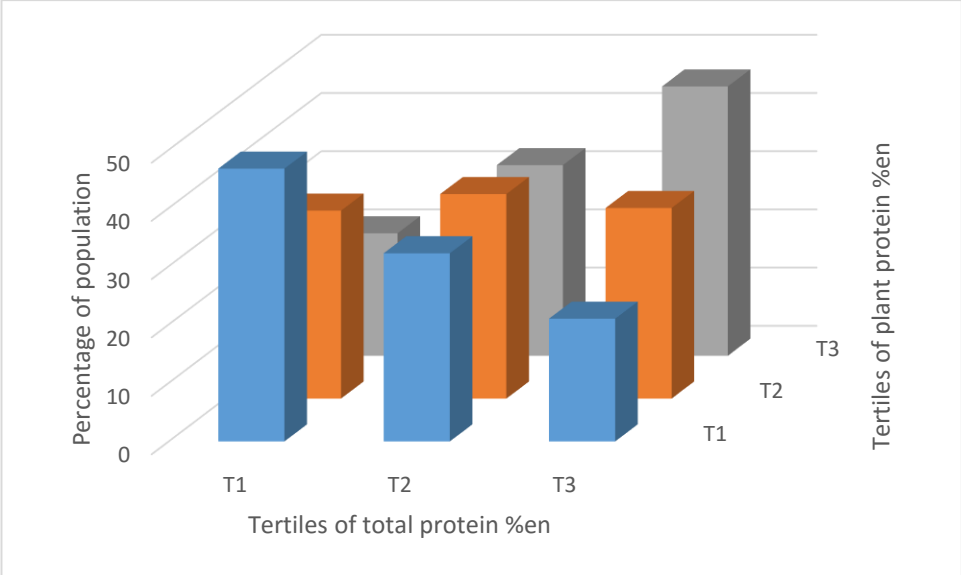


Figure 4.7 Percentage of the population in each of the tertiles of **total protein and plant protein** as a percentage of energy for 2,000 men.

Women

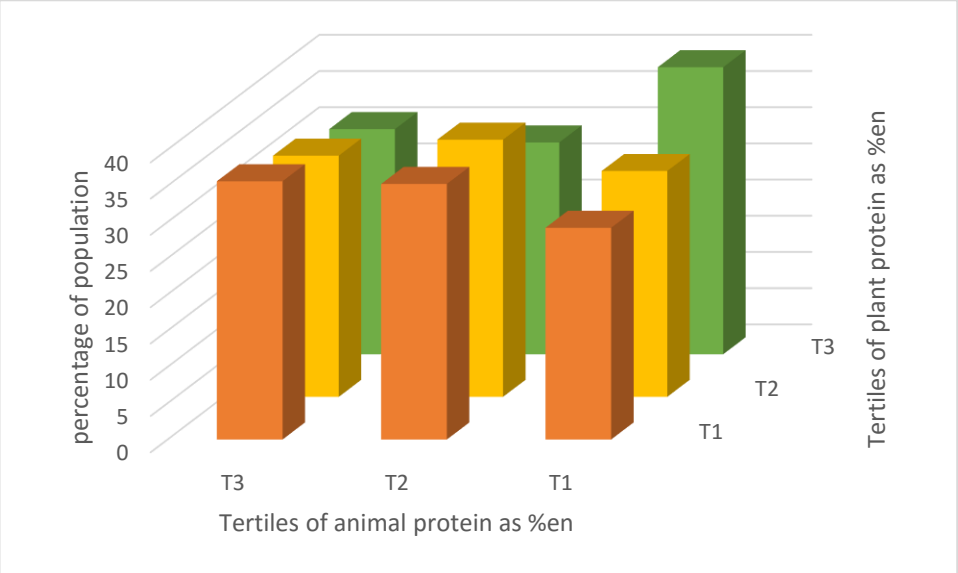


Figure 4.8 Percentage of the population in each of the tertiles of **animal and plant protein** as %en for 2,443 women.

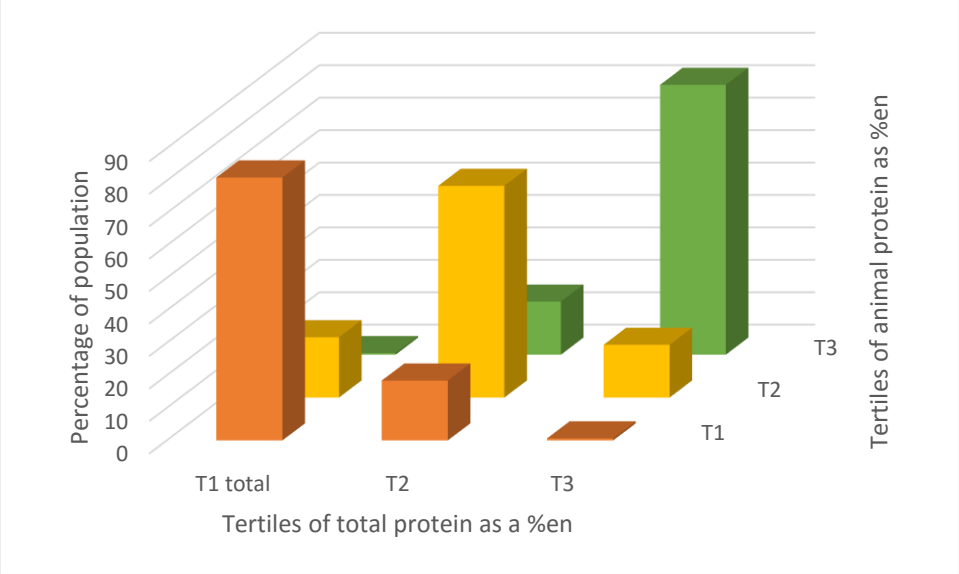


Figure 4.9 Percentage of the population in each of the tertiles of **total and animal protein** as a percentage of energy intake in 2,443 women.

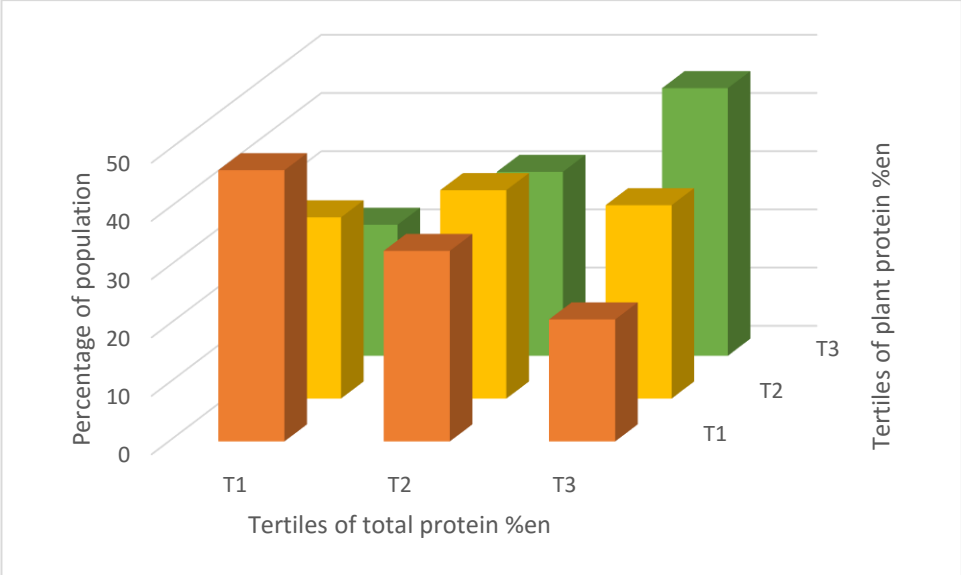


Figure 4.10 Percentage of the population in each of the tertiles of **total and plant protein** as a percentage of energy intake in 2,443 women.

4.4 Discussion

The findings of the present study indicate that as total protein intake increased there was a significant increase in protein intakes from animal-land and animal-marine based sources (P trend <0.001 for animal-land and animal-marine for both men and women) (Table 4.1). In men there was a small but significant decrease in protein from animal-derived sources (P trend 0.01) and protein from plant sources in both men and women (P trend <0.001 for men and women respectively) with increasing total protein intake (Table 4.1).

Women tended to have higher intakes of total protein %en, total animal protein %en, protein from animal-marine %en and animal-derived %en sources than men (P<0.001 for each respectively) and plant protein %en (P=0.01) (Table 4.0). There was no significant difference between protein from animal-land sources (P=0.11) or the ratio of plant:animal protein (P=0.82) in this cohort.

Previously studies in the UK and other Western and Asian countries have aimed to highlight the main sources of protein in the respective diets of these populations. Globally there are differences in the main contributing sources to protein intake, in more developed countries the primary sources of protein tend to be from meat and meat products. Whereas lower economically developed countries rely more heavily on plant protein to meet their needs.

To the best of my knowledge this thesis work describes the contributions to protein intake for the first time in the literature by investigating the differences in consumption across quintiles of different subtypes of protein intake (total, -animal, -plant and ratio plant:animal protein) as a percentage of total energy. The aim of this novel approach was to identify how different groups of protein contribute to intakes of total protein and protein subtypes. An additional aim was to determine if there is a difference in the contributing sources between men and women. Finally to establish if contributing sources differed with higher or lower intakes of protein (assessed across quintiles) and whether intakes of specific foods and nutrients also differed across quintiles of ratio of plant:animal protein, and whether these findings may partially explain potential differences in associations with stroke risk and risk factors between men and women.

Contribution of types of protein to protein intakes

The first hypothesis was that the amount of protein from different sources would differ across quintiles of intakes of total protein and subtypes and that a higher total protein intake would be associated with higher intakes of animal protein and concurrently lower intakes of plant protein.

As was hypothesised there were considerable differences in the sources of protein consumed across quintiles of different subtypes of protein. For example men in the lowest quintile of animal protein %en intake had a mean protein intake from animal-land sources of 18.5 g/d (Table 4.2) whereas those with the lowest %en from plant protein had 30.2 g/d from animal-land sources (Table 4.3). There were similar differences for women of 11.9 g/d animal-land compared with 23.0 g/d animal-land for the lowest quintiles of animal and plant protein as %en intake respectively (Tables 4.2 and 4.3 respectively). There was less difference in the amount of animal-marine and animal-derived protein sources between the quintiles of sub-types of protein. This was the case for both men and women therefore suggesting that the main contributors to the respective protein sources are animal-land and plant protein.

As would be expected across quintiles of animal protein as %en intake (Table 4.2) there was a significant trend towards higher intakes of animal-land, and animal-marine for both men and women ($P<0.001$ for all) and animal-derived for women only ($P<0.001$). In conjunction there was a significant inverse trend between animal protein as %en intake and protein from plant sources for both men and women ($P<0.001$ for both) (Table 4.2). Plant protein as %en intake was inversely associated with animal-land ($P<0.01$ and <0.001 for men and women respectively) and animal-derived intakes for both men and women ($P<0.001$ for both) (Table 4.3). Animal-marine intake was not significantly associated with plant protein as %en intake in men or women ($P=0.79$ and 0.21 respectively) (Table 4.3). Plant protein intake significantly increased across quintiles of plant protein as %en intake in both sexes ($P<0.001$ for both) (Table 4.3).

These findings may have significance in terms of stroke risk, as higher meat intake, particularly red and processed meat consumption, which was greater with higher

consumption of animal protein and specifically protein from animal-land sources, has been associated with increased risk of stroke (316, 317). Whereas there may be a beneficial effect of increased consumption of fish (318) and plant protein sources such as nuts (175) which tended to be lower in those with higher total animal protein intakes.

Differences between men and women

There were some small differences between the intakes of other foods and nutrients for men and women, which may have implications for stroke risk and risk factors (Table 4.5). In a study of French men and women, there was also a statistically significant difference between the total protein intakes of men and women in g/d and as a percentage of energy intake ($P<0.0001$ for both) (311). Similarly men in Belgium and Spain had higher protein intakes than women (310). In Belgium this included total protein, animal protein and plant protein g/d ($P<0.001$ for all). For specific food sources, red meat tended to contribute more highly to protein intake of men than women, this was also reported to be the case in other European countries (309-312) and the US (145). Whereas, fish and dairy consumption tended to be higher in women (145, 309). Although previous studies found the patterns of consumption for men and women may be similar between countries, there was wide variation in the percentage contribution that food sources made to total protein intake (145, 309) therefore it is important to understand the sources contributing to protein intake the in the UK diet as these may influence stroke risk and risk factors.

The higher intake of red meat reported in men, may also be reflected in the analysis which investigated the differences in proportion of nutrients and other foods related to stroke risk and risk factors such as dietary fat and sodium which also tended to be higher in men (Table 4.6). Across the quintiles of the ratio plant:animal protein intake men tended to have total fat intakes that were approximately 20 g/d higher than women (Table 4.6). They also had substantially higher sodium intakes which actually tended to increase with a higher ratio of plant:animal protein intake in men whereas for women sodium intake remained relatively stable across quintiles (Table 4.6).

Across quintiles of ratio of plant:animal protein in each of the quintiles men had slightly higher intakes of red meat and processed meat than women (Table 4.5). Although the

significance between sexes was not tested. For red meat the largest difference between men and women was in those with the lowest ratio of plant:animal (Table 4.5) (higher animal protein intakes). Men in quintile 1 consumed a mean of 25.7 g/d red meat compared with 17.6 g/d for women. However, in quintile 5, where there is a higher ratio of plant:animal protein, men were consuming on average 11.0 g/d and women 8.29 g/d (Table 4.5). As previously indicated, high consumption of red meat has been associated with increased risk of stroke in men and women (316, 317).

Contribution of food sources to protein intakes (including differences between men and women)

The final hypothesis was that foods contributing to protein intake would differ between quintiles of different types of protein (total, -animal, -plant, ratio plant:animal protein). However, I found this was not necessarily the case as the results of this chapter illustrate that the foods contributing to protein intake do not appear to differ substantially.

The differences in the consumption of specific food items across quintiles of the ratio of plant:animal protein were also investigated (Table 4.4). Men tended to have higher intakes of other 'Meat other'. This category includes all processed meat and meat products as well as meat dishes and offal. This would therefore indicate that men tend to have a higher consumption of processed meat, meat dishes and offal compared with women, which is not necessarily led by their consumption of plant based protein sources. The poultry intakes of men and women were relatively similar. Poultry intakes of men ranged from 11.8-18.5 g/d in the lowest to highest ratio plant:animal protein (Table 4.5). For women the range was 9.51-19.3 g/d. Women with lower ratios of plant:animal protein were consuming more poultry, a lean protein source, as opposed to red meat consumed by men (Table 4.5). In a study by Bernstein et al (175) it was reported that not only were higher intakes of red meat associated with increased risk of stroke, but also that higher poultry intakes, 1 serving/d compared with red meat, were associated with 27% decrease in stroke risk (175). Therefore the pattern of protein consumption of women in this, EPIC-Norfolk cohort, may be such that it is protective against stroke compared with men's pattern of consumption. It is important to note that these differences in types of foods consumed by men and women may influence the effects of protein intake on stroke risk factors and stroke risk.

For example, red meat and processed meat tends to be high in saturated fat. In addition processed meat products also often have a high salt content. These factors are known to influence blood pressure, lipid levels and stroke risk (319), therefore higher intakes of these products by men may influence the effects of protein on stroke risk.

In women there was little fluctuation in the consumption of eggs across the quintiles of ratio of plant:animal protein intake (Table 4.5). Although a significant trend towards lower intake was indicated across the quintiles (P trend <0.001), the actual difference was approximately 2 g/d for women (a medium egg weighs approximately 53g). In a meta-analysis Rong et al (320) concluded that there was no significant association between egg consumption and total stroke (P=0.27), and the RR associated with higher consumption of 1 egg per day was 0.91 (95% CI 0.81-1.02 P trend=0.10) but may be associated with reduced risk of haemorrhagic stroke in diabetic population specifically (320).

Men tended to have lower intakes of 'Dairy other' than women (Table 4.5). This group includes all dairy products except milk such as cheese, yogurt and cream. In men there was no significant trend between intakes of 'Dairy other' and quintiles of ratio of plant:animal protein in men (P trend 0.12) although there was a tendency towards lower intakes with higher ratio of plant:animal protein. However, in women there was a significant trend (P trend <0.001) with decreasing intakes of 'Dairy other' with an increasing ratio of plant:animal protein intake. The fat content of dairy products consumed may have an influencing factor on the effect of this group of food on stroke risk and risk factors. Previous research in a Swedish population indicated that the consumption of low fat dairy products was inversely associated with total stroke and ischaemic stroke (321). When comparing lowest intake (median 0 servings/d) with highest intakes (median 4 servings/d) of low-fat dairy products, after adjustment for confounding factors including age, sex, smoking, BMI, total energy, alcohol, fruit, vegetable and meat intakes, low fat dairy and high fat dairy intake, a RR 0.88 (95% CI 0.80-0.97 P trend=0.03) was reported for total stroke and RR 0.87 (95% CI 0.78-0.98 P trend=0.03) for ischaemic stroke (321). There was no differentiation between consumption of low and full-fat dairy products in the current analysis, however, due to the 'Dairy other' group containing cheese and cream it is likely these would be linked with high fat intake, but low-fat yogurt may have been consumed and contributed to this

category. Intakes of milk, which were also not differentiated on fat content in the present study, were relatively high for both men and women and a significant inverse trend was seen in both sexes (P<0.001 for men and women) (Table 4.5). There was a slightly wider range of milk consumption in women with a difference of 68 g/d in women compared with 38 g/d in men between the extreme quintiles.

Fruit and vegetable intakes were consistently higher in women compared with men (Table 4.5). However, in both sexes there was a difference of approximately 100 g/d between the extreme quintiles of fruit and vegetables. This is slightly more than one extra portion of fruit and vegetables per day (one portion being approximately 80g). In the US NHANES study combined fruit and vegetable intake was the second highest contributing source to plant protein intake after grains (145) and are therefore an important source of plant-based protein in the diet. It must be acknowledged that high consumption of fruit and vegetables will also incorporate a number of other health benefits, such as high fibre and micronutrient content, which may have a greater effect than the protein content of these foods. However, as little is known about the effect of protein quality on health outcomes, it may still be of importance to increase plant protein intake at the expense of less healthful protein sources such as processed meat.

4.4.1 Strengths and limitations

Strengths of the analysis include the use of 7DD to record food intake, in addition the detailed way in which food diaries were coded meant that there were a large number (~11,000) of food items and a high level of detail for classification of protein content. This sub-cohort is representative of the EPIC-Norfolk cohort which itself is representative of the overall UK population. Therefore the findings of this chapter may be applicable to the wider general population.

Differences in the classification of food items, can make direct comparisons between studies difficult. For example some studies including the NDNS report on aggregated meat and meat products whereas other studies use varying degrees of disaggregated food groups/items. Due to the groupings of some mixed food items (those which contained protein from more than one source e.g. animal-marine, animal-derived and plant protein)

it was not always possible to assign food items to one group only. Where this was the case, a decision was made based on the source of the protein that was likely to be contributing most highly to total protein intake. For example in a mixed dish such as a pastry pie, it was deemed that the meat content of the pie would contribute more highly to the total protein content of the item than the aggregated plant based products that may be included (such as vegetables and flour). Despite these limitations these findings are still of interest due to the novel approach used in assessing the contributing sources to different types of protein intake. The results of this chapter may have implications for the findings presented in the subsequent chapter 'Chapter five - Dietary protein intake and stroke risk factors and risk of stroke'. They may help to clarify potential differences in associations between protein intake and stroke risk and risk factors in men and women.

4.5 Summary

In this cohort, protein intake was above the recommended daily intakes of 55.5 and 55.3 g/d for men and women respectively. Total protein intake contributed 15.0% and 15.8% of energy for men and women respectively which compares well with other countries. The majority of total protein intake was derived from meat and meat based derivatives. However, in those with a higher ratio of plant:animal protein intake significantly less meat and meat products were consumed by both men and women. In addition higher amounts of fruit and vegetables, nuts, seeds and legumes were consumed. Women tended to have higher overall intakes of fruit and vegetables than men across all quintiles of ratio plant:animal protein intake. However, this was not the case for nuts, seeds and legumes. These differences in the contributing sources of foods to protein intake may influence disease risk between men and women.

Chapter Five

THE INFLUENCE OF DIETARY PROTEIN INTAKE ON STROKE RISK AND RISK FACTORS

5.0 Introduction

Total dietary protein intake has been indicated to beneficially influence blood pressure, a significant risk factor for stroke (140). Serum lipid levels may also be influenced by dietary protein intake (251). However, little is known about whether the type of protein (animal or plant based) affects these stroke risk factors or influences the risk of stroke. Previously reported associations between total dietary protein intake and the risk of coronary heart disease have been inconsistent (122, 322, 323). These inconsistent findings could be attributed to the consumption of different sources of protein, absolute amount of protein eaten and the foods contributing most highly to protein intake (177, 322-324). More recently work has been conducted investigating the influence of protein on stroke risk directly and whether the source of protein has an influence on stroke risk (175, 177). To the best of my knowledge no previous study has investigated the potential associations of total protein intake and effects of different sources of dietary protein %en, either plant or animal, with risk factors for stroke and stroke risk simultaneously. Previously studies have investigated the influences individually on risk factors and stroke risk (123, 124, 174, 176). Additionally few studies have investigated whether differences in the ratio of plant:animal protein in the diet has an effect on stroke risk factors and stroke risk.

Role of protein in human body

Proteins have a number of significant roles in the body, these vary depending on their structure. For example fibrous proteins with many cross-links often have structural functions such as collagen. Whereas globular proteins which have a more complex tertiary and occasionally quaternary structure include enzymes and haemoglobin (292). Protein is an essential component of cell membranes maintaining structure and regulating transport of ions, and is the primary component of all types of muscle. On a daily basis protein turnover is equivalent to approximately 300g, whilst the typical Western diet provides approximately 100g of amino acids in the form of dietary protein per day (302). Dietary protein intake may influence stroke risk factors and stroke risk via a number of mechanisms. This includes beneficial influences of increased dietary protein intakes on

insulin sensitivity, vascular function, renal function, improvements in lipid levels and blood pressure.

Stroke risk factors and stroke risk

The literature review of previous research in this chapter which follows the section on mechanisms of action will firstly start with evidence from animal studies, followed by epidemiological studies and then research from clinical trials subdivided into evidence related to total protein intakes followed by animal and plant protein intakes. Each section (animal studies, epidemiology and clinical trials) will be divided to first cover the risk factors blood pressure and lipid profile and will subsequently review the literature surrounding stroke risk.

Previous research on dietary protein intake in relation to CHD and CVD risk in general indicates that both the source and the amount consumed may have an effect on blood pressure, lipid profile and body composition among other stroke risk factors (175, 176, 251).

It is possible that the different types of protein may have different effects on risk factors and hence overall stroke risk (175, 176). The consumption of animal products, particularly red meat, has in most cases been associated with increased risk of stroke (316). However, in some instances an inverse association was identified (325). However, these studies were often conducted in Asian populations whose sources of animal protein are primarily fish, which are not only lower in saturated fats but have also been linked to reduction in risk of stroke due to the presence of n-3 PUFA (324-327).

5.0.1 Mechanisms

Reduction in stroke risk may be through in-direct effects on risk factors such as vascular health, circulating insulin and serum lipid levels (328).

Vascular health

Dietary protein intakes may influence vascular health including blood pressure and endothelial function specifically.

Several specific amino acids may have a role in influencing blood pressure. For example tyrosine, a non-essential amino acid, is required for the synthesis of catecholamines in the central nervous system. Circulating concentrations of tyrosine have been shown to be influenced by protein intake, on both an acute (single meal) and more long term (weeks) basis (329). Catecholamines have been implicated in raising blood pressure. However, their circulating presence also stimulates the synthesis and release of renalase which degrades catecholamines and therefore catecholamines may in fact have a net effect in reducing blood pressure (330). In animal studies the injection of tryptophan and tyrosine resulted in a decrease in blood pressure (331, 332). This is believed to be as a result of increased uptake in the brain leading to increased release of serotonin. However, the uptake of both of these amino acids can be influenced by the presence of other amino acids such as valine (331, 332) and thus this mechanism may or may not remain applicable for dietary intakes where the amino acids are not consumed in isolation.

Soy protein, specifically, has been associated with beneficial effects on vascular function and blood pressure which may be attributable to the higher levels of arginine that are found in such products. Soy protein is also noted for containing estrogenic isoflavones and angiotensin-converting-enzyme inhibitory peptides which can lead to a reduction in blood pressure (296, 333). Isoflavones may improve endothelial function by increasing eNOS expression thus increasing levels of NO, and leading to vasodilation as well as supressing production of endothelin-1 (296). As well as the potential beneficial effect of isoflavones from soy protein the higher content of arginine in soy protein has also been indicated to enhance NO production. Arginine is a conditionally essential amino acid present in high amounts in turkey breast and pumpkin seeds (per household measure) (334), it is a precursor in the synthesis of NO, a potent vasodilator. NO has an important role in the maintenance of vascular tone and arterial pressure and may therefore be involved in reducing blood pressure and therefore stroke risk by its role in maintenance of endothelial function (328, 335, 336). Endothelial dysfunction, results due to disruption of the balance between vasoconstrictors and vasodilators which can be due to a reduction in the bioavailability or production of NO and other vasodilators, and an increase in endothelium-

derived vasoconstrictors (337, 338). The result of this may be vasoconstriction, activation of platelets and increased coagulation, and increased inflammation which can all contribute to the development of atherosclerosis which has been implicated in the risk of stroke (337-339). The progression of atherosclerosis can increase stroke risk either through continued narrowing of the artery, which can eventually lead to complete blockage. However, the more common way that atherosclerosis can be involved in the onset of stroke is if an atherosclerotic plaque ruptures, this fragment may then travel through the blood stream and cause a blockage in a narrower vessel (54).

Insulin levels

Higher dietary intakes of non-essential amino acids have been beneficially associated with improvements in circulating insulin levels, through upregulation of gluconeogenesis. This leads to a reduction in circulating insulin levels, a risk factor for stroke (177). In addition lower intakes of essential amino acids, which would be common with vegetable protein intake is considerably higher in non-essential amino acids such as arginine, glycine, alanine and serine compared with animal protein. It also contains lower quantities of essential amino acids such as lysine, methionine and tryptophan, which may be associated with increased insulin levels (177, 340). **Table 5.0** details the differences in levels of selected amino acids of several plant and animal foods.

Table 5.0 Levels of selected essential and non-essential amino acids in a sample of animal and plant foods.

	Essential amino acid			Non-essential amino acid	
	Lysine	**Methionine**	**Tryptophan**	**Arginine**	**Glycine**
Animal foods					
Beef (mince, 7% fat, cooked)	2.39	0.74	0.15	1.88	1.96
Chicken (roast, light meat only)	2.30	0.75	0.32	1.64	1.33
Milk	0.28	0.09	0.05	0.09	0.06
Plant foods					
Potatoes (boiled)	0.11	0.03	0.03	0.09	0.06
Carrots (boiled)	0.08	0.02	0.01	0.08	0.04
Bread (whole-wheat)	0.24	0.14	0.12	0.38	0.33
Soya milk	0.13	0.03	0.04	0.19	0.10

Based on USDA National Nutrient Database for Standard Reference, Release 28 (341).

Serum lipids

Higher protein intakes have also been reported to reduce circulating cholesterol levels (342) and it has been suggested that protein from plant sources may exert more beneficial effects than protein from animal sources (343). This may be due to a decrease in the formation of micelles which leads to a reduction in the absorption of cholesterol in the intestine, which is then excreted in faeces (344). Dietary proteins and their peptides may also reduce cholesterol synthesis by acting as an inhibitor to HMG-CoA reductase which is required during the synthesis of cholesterol (344).

Although not a mechanism it is also possible that protein intake has indirect effects through modulation of the whole diet. For example a higher dietary protein intake may replace or reduce the intake of carbohydrate or fat content of the habitual diet and may therefore help with weight management, a contributing factor to raised BP and poor serum lipid profile (147).

Mechanisms of action of dietary magnesium on stroke risk via effect on risk factors blood pressure and lipid levels are summarised in **Figure 5.0.** The following narrative literature review was obtained through searches of MEDLINE (Ovid) and PubMed. The search term dietary was combined with; protein, total protein, plant protein, animal protein and relevant key words such as blood pressure, systolic blood pressure, diastolic blood pressure, cholesterol, LDL, HDL, and stroke risk. Titles and abstracts of returned search items were reviewed and the full text of relevant articles was obtained. The reference lists of full text articles were subsequently reviewed to identify additional relevant publications.

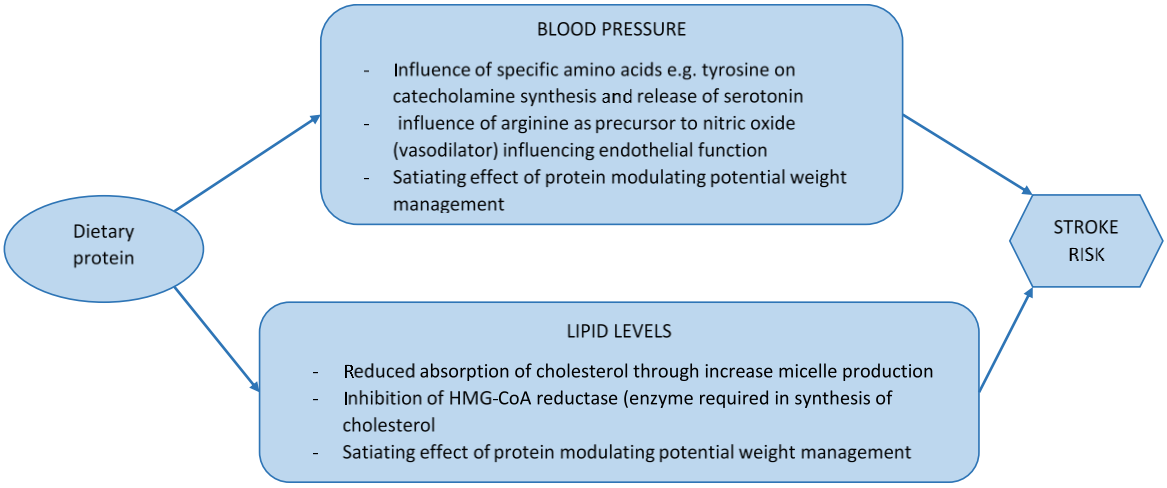


Figure 5.0. Summary of mechanisms of action of dietary protein intake on stroke risk via effects on risk factors blood pressure and lipid levels.

5.0.2 Animal models

Blood pressure

Total protein

Few studies in animal models have investigated the overall impact of total protein on blood pressure (**Table 5.1)**. Studies largely use either a specific protein source in varying concentrations (333, 345) or compare different sources of protein (346-348).

Studies on adult rats frequently investigate protein intake and blood pressure in models with renal dysfunction. Differences in blood pressure were reported in Dahl salt-sensitive rats fed a low (6% of energy), normal (18% of energy) or high casein protein diet (30% of energy) (345). The high protein diet was associated with a significant increase in blood pressure compared to rats on the low and normal protein diet. However, there was no significant difference in the blood pressure of rats on the low and normal diets. The mechanisms of action remain unclear but may be related to renal damage (345).

Plant and animal protein

The type of protein, whether animal or plant based, may influence risk factors for stroke via effects on endothelial function.

Magne et al (339) studied rats, fed a high saturated fat diet, with either milk protein (MP), rapeseed protein isolate (RP) or milk protein with the addition of L-cycsteine and L-arginine (MP+AA) in concentrations similar to that present in rapeseed protein isolate. Diet composition was 60% fat, 20% carbohydrate and 20% protein. Postprandial plasma amino acid levels were measured. During the RP and MP+AA diets compared with the MP diet, there were increases in the concentration of many amino acids at 4, 6 and 8h after the meal including arginine, aspartate, glutamate and methionine. However, there was no significant difference in plasma total cysteine across the diets. This could indicate that these amino acids have a higher bioavailability from the RP and MP+AA diets than the MP diet. There was a decrease in vascular reactivity, indicated by increase in blood pressure, after consumption of MP meal and this decrease in vascular reactivity was less pronounced after MP+AA and inhibited with consumption of RP. This may be attributable to the presence of L-arginine. Previous research that has also focussed on L-arginine supplementation has shown a reduction in endothelial dysfunction in human participants (349, 350). L-arginine is a precursor to NO which is a potent vasodilator. Therefore it is likely that the beneficial influences on vascular function that are indicated with supplementation with this amino acid stem from this effect. In the study by Magne et al (339) plasma triglyceride levels increased after consumption of the high fat meal but were not influenced by the composition of the protein component of the diet. However, the effects may not be the same for a different meal composition for example higher in carbohydrate. Although, it could be hypothesised that any potential effect on plasma triglyceride levels would be more pronounced after the consumption of high fat meal.

In a study on soyabean protein hydrolysate (SPH) Yang et al (333) reported significantly lower blood pressure in rats fed SPH compared with those without. Twenty four spontaneously hypertensive rats were fed a high sodium diet containing 0% SPH, 0.5% SPH or 1% SPH. A control group of 8 Wistar-Kyoto rats were also included in the study. Every 4 weeks (for 12 weeks total), fasting blood samples were taken and plasma total cholesterol,

triglyceride, sodium, potassium and chlorine levels were determined. Blood pressure was also measured at 4 weekly intervals. At 4 and 8 weeks of the diet, rats fed either 0.5% SPH or 1% SPH had significantly lower SBP than the spontaneously hypertensive rats fed 0% SPH. The SBP of these two groups (0.5% and 15% SPH diets) was significantly higher than the control group of Wistar-Kyoto rats. At 12 weeks SBP and mean BP of rats on the 0.5% SPH and 1% SPH diets were similar to that of the control group and significantly lower than the spontaneously hypertensive rats fed 0% SPH diet. Thus indicating that the addition of SPH in the diet may delay the onset of hypertension.

Consumption of a fish protein diet in spontaneously hypertensive rat species has been shown to lead to a reduction in blood pressure compared with rats consuming a casein diet (351). The fish protein diet was also related to a decrease in plasma total cholesterol as well as liver triacylglycerol and total cholesterol. These findings have relevance in terms of total animal protein intake, as they may explain potential discrepancies between findings reported in studies investigating Western populations and Asian populations. Whereby, in Western populations, high animal protein intake is often negatively associated with health status including blood pressure, lipid profile and CVD risk. However, this is not always the case in Asian populations where their animal protein may be from fish and lean meats such as poultry rather than red meat, more typically consumed in Western diets.

Lipid profile

Dietary protein intakes may also influence circulating lipid levels, a risk factor for stroke. There is conflicting evidence as to whether the effects are attributable to animal or plant-based protein sources or overall protein intake.

Plant and animal protein

Two studies by Tomotake et al (346, 347) investigated the effects of different protein sources on the lipid profile in a number of animal models; hamsters, rats and mice **(Table 5.2)**. They indicated that the plant-based sources, buckwheat protein (BWP) or soy protein isolate (SPI), significantly improved lipid profile in the rat and hamster studies compared with the animal protein diet (casein), with the exception of TC:HDL in hamsters. In hamsters

fed a diet with 5 g/kg cholesterol and 200 g/kg of casein, SPI or BWP, both SPI and BWP reduced cholesterol levels in gallbladder bile.

Aziz et al (348) however, reported contradictory results regarding protein source and lipid profile in hamsters. Both wheat gluten and casein diets significantly lowered HDL levels when compared with beef protein or soya protein diets. These findings may indicate that it is potentially the specific amino acid content of diets that influences stroke risk factors. However, to date there has been limited research in this area.

In the study previously described by Yang et al (333) in relation to soy protein intake and blood pressure, the authors reported measures of total cholesterol and triglyceride levels. There was no significant difference in levels of total cholesterol or triglycerides between the different levels of soyabean protein hydrolysate supplementation (0%, 0.5% and 1% diets) in the stroke prone spontaneously hypertensive rat species. However, total cholesterol and triglyceride levels were significantly lower ($P<0.05$) in each of the SPH supplementation groups than those of the control group of Wistar Kyoto rats. Despite no significant difference being reported between the SPH supplementation groups, there was a non-significant difference of 0.20 mmol/L in total cholesterol between the lowest SPH supplementation group (0%) and highest supplementation group (1% SPH). It should be noted that rats on 0.5% and 1.0% SPH diets had significantly higher weight gain across the course of the study compared with both the 0% SPH diet group and that total cholesterol levels were still lower despite this.

Stroke risk

Several animal studies have indicated a potential association between dietary protein intake and onset of stroke, in stroke prone spontaneously hypertensive rat species (SPSHRs) **(Table 5.3)** (296, 352). Chiba et al (2009) reported a significant delay in the onset of stroke in rats fed an experimental diet, high in protein, compared with placebo diet. They aimed to determine the macronutrient influencing stroke onset by changing the ratios of two macronutrients whilst keeping the third constant. In high protein (45% and 55% of total energy) low carbohydrate (45% and 35% respectively) diets, where fat content remained constant at 10% there was a significant delay in onset of stroke compared with the placebo

diet (20% protein, 70% CHO, 10% fat) (P=0.009 for 45% protein diet and P<0.001 for 55% protein diet).

In addition, when rats were fed a low protein diet (5% protein, 85% CHO 10% fat) onset of stroke occurred more rapidly, 16 days compared with 55 days for the rats fed a diet 55% protein. In the diet with protein intakes closest to those recommended in humans (10% of energy from protein) the number of days to onset of stroke was 22, which was the same mean time to stroke onset as the control group (352). A higher protein diet (60%) with only 30% of energy from carbohydrates was associated with a delay in stroke onset. Additionally a low protein diet (7.5%) and high carbohydrate (85%) led to earlier onset of stroke (23 days compared with 29 days) when compared with a diet comprised of 15% protein. These findings further indicate that the effect was specifically in relation to the protein composition of the diet.

Table 5.1. Overview of animal studies investigating the effects of protein on blood pressure in rat models.

Author Model	Treatment and duration	Results
Yang (2004) (333) M SHR or Wistar-Kyoto rats	Control wistar rats 0% soyabean protein hydrolysate (SPH). All other SHR rats 1. 0% SPH, 200 g/kg casein 2. 0.5% SPH, 3. 1% SPH All fed a high Na diet	SBP significantly lower in diets higher in SPH compared with control
Ait-Yahia (2003) (353) M SHR	200 g/kg casein or fish protein 2 months	Fish protein significantly lowered SBP compared with casein diet. -14% (P<0.05)
Endoh (2001) (354) M Sprague-Dawley rats	1. 23% casein, 28% sucrose, 8% α-corn starch, 24% β-corn starch, 7% corn oil, 2. 6% casein, 35% sucrose,10% α-corn starch, 31% β-corn starch, 7% corn oil, 3. 6% casein, 28% sucrose, 10% α-corn starch, 38% β-corn starch, 7% corn oil 4. 3 & 1 mg/kg prazocin hydrochide, 5. 3 & reduced NaCl, 6. 3 & 2% L-arginine 8 weeks	Diets 2, 5 & 6 significantly increased SBP compared with 1, 3 & 4 (P<0.05) by 10-15 mmHg. High sucrose low protein significantly increases SBP over low sucrose low protein (P<0.01).
Martin (2001) (355) F SHR or Wistar-Kyoto rats	Control: casein protein or 19% soy protein diet Minimum 8 weeks	NS change in mean arterial pressure in sham wistar-Kyoto or SHR, and ovariectomized wistar rats between casein and soy diets.

	Significant decrease in mean arterial pressure ~14 mmHg, in SHR ovariectomized rats with soy diet ($p<0.05$).

Abbreviations

NS, non-significant; SBP, systolic blood pressure; SPH, soy protein hydrolysate; SHRs, spontaneously hypertensive rat species;

Table 5.2. Overview of animal studies investigating the effect of protein on lipid profile in hamster and rat models.

Author Model	Treatment and duration	Results
Aziz (2008) (348) M Golden Syrian hamsters	20% diet: casein, beef protein, wheat gluten, or soya protein. 15.5% diet fat 8 weeks.	Casein, wheat gluten and soya protein were sig different to beef protein for TC and LDL and Casein and wheat gluten for HDL (P<0.05).
Tomotake (2007) (347) M Sprague-Dawley rats M ddY mice	20% of protein from: casein, tartary buckwheat or buckwheat protein With increasing conc of cholesterol to induce hypercholesterolemia in rats and gallstones in mice 27 days.	Rats: serum TC sig lower (P≤.05) w/ buckwheat or tartery buckwheat vs casein Mice: NS diff in TC
Tomotake (2000) (346) M Golden Syrian hamsters	1. 230 g/kg casein, 2. 238 g/kg SPI, 3. 381 g/kg buckwheat 2 weeks.	Sig lower plasma TC & HDL (P≤0.05) w/BWP vs SPI and casein. Sig lower TC:HDL (P≤0.05) w/ casein vs SPI and BWP. Sig lower TG (P≤0.05) w/ SPI or BWP vs casein.

Abbreviations

HDL, high density lipoprotein; LDL, low density lipoprotein; NS, non-significant; TC, total cholesterol; TG, triglyceride;

Table 5.3. Overview of animal studies investigating the effects of protein on stroke risk in stroke prone spontaneously hypertensive rat models

Author Model	Treatment and duration	Results
Chiba (2012) (356) Male SPSHRs	Control: 20% protein, 10% fat, 70% CHO Four experiments: (all diets 10% fat) 1. 5%, 20% or 55% total energy from casein (20% control) or soybean 2. 55% total energy from casein (control), whey, soybean or egg white 3. 20% casein (control) 55% casein or 55% amino acid (casein composition) 4. 10% butter & 20% casein (control), 50% butter, 50% beef tallow, 50% cocoa butter Duration - not specified	1. Delayed onset stroke with 20% and 55% casein vs 5% (P=0.016 and P<0.001), similar for 55% soybean (P=0.018). 55% Casein delayed onset more than soybean (P=0.019). NS diff in BP between 20 and 55% casein diets at 1 or 2 wks. 2. Delayed onset order (least effect first): soybean, egg white, casein, whey. 3. delayed onset in rats fed 55% casein but not 55% aa vs 20% casein (P=0.002 and P=0.069) 4. NS diff in onset of stroke with 50% butter, beef tallow, or cocoa butter.
Chiba 2009 (352) Male SPSHRs	Percentage energy from diet. All diets 10% fat 5% protein, 85% CHO. 2. 10% protein, 80% CHO. 3. Placebo 20% protein, 70% CHO. 4. 45% protein, 45%CHO. 5. 55% protein, 35%CHO	Rats fed high protein low CHO diet (45% and 55% protein) had delayed onset of stroke P=0.009 and P<0.001 respectively. Low protein high CHO (5% protein).
Gilani (2009) (296) Male SPSHRs1	1. Casein, 2. casein + isoflavones 500 mg/kg, 3. casein + anthocyanins 500 mg/kg, 4. Soy protein isolate (SPI), 5. SPI + isoflavones 500 mg/kg, 6. SPI + anthocyanins 500 mg/kg (31 days)	NS difference in mean survival time between casein and SPI diets. Addition of anthocyanins and isoflavones was associated with significantly shorter time to death (P<0.05)

Gilani 2006 (357) Male SPSHRs	Casein (222 g/kg) & cysteine (232 mg/MJ) , casein & methionine (550 mg/MJ), SPI (222 g/kg) & cysteine (340 mg/MJ), SPI & methionine (340 mg/MJ)	Type of protein casein vs SPI had no effect on survival time. Methionine supplemented group had sig longer mean survival time over cysteine group ($P<0.05$)

Abbreviations

CHO, carbohydrate; SPI, soy protein isolate; SPSHRs, stroke prone spontaneously hypertensive rat species

5.0.3 Epidemiological studies

Blood pressure

Total protein

A number of studies have prospectively investigated associations between dietary total protein intake and blood pressure and hypertension **(Table 5.4)** (123, 124, 149, 358-361). However, only a small number of studies were conducted in European populations. In addition the majority of studies use either 24hr recall or FFQs to determine dietary intakes. Studies validating methods of recording dietary intake have indicated that dietary protein intake is more accurately determined using weighed or estimated food records than FFQs or 24hr recall which tend to overestimate protein intakes (183, 362, 363). Two systematic reviews have also been conducted, including most of these studies, aiming to determine the relationship between dietary protein intake and blood pressure. The first of these by Altorf-van der Kuil et al (140) which included 12 observational studies concluded that total protein intake tended to be weakly inversely associated with blood pressure and that there was no clear association between total protein intake and development of hypertension in two separate studies (364, 365). A more recent meta-analysis by Tielemans et al (174) on observational studies and RCTs aimed to determine relationships between dietary protein intake and blood pressure and development of hypertension. They included 6 cross-sectional studies (n=48,985 participants) in their analyses of total protein and blood pressure which included cohorts from China, Japan, USA, Italy and The Netherlands. There was a small, but significant, inverse association between dietary total protein intake, per ~25g increments, and SBP with an effect size of -0.20 mmHg SBP (95% CI -0.39—0.01) with non-significant variability using random effects model. There was no significant association between increasing total protein intake (~25 g increments) and development of hypertension across three prospective studies (n=11,761 participants) RR 0.99 (95% CI 0.96-1.02) (366).

Plant and animal protein

The previously mentioned systematic reviews (140) and meta-analyses (174) also sought to determine the independent effects of plant and animal protein intake on blood pressure

(Table 5.4). Altorf-van der Kuil (140) identified a total of 10 cross-sectional and prospective studies between 1987 and 2008 which recorded information on plant and animal protein intake. Only one of these studies (INTERMAP) included a UK cohort (141). The INTERMAP study included a sample of n=501 UK participants who were middle aged 40-59 years with a mean blood pressure of 120.4/77.3 mmHg. Mean protein intakes were 15.8% (±3.1) of total energy intake and plant and animal protein accounted for 6.1% (±1.4) and 9.8% (±3.3) respectively. Dietary protein intake was estimated using four repeated 24hr dietary recalls. The fully adjusted multivariable model included; sample, age, sex, special diet, history CVD or DM, family history of hypertension, physical activity, dietary supplement use, 24hr urinary sodium and potassium, alcohol intake, calcium, SFA, PUFA, dietary cholesterol, magnesium, and fibre intakes. Prior to adjusting for height and weight there was a significant difference in estimated blood pressure per two standard deviations of plant protein intake (-1.29 mmHg $P<0.05$ for SBP and -1.12 mmHg $P<0.01$). However, after adjusting for height and weight the values for SBP were -1.01 and non-significant. Despite the estimated decrease being less, DBP remained significant with an estimated difference of -0.95 ($P<0.05$) per two standard deviations of plant protein intake. Animal protein was not significantly associated with blood pressure in the fully adjusted model, with the exception of DBP prior to adjustment for height and weight, where an estimated difference of 0.70 ($P<0.05$) was reported. However, there did appear to be a trend towards an estimated increase in blood pressure in many earlier models, but no estimated differences were significant after adjustment for height and weight (141).

In a population of 20,820 Dutch adults after adjustment for a number of relevant confounding factors including; age, sex, BMI, total energy, magnesium and other protein sources amongst others there was no significant association between animal protein intakes and SBP or DBP (P trend =0.39 and 0.71 respectively) (124). However, a significant inverse association was seen in relation to plant protein intake and SBP and DBP ($P<0.001$ for both). In addition the authors aimed to identify if modelled substitution of 3% of energy from protein intake for 3% energy from another macronutrient (carbohydrate or MUFA source) was associated with blood pressure. There was no reported benefit of substituting animal protein for 3% of energy intake from either carbohydrate or MUFA sources. There

is no justification for the choice of MUFA as the 'fat' source to replace, and there may therefore be different results for substituting protein for other fat sources such as saturated fat intake. However, when substituting 3% of total energy from either carbohydrate, or MUFA sources with plant protein sources a decrease in SBP and DBP was reported. Analyses substituting plant protein for carbohydrate estimated a decrease in BP of -2.1/-1.0 mmHg (P trend<0.01). An estimated decrease in BP of -1.3/-1.2 mmHg was reported when plant protein was substituted for MUFA (P trend<0.05). These findings suggest that the effect on blood pressure reduction by increased protein intake may be exclusively from plant protein (124). However, the study utilised FFQs to determine dietary intakes, and whilst total protein intake was validated by 24h dietary recalls and urinary nitrogen, there was no validation for individual protein sources.

In the INTERMAP study cohort, an inverse association between vegetable protein intake and blood pressure was identified (141). It should also be noted that this study reported no significant association between animal protein or total protein intakes and blood pressure. Thus suggesting that it may be the non-animal (vegetable/plant) component of the diet that may have a benefit on blood pressure. It was also reported that that individuals consuming higher intakes of vegetable protein as opposed to animal sources had increased circulating concentrations of a number of amino acids. This included; glutamic acid, cystine, proline, and serine, which are all non-essential amino acids, these reportedly do not elevate insulin levels, as opposed to essential amino acids, which are abundantly present in animal foods, which have been associated with increasing insulin levels (141, 177). Conversely Altorf-van der Kuil et al (123) reported no significant trend between plant protein and hypertension (SBP ≥140 mmHg and/or DBP ≥90 mmHg), but higher intakes (≥18 g/d) vs lower intakes (≤17 g) of protein from grains was associated with a significant decrease in risk of hypertension in point estimate, albeit with only a marginal significance, HR 0.85 (95% CI 0.73-1.00 P trend=0.04). However, the FFQ used in this study was not validated for the specific types of protein intake including grains (123). Participants in the cohort were also relatively healthy with a mean blood pressure of 118/76 mmHg. It is likely that the effects of protein source on blood pressure may be more pronounced in pre-hypertensive (SBP 120-139 mmHg and DBP 80-89 mmHg) or hypertensive (SBP >140

and DBP >60 mmHg) participants, therefore, it is possible that the magnitude of difference in blood pressure inferred by plant-based protein was not obvious within this study population (149).

The previously mentioned systematic review and meta-analyses by Tielemans et al (174) also aimed to determine if dietary intakes of plant or animal protein were associated with blood pressure. From 7 cross-sectional studies, including 42,938 participants, a non-significant inverse association with plant protein intake, per ~11 g increments, was identified (-0.52 mmHg SBP 95% CI -1.10-0.05). There was significant heterogeneity reported (I^2=75%, P=0.01) which was deemed to be attributable to one study by Umesawa et al (361). After removing this study the findings in association with plant protein were strengthened -0.73 mmHg SBP (95% CI -1.08- -0.38) and animal protein intake was associated with an estimated increase in +0.24 mmHg SBP (95% CI -0.09-0.57).

This difference in findings in a Japanese population may be due to the different contributing food sources to plant and animal protein intakes. For example Japanese populations consume more fish than Western populations. In this instance it may be that animal protein intake is a surrogate of other nutrient intakes which can also affect blood pressure, saturated fat which is found in higher levels in red meat which is consumed in higher quantities in Western countries. Additionally, traditional Japanese cuisine can be very high in salt, and this includes vegetables which are often pickled or eaten with high salt seasonings (361, 367). Therefore if this were the case in this Japanese population it may be that the relatively high salt intakes that accompanied vegetable intake had a greater influence on blood pressure than protein component.

Lipid profile

The majority of studies investigating the effect of dietary protein intake on lipid levels have focused specifically on the effect of soy protein intake. Findings generally indicate an inverse relationship between increased intakes of soy protein and TC and LDL levels. More inconsistent findings are reported on the potential effects on HDL and TG levels **(Table 5.5)** (27-29, 368).

Total protein

Recently Mirmiran et al (173) evaluated the effects of different protein sources on lipid profile, specifically HDL. This cohort consisted of 2,537 men and women, part of the Tehran Lipid and Glucose Study, who were aged 19-70 years. Dietary protein intake was estimated using a validated semi-quantitative FFQ. The mean protein %en was 13.7% for men and 13.6% for women.

They reported a significant positive association with total protein intake and HDL levels in both men and women ($P<0.05$ for both) after adjustment for a number of covariates including; age, physical activity, BMI, smoking, SBP, DBP and dietary factors such as total energy (despite nutrients already being energy adjusted per 4186.8 KJ), dietary fat and carbohydrate intakes as percentage of energy, saturated fat, MUFA, PUFA, cholesterol, fibre and sodium (adjusted grams per 4168.8 KJ). However, no significant association between total protein and triglyceride levels was reported (173). These findings are in contrast to previous work by Smit et al (369) which indicated no significant association. In the study by Smit et al (369) lower serum TC was associated with higher percentage energy from protein.

These inconsistent results may be attributable to differences in the characteristics of study populations, one an Iranian population, the second US, study methodologies, Mirmiran et al (173) estimated intakes from FFQ whilst Smit et al (369) used 24hr dietary recall, or the confounding variables included in adjusted models.

Plant and animal protein

In the previously mentioned study by Mirmiran et al (173) (total protein and lipids section) they also investigated the effects of the ratio of animal:plant protein intake with lipid levels. The mean ratio of animal:plant protein intake was 1.4 (±0.9) for men and 1.8 (±1.4) for women, which was significantly higher in women than men. They reported no significant difference in the sources of protein consumed by men and women, with the highest contributing sources to protein intake being grains which accounted for approximately 29.7% of protein intake. Meat intakes were segregated into specific types for example red

meat and poultry, and therefore their individual contributions were lower. However, if total meat intake was a combined variable this would have been the highest source of protein.

There was no significant association with increasing ratio of animal:plant protein intake and increasing HDL or triglyceride levels after adjustment for a number of covariates, previously mentioned. This finding is in accordance with the study by Smit et al (369) which also reported no significant association between the ratio of animal:plant protein intake and HDL after adjustment for saturated fat intake. Total animal protein intake was significantly different in the highest quartile of serum total cholesterol (≥5.68 mmol/L) compared with the lowest quartile (≤4.29 mmol/L). Age, sex and race adjusted mean intakes of animal protein were 65.3% and 67.7% for the lowest and highest quartiles of serum cholesterol levels ($P<0.01$). In contrast plant protein intake was significantly lower in those in the highest quartile of serum total cholesterol compared with the lowest quartile. Age, sex and race adjusted mean plant protein intakes were 34.7% and 32.3% for the lowest and highest quartiles of serum total cholesterol respectively ($P<0.01$). Smit et al (369) also failed to identify an association between source of protein intake and LDL levels.

Stroke risk

Fung et al (370) compared the risk associated with consumption of a prudent pattern – characterised by higher intakes of fruit, vegetables, fish and whole grains vs a Western pattern – higher in red and processed meats, processed grains and full-fat dairy products. They identified in women that a typical Western dietary pattern is associated with an increased risk of total and ischaemic stroke RR of 1.58 and 1.56 respectively. A prudent dietary pattern on the other hand may infer a protective effect RR 0.78 for total stroke (370). There may be a benefit from swapping one protein source for another, Bernstein et al (175) identified that substituting one serving/d of red meat for poultry, nuts or fish equated to a 27% (95% CI, 12-39%), 17% (95% CI, 4%-27%) and 17% (95% CI 5-17%) reduction in stroke risk respectively.

Total protein

In a biomarker calibrated study of n=80,370 postmenopausal women, part of the Women's Health Initiative Dietary Modification Trial between 2004 and 2005 and Women's Health

Initiative Observational Study, an inverse association was reported between protein intake and ischaemic stroke **(Table 5.6)** (371). There was no association with non-calibrated protein intakes, however, the authors reported that the dietary FFQ tended to overestimate protein intake in grams per day and to underestimate protein as a percentage of energy intake (protein %en). Therefore the calibrated protein intake may more accurately predict associations between dietary protein intake and stroke risk. There was a total of 1224 stroke cases reported (714 ischaemic stroke and 217 haemorrhagic stroke). Calibrated protein intakes were 78.1 g/d (95% CI 58.5-104.4) for the Dietary Modification Trial Comparison Group and 74.3 g/d (95% CI 55.0-100.5) and Observational Study. The calibrated protein %en was 14.4 (95% CI 12.0-17.3) and 14.4 (9%% CI 11.9-17.6) for the two study groups respectively. The HR for each 20% increment increase in calibrated protein intake and total stroke was 0.87 (95% CI 0.78-0.98) and similar significant HR was reported for ischaemic stroke 0.87 (95% CI0.75-1.00) after adjusting for ethnicity, education, history of CVD, family history premature CVD, smoking, hypertension, diabetes, statin use, aspirin use, previous hormone use, leisure-time physical activity and BMI. They did not however, adjust for some key risk factors for stroke; blood pressure and serum lipid levels or any other dietary factors which may also influence stroke risk such as sodium, alcohol and dietary fat intakes. In addition haemorrhagic stroke risk was not significantly associated with increasing calibrated protein intake (in 20% increments) after adjusting for confounding variables. This may in part have been due to the smaller number of incident cases of haemorrhagic stroke, thus making it difficult to establish associations.

A study of 34,670 Swedish women, part of the Swedish Mammography Study, sought to establish potential associations between dietary protein intake and stroke risk (176). Women were free from cancer and CVD at the start of the study (in 1997). Dietary protein intake was estimated using FFQ, and all nutrients were residually energy adjusted. During a mean follow up of 10.4 years there were 1,680 incident stroke cases, of which 1,310 were ischaemic in origin and 216 haemorrhagic strokes, a further 137 cases were unclassified. There was a higher reported history of hypertension with higher intakes of total protein in g/d. Higher total protein intakes were associated with a significant trend towards lower risk of total stroke and ischaemic stroke after adjustment for relevant confounding

variables including; age, smoking (and number of years smoking), education, BMI, physical activity, history hypertension, history diabetes, aspirin use, family history MI, dietary total energy, calcium, cholesterol, total fat, fruit, and vegetables intakes. The RR for total stroke risk in those with highest total protein intakes (≥78.7 g/d) was 0.74 (95% CI 0.61-0.91) compared with lowest total protein intakes (<61.8 g/d) which was the reference category. P trend across quintiles of total protein intake was 0.006. For ischaemic stroke specifically the P trend was 0.008 and the RR for the highest total protein intakes was 0.72 (95% CI 0.58-0.90) compared with the lowest total protein intakes.

Plant and animal protein

In the previously mentioned study on Swedish women (176) analyses were also conducted for plant and animal protein and risk of stroke (Table 5.6). There was a significant trend towards lower risk of stroke across increasing quintiles of animal protein for total stroke and ischaemic stroke (P trend 0.01 and 0.004 respectively). A RR for total stroke of 0.71 (95% CI 0.57-0.88) was reported for those with the highest intakes of animal protein (≥58.0 g/d) compared with the lowest animal protein intakes (<38.3 g/d). Ischaemic stroke was associated with a RR of 0.65 (95% CI 0.51-0.84) in the highest quintile of animal protein compared with the lowest quintile. Plant protein intake was not significantly associated with total stroke or ischaemic stroke (P trend 0.22 and 0.07 respectively). However, in sub analysis of those without a history of hypertension neither animal protein nor plant protein intakes were significantly associated with total stroke risk (P trend 0.23 and 0.60 respectively) or ischaemic stroke risk (P trend 0.11 and 0.14 for animal and plant protein respectively). Thus suggesting that history of hypertension, which is likely to be associated with elevated blood pressure – a significant risk factor for stroke, has a greater influence on stroke risk than dietary intakes of animal and plant protein.

Specific foods and stroke risk

This section aims to provide a brief overview of studies that have investigated intakes of specific high protein food items and stroke risk.

Eggs

A number of studies have investigated the specific effects of egg consumption on stroke risk (324, 372-375). A recent meta-analysis by Rong et al (320) concluded that higher egg consumption, increase consumption of one egg/d, was not associated with increased stroke risk. However, in sub-group analyses of a diabetic population a higher consumption of eggs, compared with lowest intakes, was associated with a reduced risk of haemorrhagic stroke (0.75 95% CI 0.57-0.99). These findings are however, based on very small numbers of cases, n=8 and n=3 respectively for total stroke and diabetic sub-group analysis and may not be able to be extrapolated to the wider diabetic or healthy population.

The potential mechanism by which higher intakes of eggs may increase stroke risk is likely linked to the cholesterol content of eggs, however, it has been reported that dietary cholesterol intake only has a small impact on circulating levels (376). Egg consumption may actually reduce the development of atherosclerotic plaques as evidence suggests egg consumption leads to the formation of larger HDL and LDL particles which may infer a protective effect (377, 378). Higher egg consumption may also be of benefit by acting as a substitute for other dietary constituents such as carbohydrates and fat. Protein is also satiating and therefore may lead to overall reduced calorie intake which may initiate the benefit. The satiating effect may be mediated via slight increases in thermogenesis of protein compared with carbohydrates. The benefits or negative effects of egg consumption on health may also be dependent on the cooking method used and this was not stated. For example fried or scrambled eggs would usually incorporate additional ingredients such as fat or dairy products which may affect the relationship between the food and health.

The effect of higher egg consumption on haemorrhagic stroke in diabetics may be due to the reduced insulin sensitivity of this population group. This leads to decrease in the sequestration of circulating cholesterol which in the case of HS may actually be beneficial (with lower TC levels related to increase risk of haemorrhagic stroke). Therefore, this

finding may only be applicable to this particular population group and the findings not directly extractable to the wider population. Another potential mechanism of action, may be related to the vitamin D content of eggs. Eggs are one of only few dietary sources of vitamin D and higher vitamin D intakes may reduce visceral adipose tissue and other CVD risk factors (379, 380).

Meat

Western diets, characterised by higher intakes of meat have been associated with increased risk of stroke (381). The risk of stroke can differ between sources of meat and meat products. For example processed meats infer an increase in stroke risk, whilst poultry and fish have been associated with decrease in stroke risk (175). This may be due to differences in nutritional composition of meat and meat products. For example processed meat, is typically more calorie dense and has a higher sodium content than red meat or poultry. Processed meats also tend to be lower in both protein and iron compared with red and white meats such as poultry (381). The overall increase in risk seen in higher meat eaters, such as seen in the Western dietary pattern may be in part attributable to the iron content. Haem iron, the form present, in meat has been associated with an increased risk of CVD mortality (175). Whereas foods that are rich in non-haem iron, such as fruit and vegetables, cereals and wine, may reduce risk. However, this may be associated with other beneficial properties of these food items, such as phytochemicals, antioxidants, fibre and other vitamins and minerals.

Where comparisons are made between dietary protein sources the difference in stroke risk inferred by each product may result from differences in nutritional composition including saturated fat and haem iron content as well as traditionally different cooking methods (175).

Table 5.4. Overview of epidemiological studies investigating the associations between dietary protein intake and blood pressure.

Author Study Popn-	Sex, Age (y) Dietary Method	Type of Protein	Adjustments	Size of Effect
Altorf-van der Kuil (2012) (123) Doetinchem cohort (n=3,588) Netherlands	M/W, 26-65, FFQ and 24h recall	Total Animal Plant Dairy Meat and grain	Total energy (residual method), age, sex, BMI, education, smoking, alcohol, SBP, energy, SFA, PUFA, CHO, fibre, Ca, Mg, and K, and protein from other sources.	Total, plant and animal protein was not significantly associated with incident hypertension. Grain protein 15% reduction in hypertension HR 0.85 (95% CI 0.73-1.00 P trend=0.04) highest vs. lowest tertile. Other sources of plant protein were not associated with hypertension risk
Altorf-van der Kuil (2012) (124) MORGEN (n=20,820) Netherlands	M/W, 20-65, FFQ	Total Animal Plant Dairy Meat and grain	Protein adjusted for total energy (residual method), age, sex, BMI, education, smoking, alcohol, total energy, SFA, CHO, fibre, Ca, Mg, K, and protein from other sources.	No significant association between total and animal protein and blood pressure. Plant protein -1.8 mmHg lower SBP and -1.0 mmHg lower DBP highest vs lowest quintile (P trend<0.01).
Umesawa 2009 (361) CIRCS (n=7,585) Japan	M/W, 40-69, 24h recall	Total Animal Plant	Age, sex, community, BMI, antihypertensive medication, alcohol, smoking Na, K, Ca.	NS association in M or W for SBP/DBP after dietary Na, K, and Ca added to model. P trend M: 0.466/0.268 and W:0.305/0.128.

Elliot (141) (2006) INTERMAP (n=4,680) Japan, China, UK, USA	M/W, 40-59, 24h recalls	Total Animal Veg	Height, weight, sample, age, sex, special diet, history CVD/DM, family history hypertension, moderate/heavy PA, supplement, 24h urinary Na & K, alcohol, PUFA, dietary cholesterol, Ca, SFA, Mg, fibre. Age and sex interaction terms	Inverse association between vegetable protein and BP but not total or animal protein.
Stamler (364) (2002) Chicago Western Electric Study (n=1,714)	M, 40-55, Interview & FFQ	Total Animal Veg	Time, age, height, education, smoking, alcohol, and variables to average BP.	Positive association between total and animal protein with change in SBP, and inverse association for vegetable protein. Inverse association between vegetable protein and change in DBP No associations with change in DBP and total or animal protein.

Abbreviations: BMI, body mass index; Ca, calcium; CHO, carbohydrate; CVD, cardiovascular disease; D, dairy protein; DBP, diastolic blood pressure; DM, diabetes; FFQ, food frequency questionnaire; k, potassium; Mg, magnesium; Na, sodium; PA, physical activity; PUFA, polyunsaturated fat; SBP, systolic blood pressure; SFA, saturated fat.

Table 5.5. Overview epidemiological studies investigating the associations between dietary protein intakes and circulating lipid levels.

Author Study Population	Sex, Age (y) Dietary Method	Adjustments	Size of Effect
Mirmiran (173) (2012) Tehran Lipid and Glucose Study (n=2537)	M/W, 19-70, FFQ	Energy adjusted nutrients, age, PA, smoking, BMI, total energy, dietary fat, CHO, SFA, MUFA, PUFA, cholesterol, fibre, Na	Higher total protein (but not animal:plant) intake positively associated with HDL in M&W (P trend <0.05). NS association with TG in M/W for total or animal:plant protein
Zhang (27) (2008) Chinese (n=406)	M/W, 40-65, FFQ	Age, BMI, waist circumference, smoking, total energy, SFA, dietary fibre,	Sig inverse association between soy intake and TC (P trend 0.003 & 0.016) and LDL (P trend 0.002 & 0.028) in men and women. HDL and TG NS in both sexes.
Rosell (28) (2004) EPIC-Oxford (n=1033)	W, ≥20, FFQ	Age, menopausal status, smoking, alcohol, HRT use, BMI, total energy, % energy SFA, % energy PUFA, dietary cholesterol, fibre, diet group (non-vegetarian, vegetarian, vegan).	Sig inverse association between soy intake and TC (P=0.048 &0.002) and LDL (0.029 & <0.001) in pre and postmenopausal women Sig inverse association Total:HDL in postmenopausal women (P=0.006) NS association with HDL
Ho 2000 (29)	M/W, 24-74, Questionnaire	Age, education, dietary cholesterol, dietary fats, SFA.	Sig inverse association TC and LDL in males and women <50. Women ≥ not adjusted. NS association with HDL (data not presented)

Chinese (n=1010)	& FFQ		
Smit 1999 (369) NHANES III (n=6228)	M/W, ≥20, 24h recall	Age, sex, race, recall day, BMI, smoking, income, dietary variables incl SFA,	Higher % energy from plant protein in lower quartiles serum cholesterol. Animal:plant NS after adjustment for SFA NS association with LDL or HDL
Nagata (368) (1998) Takayama Study (n=4837)	M/W, ≥35, FFQ	Total energy, age, BMI, coffee consumption, vitamin C, smoking (W only), alcohol, green tea, PA, dietary variables associated with serum cholesterol or soy intake	Sig inverse association with TC and soy intake in M&W (P trend 0.0001 both sexes)

Abbreviations:

BMI, body mass index; Ca, calcium; CHO, carbohydrate; FFQ, food frequency questionnaire; HDL, high density lipoprotein; HRT, hormone replacement therapy; k, potassium; LDL, low density lipoprotein; Mg, magnesium; MUFA, monounsaturated fat; Na, sodium; NS, non-significant; PA, physical activity; PUFA, polyunsaturated fat; SBP, systolic blood pressure; SFA, saturated fat; TC, total cholesterol.

Table 5.6. Overview of epidemiological studies investigating associations between dietary protein intake and risk of stroke.

Author Study Popn-	Sex, Age (y) Dietary Method	Type of Protein	Adjustments	Size of Effect
Larsson (176) (2012) Swedish Mammography Cohort (n=34,670)	W 49-83 FFQ	Total	Age, smoking, eduction, BMI, PA, hypertension, DM, aspirin, family history MI, dietary: total energy, Ca, cholesterol, total fat, fruits and vegetables, animal protein, plant protein.	Analyses n=1680 Total stroke 0.74 (95% CI 0.61-0.91) P trend=0.006
Park (382) (2010) Stroke cases (n=69) vs Control (n=69) Korean	M/W Mean age 57.7 24h recall	Total Plant Animal	Age, sex, BMI, family history stroke, total energy	Non-significant association for **Total** (P=0.087), **Plant** (P=0.801), **Animal** (P=0.338)
Preis (177) (2010) Health Professionals Follow-Up (n=43,960)	M, 40-75, FFQ	Total Animal Veg	Quintiles of % energy from protein, SFA, MUFA, PUFA and trans fat, total energy, BMI, smoking, parental MI <65, alcohol, multivitamin use, quintiles; PA, GI, folate, B6, B12, vitamin C, K, Mg, and total omega-3 FAs. In addition baseline hypertension, diabetes and hypercholesterolemia status)	Extreme quintiles **Total Protein**;TS 1.14 (95% CI 0.90-1.43 P trend=0.43) **Animal Protein**; TS 1.11 (95% CI 0.87-1.41 P trend= 0.52) **Vegetable Protein**: TS 0.82 (95% CI 0.60-1.12 P trend= 0.17)

Iso (383) (2003) Japanese (n=4,775)	M/W, 40-69 24hr recall	Total	Age, sex, quartiles of total energy and BMI, hypertension category, DM, cholesterol categories, smoking status, alcohol intake and menopausal status (in women)	N.B analyses n=68 Comparison extreme quintiles fully adjusted RR for intraparenchymal haemorrhage RR 0.58 (0.26-1.28) P trend=0.14
Sauvaget (324) (2003) The Life Span Study (n=37,130)	M/W, 34-103, FFQ	Animal	Age, stratified by sex and birth cohort. adjustment; smoking, alcohol, BMI (sr), education, DM, hypertension, radiation dose, city	12% reduction in stroke mortality. After adjustment for fruit and veg intake, HR 0.96 (95% CI 0.84, 1.09) for stroke mortality

Abbreviations: BMI, body mass index; Ca, calcium; CHO, carbohydrate; DM, diabetes; FFQ, food frequency questionnaire; GI, glycaemic index; K, potassium; MI, myocardial infarction; Mg, magnesium; MUFA, monounsaturated fat; Na, sodium; PA, physical activity; PUFA, polyunsaturated fat; SFA, saturated fat; sr, self-reported;.

Table 5.7. Overview of epidemiological studies investigating the associations between dietary patters and risk of stroke.

Author Study Popn-	Sex, Age (y) Dietary Method	Dietary Pattern	Adjustments	Size of Effect
Fung (384) (2009) Nurse's Health Study (n=74,886)	F 38-63, FFQ	Mediterranean	Age, smoking, BMI, menopausal status (incl HRT use), aspirin use, energy intake, alcohol, hours of PA, multivitamin use, family history	Extreme quintiles RR 0.87 (95% CI 0.73- 1.02 P trend = 0.03) total stroke
Fung (385) (2008) Nurse's Health Study (n=88,517)	F 38-63, FFQ	DASH	Age, smoking, BMI, menopausal status (incl HRT use), aspirin use, energy intake, alcohol, hours of PA, multivitamin use, family history	Extreme quintiles RR 0.83 (95% CI 0.71- 0.96 P trend = 0.007) total stroke
Fung (370) (2004) Nurse's Health Study (n=71,768)	F, 30-55, FFQ	Prudent vs. Western	Age, smoking, BMI, menopausal status (incl HRT use), aspirin use, energy intake, alcohol, hours of PA	Extreme quintiles Prudent RR 0.78 (95% CI 0.61-1.01 P trend= 0.13) total stroke

				Western RR 1.58 (95% CI, 1.15-2.15 P trend=0.0002) total stroke
Key (386) (1999) Multiple*[1] (n=76,172)	M/w, 16-89, FFQ	Vegetarian vs non-vegetarian	Age (per 5yr), sex, smoking. N.B some data was missing for different cohorts e.g. BMI, alcohol, education, PA	NS difference in stroke mortality.

Abbreviations: BMI, body mass index; FFQ, food frequency questionnaire; HRT, hormone replacement therapy; NS, non-significant; PA, physical activity.

[1] The Adventist Mortality Study, Health Food Shoppers Study, The Adventist Health Study, The Heidleberg Study, The Oxford Vegetarian Study

5.0.4 Randomised controlled trials

Blood pressure

A number of clinical trials have been conducted investigating the effect of supplementation with protein on blood pressure and lipid profile. However, the majority of these have involved specific populations comprised of participants with existing risk factors including; prehypertension, stage one hypertension, hyperlipidaemia or obesity. They have also primarily assessed the effects of soy protein supplementation (127, 128, 387-390).

A number of studies have identified a significant association with diets higher in protein and also specifically soy protein (388-390) **(Table 5.8)**. The difference between sources of protein, animal/dairy compared with soy may also result from different amino acid composition of the diets, or from other compositional changes to the diet associated with changes in protein content e.g. carbohydrate and fat content, although there is a lack of conclusive evidence in this area.

Plant and animal protein

Recently Teunissen-Beekman et al (391) investigated the effect of consumption of either a mixed protein drink, comprised of 20% each of soy and pea, and 30% each of egg and milk protein-isolate, or maltodextrin drink 20g 3 times/d on blood pressure in overweight Dutch men and women with prehypertension or stage I hypertension. This ratio of plant to animal protein (40:60) was said to reflect the pattern of protein consumption in the Dutch population. Their main findings indicate that additional mixed protein supplementation (pea, soy, egg, and milk-protein isolate) can significantly reduce in-office and daytime ambulatory BP. An average change after four weeks intervention of -4.9±1.7 mmHg (95% CI 1.65-8.2, P=0.005) and -4.6±1.7 mmHg (95% CI 1.3-7.9, P=0.006) for SBP for in-office and daytime ambulatory respectively. A change of-2.7±1.3 mmHg (95% CI0.1-5.4, P=0.05) was reported for in office DBP. There was no significant change in daytime ambulatory DBP. Previously reductions of 1-3 mmHg and 4mmHg in SBP and DBP have been associated with 20-30% and 23% reductions in overall stroke risk (16, 70, 71). However, the protein supplementation appeared to cause greater daily fluctuations in BP, up to 4 mmHg from one day to the next, compared with supplementation with maltodextrin. Whether these

fluctuations would settle with prolonged intake of these diets is unclear. There is evidence to suggest that long-term intra-individual variation in BP is an independent predictor of stroke risk (392, 393) and therefore may indicate that supplementation of this nature actually increases stroke risk.

In addition a recent systematic review and meta-analysis on RCTs investigating dietary protein intake and blood pressure identified 40 relevant trials, published between1980 and 2011, to include in their analysis (366). The majority of the RCTs compared protein intake versus carbohydrate intake (32 studies). Twelve of the RCTs also specifically investigated differences between animal and plant protein. Only two of the identified studies were conducted in UK populations (Scotland) (394, 395), the majority were from the United States (11 studies) and Australia (13 studies). Both of the Scottish studies used supplementation to achieve differences in protein intakes of the control and intervention groups and in addition only investigated vegetable protein intake compared with carbohydrate intake. Only the study by Sagara et al (395) which supplemented 20 g protein/d for 5 weeks showed a significant decrease in blood pressure of -7.2 mmHg and -3.4 mmHg for SBP and DBP. In the study by Harrison et al (394) where 25 g/d of vegetable protein supplement was consumed for 5 weeks there was a slight net increase in SBP of 0.95 mmHg and a small reduction in DBP of -1.0 mmHg. Participants in both studies had an average blood pressure in the pre-hypertensive range.

The authors concluded that the pooled results of all 40 RCTs suggested that an increase in total protein led to an estimated decrease of -1.76 mmHg (-2.33 to -1.20) for SBP and -1.15 (-1.59 to -0.71) for DBP. There was no statistically significant difference in blood pressure in studies assessing the effect of animal compared with plant protein.

Lipid profile

Plant protein

Clinical trials have predominately focused on investigating the effects of differing amounts of soy or dairy protein on changes in lipid profile. Most indicate no significant association between soy protein intake and HDL levels, but a significant inverse association with TC, LDL, TC:HDL and LDL:HDL **(Table 5.9)** (127, 128, 387, 396). Although, findings in relation to

other lipid profile variables are more inconsistent. Appel et al (251) looking at the impact of a mixed protein diet (including some soy) compared with a high CHO diet on lipid profile reported a negative effect. A small (0.03 mmol) but significant decrease in HDL (P=0.02) was reported in those on the protein diet.

Jenkins et al (396) investigated the effect of replacing 93% of animal protein with plant protein (including some soy). They reported significant differences in TC, LDL, TC:HDL and LDL:HDL ratios. This indicates that diets higher in plant protein as opposed to animal protein may exert beneficial effects on lipid profile. However, it cannot be ruled out that other factors associated with this diet including a high fibre content and increase in micronutrients were contributing to the differences exhibited.

One important factor that should be noted is that these studies were conducted in specific populations with many of the participants having existing dyslipidaemia or high blood pressure. Therefore the findings may not be directly comparable to the effects that would be seen in the general population.

Table 5.8. Overview of intervention studies investigating the effect of protein supplementation on blood pressure.

Author	Sex, Age (y)	Intervention (Design, blinding, duration, protocol)	Change in BP (mmHg) SBP	DBP
Teunissen-Beekman (391) (2012) Prehypertensives or stage 1 hypertension PROPRES (n=94) Dutch	M/W, 20-70,	Randomised parallel Double blind 2 wks run in 4 wks Base diet 15% energy protein, 30% fat, 55% CHO. 20 g malodextrin (control) or 20 g mixed protein in 200 ml water at each meal.	Protein vs malodextrin Office: -4.9±1.7 mmHg (95% CI 1.65-8.2) P=0.005 Daytime -4.6±1.7 mmHg (95% CI 1.3-7.9) P=0.006	Office: -2.7±1.3 mmHg (95% CI0.1-5.4) P=0.05 Daytime: NS
Azadbakhtn (389) (2011) Overweight + Obese (n=23)	W, 18-30	Crossover No blinding 6 wks (3 wks washout) Soy drink replacement (one glass replacement of cow's milk) control; only cow's milk	% change Soy -4.0±0.9 Cow's -1.7±0.5 P trend 0.4	% change Soy -0.4±0.1 Cow's 0.4±0.1 P trend 0.4
He (390) (2011) Prehypertensives or stage 1 hypertension (n=352)	M/W, ≥22,	Crossover Double blind 8 wks (3 wks washout) 40 g/d soy protein, milk protein or CHO supplementation (control)	Soy protein -2.0 Milk protein -2.3	NS

USA Appel (251) (2005) Omni-Heart (n=164) US	M/W, ≥30,	Randomised Crossover Single blind 6 days run in 6 wks each CHO rich (58%), protein (mixed) rich (25%), unsaturated fat rich (mainly MUFA)	Mean change w/ protein diet All: -9.5 (95% CI -10.9 to -8.2) Stage 1 hypertensive: -16.1 (95% CI -19.7 to-12.5) Prehypertensive: -8.0 (95% CI -9.3 to -6.6)	Mean change w/ protein diet All: -5.1 (95% CI -6.1 to -4.4) Stage 1 hypertensive: -8.6 (95% CI -10.9 to -6.4) Prehypertensive: -4.4 (95% CI -5.3 to -3.6)
Jenkins (388) (2002) Hyperlipidemic M and postmenopausal W (n=41 (23/18))	M/W mean age 62,	Randomised Crossover Single blind 1 mo each (2 wk washout between) High isoflavone 50 g/d soy & 73 mg/d isoflavones, low isoflavone 52 g/d soy & 10 mg/d isoflavones) control low fat dairy food.	NS difference in SBP or DBP in combined M&W analyses. M only isoflavone diets lower blood pressure than control P=0.065 and 0.007 for low and high respectively.	

Abbreviations: CHO, carbohydrate; DBP; diastolic blood pressure; MUFA, monounsaturated fat; NS, non-significant; SBP, systolic blood pressure

Table 5.9. Overview of intervention studies investigating the effect of protein supplementation on circulating lipid levels.

Author Study Popn	Sex, Age (y)	Intervention (duration, protocol)	Design and Blinding	Results
Thorp (127) (2008) Raised TC (n=91 (33/58))	M/W, 18-80	6 wks each (3 wk washout) 1. 24 g/d soy protein, 2. 12 g/d dairy % 12 g/d soy, 3. Control: 24 g/d dairy.	Randomised Crossover Double Blind	Sig diff w/ soy diet for TC (P=0.014) soy & dairy diet for TG NS effect on LDL or HDL (P<0.001).
Gardner (128) (2007) Hyperlipidemic (n=28)	M/W, 30-65	4 wks each (4 wk washout) 25 g/d from 1. Whole soy bean milk, 2. SPI milk, 3. Low-fat dairy milk	Randomised Crossover Single Blind	Sig decrease in LDL with whole soy bean and SPI compared with dairy milk (P=0.02). NS TG and HDL
Appel (251) (2005) Omni-Heart (n=164) US	M/W ≥30	6 days run in 6 wks each CHO rich (58%), protein rich (25%), unsaturated fat rich (mainly MUFA)	Randomised Crossover Single Blind	Protein sig decrease TG by 0.18 mmol (P<0.001) LDL by 0.09 (P=0.01), and HDL by 0.03 (P=0.02) compared to CHO diet.
Wang (387) (2004)	M/W >50	6 wks each 1. SPI (25 g/1000 kcal) minus isoflavones, 2. Isoflavone (49 mg/1000 kcal) enriched SPI,	Randomised Crossover Single Blind	Sig decrease with soy protein vs animal protein TC (P<0.001), TG (P<0.001), LDL (P<0.003), TC:HDL (P=0.037) and LDL:HDL (P=0.018). But not HDL or VLDL.

Hyperlipidemic(n=20 (6/14)) US		3. Animal protein (25 g/1000 kcal), 4. Animal protein with isoflavones (49 mg/1000 kcal).		NS effect of isoflavone enriched diet, except TC (P=0.009).
Jenkins (388) (2002) Hyperlipidemic M and postmenopausal W (n=41 (23/18))	M/W mean 62	1 mo each (2 wk washout) High isoflavone 50 g/d soy & 73 mg/d isoflavones, low isoflavone 52 g/d soy & 10m g/d isoflavones) control low fat diary food.	Randomised Crossover Single Blind	NS difference between high and low isoflavone diets and lipid levels. Sig difference between control and isoflavone diets (low high respectively) TC P=0.016 & 0.045 Total:HDL P=0.001 & 0.018 LDL:HDL P=0.001 & 0.004
Jenkins (396) (1999) Hyperlipidemic M and postmenopausal W (n=31 (19/12))	M/W, 31-70	1 mo each (2 wk washout) Test diet: 93% animal protein replaced with vegetable-soy, other legumes, and cereals, high soluble fibre content. Control: lacto-ovo vegetarian incl low fat dairy. Cholesterol content same in both diets	Randomised Crossover No Blinding	Sig difference in TC, LDL, TC:HDL, and LDL:HDL P=<0.001, <0.001, 0.002 and 0.004 respectively. NS difference HDL or TG with treatment.

Abbreviations: CHO, carbohydrate; HDL, high density lipoprotein; LDL, low density lipoprotein; MUFA, monounsaturated fat; NS, non-significant; SPI, soy protein isolate; TC, total cholesterol; TG, triglyceride; VLDL, very low density lipoprotein.

To summarise the findings of the literature review indicated that there were inconsistent reports of the influence of protein on stroke risk and risk factors. In addition there has been limited research conducted in UK populations, although a UK cohort was included in the INTERMAP study investigating the effects of dietary protein on blood pressure. Previous research suggests that there may be a benefit of increased consumption of dietary protein on stroke risk factors blood pressure and lipid profile and also directly on stroke risk. Some reports have provided conflicting findings, often potentially due to differences in the source of protein investigated which warranted further investigation.

With this in mind, it is important to elucidate the potential associations between dietary protein intake, including the effects of different sources, on blood pressure, lipid levels and stroke risk in a UK population. To date to my knowledge, no other study has simultaneously investigated the associations between dietary protein intake and stroke risk and risk factors in a UK population of men and women. This may elucidate if the beneficial effects previously reported are due to protein content (composition) or other factors associated with the intake of the dietary pattern or specific protein source.

5.1 Aims and hypotheses

This chapter aims to address research question 3, outlined in the Introductory Chapter, Chapter One.

3. What are the relationships between dietary protein intake, including that from different types (animal and plant) and stroke risk factors, blood pressure and serum lipid levels and the risk of stroke in middle and older aged men and women?

The hypothesis was that overall higher total protein intake would be inversely associated with blood pressure, a more favourable lipid profile and lower risk of stroke.

For subtypes of protein intake it was hypothesised that higher animal protein intake would be associated with higher blood pressure, less favourable lipid profile and overall increase in stroke risk. Whereas for plant protein, the hypothesis was that higher plant protein intakes would be inversely associated with blood pressure and a more favourable lipid profile and lower stroke risk.

5.2 Methods

Methods are provided in full in Chapter Two – Subjects and methods.

Briefly 4,361 participants were included in these analyses after subjects with missing data for one or more of the following variables were excluded; BMI, WHR, SBP, DBP, LDL, HDL, or TG levels, education status, baseline prevalent stroke, MI, DM, family history of stroke or MI (n=559). Individuals with missing data for cigarette smoking were coded as current smoker (n=37) and no response to the question 'Have you taken aspirin continuously for 3 months or more' where recoded into the 'no' category (n=710).

Protein categorisation

Protein was classified into total protein, animal protein, plant protein and unclassified. Total protein was all protein intake. Animal protein was a combination of three protein groups, animal-land, animal-marine and animal-derived. In addition a number of mixed foods where the main bulk of protein was derived from animal sources were also added to this group, such as food in milk or cheese based sauces. Plant protein contains anything of plant origin including fruit, vegetables and grains. An 'unclassified' category was created for items which did not fit into either the plant or animal group. This included items such as water, artificial sweeteners, spirits, yeast and honey, as these do not contribute highly to protein intake, they are therefore unlikely to influence the findings. Further details of the categorisation of protein is included in the previous chapter; Chapter Four – Contribution to dietary protein intake.

Protein intakes were expressed as a percentage of total energy intake. This unit was chosen to account, to some extent, for total energy intake as protein intake is strongly correlated with total energy. Expressing intakes as a percentage of energy also allows comparison between sexes and populations. Variables of protein intake as a percentage of total energy were created by multiplying the protein intake in g/d by 4 (the number of kcal in one gram of protein), divided by total energy intake and multiplying by 100:

$$(\text{protein}*4/\text{total energy})*100$$

The ratio of plant:animal protein intake was calculated by dividing plant protein by animal.

Statistical analyses

The risk factors analysed for associations with percentage energy from protein were blood pressure and blood lipids. These risk factors were chosen as raised blood pressure and abnormal lipid profile are significant independent risk factors for stroke.

Analyses were conducted by sex-specific quintiles of percentage energy from protein intake. Percentage of energy from protein was used as this provides a better representation of protein intake independent of total energy intake. Multiple regression analysis with multivariable adjustment was used to identify potential associations between percentage energy from protein intake in relation to blood pressure (SBP and DBP) and lipid profile (TC, LDL, HDL and TG) in men and women aged 40-75 years.

5.3 Results EPIC

Table 5.10 Baseline characteristics of men and women aged 39-80 years in the EPIC-Norfolk sub-cohort.

	Men n=2000	**Women n=2443**	**P-value[1]**
Age (years)	61.1 (±9.53)	60.4 (±9.7)	0.02
BMI (kg/m$^{2)}$	26.5 (±3.18)	26.2 (±4.24)	0.01
Stroke Cases (%)	449 (22.5%)	479 (19.6%)	0.02
Family History Stroke (%)	465 (23.3%)	601 (24.6%)	0.29
Family History MI (%)	720 (36.0%)	934 (38.2%)	0.13
Family History DM (%)	222 (11.1%)	305 (12.5%)	0.16
Blood Pressure mmHg			
SBP	140 (±18.5)	136 (±19.5)	<0.001
DBP	85.3 (±11.5)	81.8 (±11.4)	<0.001
Antihypertensive Use (%)	417 (20.9%)	516 (21.1%)	0.83
Aspirin Use (%)	271 (13.6%)	197 (8.06%)	<0.001
Blood Lipids mmol/l			
Total Cholesterol	6.07 (±1.10)	6.36 (±1.22)	<0.001
Statin use (%)	27 (1.35%)	43 (1.76%)	0.28
Smoking (%)			<0.001
Current	234 (11.7%)	314 (12.9%)	
Former	1,114 (55.7%)	774 (31.7%)	
Never	652 (32.6%)	1,355 (55.5%)	
Physical Activity (%)			<0.001
Inactive	644 (32.2%)	800 (32.8%)	
Moderately Inactive	476 (23.8%)	790 (32.3%)	
Moderately Active	440 (22.0%)	514 (21.0%)	
Active	440 (22.0%)	339 (13.9%)	
Education Level (%)			<0.001
0 – No Qualifications	667 (33.4%)	1,086 (44.5%)	
1 – O-Level or Equivalent	165 (8.25%)	249 (10.2%)	
2 – A-Level or Equivalent	887 (44.4%)	822 (33.7%)	
3 – Degree or Equivalent	281 (14.1%)	286 (11.7%)	
Dietary Factors			
Total Energy (kcal/d)	2218 (±505)	1685 (±384)	<0.001

Protein percentage energy (%)	15.0 (±2.58)	15.8 (±2.87)	<0.001
Plant protein (g/d)	25.5 (±7.94)	19.6 (±5.87)	<0.001
Animal protein (g/d)	55.0 (±14.8)	44.5 (±12.4)	<0.001
Ratio plant:animal (g/d)	0.50 (±0.23)	0.49 (±0.54)	0.82
Unclassified protein (g/d)	1.03 (±0.65)	0.94 (±0.59)	<0.001
Alcohol (g/d)	15.9 (±20.8)	7.70 (±11.7)	<0.001
Total fat (g/d)	85.1 (±25.9)	64.6 (±20.6)	<0.001
Sodium (mg/d)	3150 (±864)	2405 (±660)	<0.001

Table 5.11 Association of quintiles of percentage energy from protein (range and mean quintile intake) and blood pressure (mean and SE) in 2,000 men, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Total protein intake as %en					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		7.18-12.93%	**12.94-14.16%**	**14.17-15.26%**	**15.27-16.95%**	**16.96-32.16%**	**P trend**
		11.8%	**13.7%**	**14.7%**	**16.0%**	**18.8%**	
		n=400	**n=400**	**n=400**	**n=400**	**n=400**	
SBP	Unadjusted	139 (±0.86)	138 (±0.87)	140 (±0.95)	140 (±0.94)	141 (±1.00)	0.03
	Model 1[1]	140 (±0.86)	139 (±0.86)	140 (±0.86)	140 (±0.86)	139 (±0.86)	0.35
	Model 2[2]	140 (±0.91)	139 (±0.86)	140 (±0.85)	140 (±0.86)	139 (±0.92)	0.95
DBP	Unadjusted	85.6 (±0.56)	84.2 (±0.53)	85.8 (±0.60)	85.6 (±0.59)	85.5 (±0.59)	0.54
	Model 1[1]	86.3 (±0.56)	84.9 (±0.56)	85.7 (±0.56)	85.4 (±0.56)	84.4 (±0.56)*[3]	0.07
	Model 2[2]	85.8 (±0.59)	84.8 (±0.56)	85.8 (±0.55)	85.4 (±0.56)	84.9 (±0.60)	0.60

[1]Model 1: age, BMI, smoking status, physical activity, education level, antihypertensive medication use

[2]Model 2: model 1 + baseline MI or diabetes, family history stroke, family history MI, alcohol intake, dietary sodium, total energy

[3]P value ≤0.05 compared with Q1

Table 5.12 Association of quintiles of percentage energy from protein (range and mean quintile intake) and blood pressure (mean and SE) in 2,443 women, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Total protein intake as %en					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		8.13-13.33%	**13.34-14.82%**	**14.83-16.16%**	**16.17-17.91%**	**17.92-30.79%**	**P trend**
		12.1%	**14.2%**	**15.5%**	**16.9%**	**20.0%**	
		n=489	**n=489**	**n=488**	**n=489**	**n=488**	
SBP	Unadjusted	135 (±0.92)	135 (±0.89)	136 (±0.85)	137 (±0.90)	136 (±0.86)	0.07
	Model 1[1]	137 (±0.77)	136 (±0.77)	136 (±0.77)	136 (±0.77)	134 (±0.78)**[3]	0.01
	Model 2[2]	137 (±0.82)	136 (±0.78)	136 (±0.77)	136 (±0.77)	134 (±0.83)**	0.02
DBP	Unadjusted	81.4 (±0.53)	81.5 (±0.49)	82.0 (±0.54)	82.8 (±0.53)	81.2 (±0.49)	0.59
	Model 1[1]	82.7 (±0.48)	81.9 (±0.48)	82.1 (±0.48)	82.3 (±0.48)	79.9 (±0.48)***	0.001
	Model 2[2]	82.7 (±0.51)	82.0 (±0.49)	82.0 (±0.48)	82.3 (±0.48)	79.9 (±0.52)***	0.01

[1]Model 1: age, BMI, smoking status, physical activity, education level, antihypertensive medication use

[2]Model 2: model 1 + baseline MI or diabetes, family history stroke, family history MI, alcohol intake, dietary sodium, total energy

[3]P value ≤0.05, ** P value ≤0.01 ***≤0.001 compared with Q1

Total protein %en

There was no significant association between the percentage of energy from protein and blood pressure in 2,000 men **(Table 5.11)** after adjusting for relevant confounding variables including age, BMI, use of antihypertensive medication and dietary factors; total energy and sodium intake. There was however, a significant difference in DBP between extreme quintiles of -1.9 mmHg (P≤0.05) which was identified after adjustment for factors including age and physical activity levels. This significance was attenuated to -0.9 mmHg, with the addition of family history of stroke or MI, prevalent MI or DM and dietary factors; alcohol intake, total energy intake and sodium intake. In women **(Table 5.12)** a significant inverse trend was seen across quintiles of protein %en for both SBP and DBP after full adjustment for confounding variables (P trend =0.02 and 0.01 respectively). Significant differences of -3 mmHg (P≤0.01) and -2.8 mmHg (P≤0.001) in mean SBP and DBP were identified between the extreme quintiles.

Table 5.13 Association of quintiles of dietary plant protein intakes (range and mean quintile intake) and blood pressure (mean and SE) in 2,000 men, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary plant protein intake as %en					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		1.44-3.67%	**3.68-4.23%**	**4.24-4.75%**	**4.76-5.51%**	**5.52-11.0%**	**P trend**
		3.20%	**3.98%**	**4.49%**	**5.11%**	**6.43%**	
		n=400	**n=400**	**n=400**	**n=400**	**n=400**	
SBP	Unadjusted	142 (±0.91)	140 (±0.96)	139 (±0.91)	139 (±0.93)	138 (±0.92)	<0.01
	Model 1[1]	141 (±0.86)	140 (±0.85)	139 (±0.85)	138 (±0.85)*[3]	139 (±0.85)*	0.01
	Model 2[2]	141 (±0.88)	140 (±0.85)	139 (±0.85)	139 (±0.85)	139 (±0.89)	0.06
DBP	Unadjusted	86.3 (±0.56)	86.0 (±0.60)	85.0 (±0.55)	84.5 (±0.59)	84.8 (±0.57)	0.01
	Model 1[1]	86.6 (±0.56)	85.9 (±0.56)	85.1 (±0.56)*	84.2 (±0.56)**	84.9 (±0.56)*	<0.01
	Model 2[2]	86.4 (±0.58)	85.7 (±0.56)	85.1 (±0.55)	84.4 (±0.57)*	84.9 (±0.58)	0.03

[1]Model 1: age, BMI, smoking status, physical activity, education level, antihypertensive medication use

[2]Model 2: model 1 + baseline MI or diabetes, family history stroke, family history MI, alcohol intake, dietary sodium, total energy, animal protein

[3]P value ≤0.05 **≤0.01 compared with Q1

Table 5.14 Association of quintiles of plant protein intake (range and mean quintile intake) and blood pressure (mean and SE) in 2,443 women, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary plant protein intake as %en					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		0.00-3.75%	**3.76-4.34%**	**4.35-4.91%**	**4.92-5.58%**	**5.59-13.2%**	**P trend**
		3.22%	**4.07%**	**4.63%**	**5.23%**	**6.51%**	
		n=489	**n=489**	**n=488**	**n=489**	**n=488**	
SBP	Unadjusted	136 (±0.88)	137 (±0.92)	136 (±0.89)	136 (±0.85)	134 (±0.89)	0.10
	Model 1[1]	136 (±0.77)	137 (±0.77)	136 (±0.77)	136 (±0.77)	135 (±0.77)	0.23
	Model 2[2]	136 (±0.80)	137 (±0.77)	136 (±0.77)	136 (±0.77)	134 (±0.81)	0.09
DBP	Unadjusted	81.8 (±0.50)	82.5 (±0.54)	81.7 (±0.49)	82.1 (±0.52)	80.8 (±0.52)	0.13
	Model 1[1]	81.7 (±0.48)	82.6 (±0.48)	81.8 (±0.48)	81.9 (±0.48)	80.8 (±0.48)	0.10
	Model 2[2]	81.9 (±0.50)	82.7 (±0.48)	81.8 (±0.48)	81.9 (±0.48)	80.6 (±0.51)	0.05

[1]Model 1: age, BMI, smoking status, physical activity, education level, antihypertensive medication use

[2]Model 2: model 1 + baseline MI or diabetes, family history stroke, family history MI, alcohol intake, dietary sodium, total energy, animal protein.

Plant protein %en

In men no significant association was identified between quintiles of dietary plant protein %en and SBP after full adjustment (P trend =0.06) **(Table 5.13)**. Prior to adjusting for medical history and dietary variables there was a significant inverse trend (P trend =0.01) between plant protein intake and SBP with a significant difference of -2 mmHg between extreme quintiles (P≤0.05). A non-significant difference of -1.5 mmHg was present between extreme quintiles for DBP after full adjustment including age, BMI, and dietary factors; total energy and intake of animal protein and there was a significant trend towards lower DBP with increasing intakes of plant protein %en (P trend =0.03). For women, there was no significant association between SBP and plant protein %en (P trend =0.09) **(Table 5.14)**, there was however, a non-significant difference of -2.0 mmHg between the extreme quintiles in the fully adjusted model, which also adjusted for animal protein intakes in addition to other dietary factors, medical history and lifestyle factors. There was a weakly significant (P trend =0.05) trend towards lower DBP with increasing plant protein %en in the fully adjusted model.

Table 5.15 Association of quintiles of animal protein intake (range and mean quintile intake) and blood pressure (mean and SE) in 2,000 men, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary animal protein intake as %en					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		2.71-8.23%	**8.24-9.41%**	**9.42-10.4%**	**10.5-11.8%**	**11.9-27.4%**	**P trend**
		6.96%	**8.87%**	**9.94%**	**9.94%**	**13.7%**	
		n=400	**n=400**	**n=400**	**n=400**	**n=400**	
SBP	Unadjusted	138 (±0.90)	139 (±0.88)	138 (±0.90)	142 (±0.95)	140 (±0.99)	0.02
	Model 1[1]	140 (±0.86)	140 (±0.86)	138 (±0.85)	141 (±0.85)	138 (±0.86)	0.43
	Model 2[2]	140 (±0.91)	140 (±0.85)	138 (±0.85)	141 (±0.85)	139 (±0.90)	0.64
DBP	Unadjusted	84.7 (±0.58)	85.3 (±0.56)	85.1 (±0.56)	86.2 (±0.58)	85.3 (±0.60)	0.24
	Model 1[1]	85.5 (±0.56)	85.8 (±0.56)	85.0 (±0.56)	86.1 (±0.56)	84.3 (±0.56)	0.26
	Model 2[2]	85.3 (±0.59)	85.6 (±0.56)	85.1 (±0.55)	86.1 (±0.56)	84.5 (±0.59)	0.61

[1]Model 1: age, BMI, smoking status, physical activity, education level, antihypertensive medication use

[2]Model 2: model 1 + baseline MI or diabetes, family history stroke, family history MI, alcohol intake, dietary sodium, total energy, plant protein

Table 5.16 Association of quintiles of animal protein intake (range and mean quintile intake) and blood pressure (mean and SE) in 2,443 women, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary animal protein intake as %en					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		0.43-8.58%	**8.56-9.99%**	**10.0-11.2%**	**11.3-12.8%**	**12.9-26.0%**	**P trend**
		7.14%	**9.37%**	**10.6%**	**12.0%**	**14.9%**	
		n=489	**n=489**	**n=488**	**n=489**	**n=488**	
SBP	Unadjusted	134 (±0.90)	136 (±0.91)	136 (±0.83)	137 (±0.90)	137 (±0.87)	0.01
	Model 1[1]	137 (±0.77)	136 (±0.77)	136 (±0.77)	135 (±0.77)	135 (±0.77)	0.04
	Model 2[2]	137 (±0.82)	136 (±0.78)	136 (±0.77)	135 (±0.77)	135 (±0.82)	0.04
DBP	Unadjusted	81.1 (±0.52)	81.7 (±0.52)	82.0 (±0.50)	82.4 (±0.53)	81.8 (±0.50)	0.19
	Model 1[1]	82.4 (±0.48)	82.0 (±0.48)	82.2 (±0.48)	81.6 (±0.48)	80.6 (±0.49)**[3]	0.01
	Model 2[2]	82.6 (±0.51)	82.0 (±0.48)	82.1 (±0.48)	81.6 (±0.48)	80.6 (±0.51)**	0.01

[1]Model 1: age, BMI, smoking status, physical activity, education level, antihypertensive medication use

[2]Model 2: model 1 + baseline MI or diabetes, family history stroke, family history MI, alcohol intake, dietary sodium, total energy, plant protein

[3]P value ≤0.05 **≤0.01 compared with Q1

Animal Protein %en

No significant associations were identified between animal protein %en and blood pressure in men **(Table 5.15)**. However, for women **(Table 5.16)** there was a significant inverse association between dietary animal protein %en and SBP and DBP (P trend = 0.04 and 0.01 respectively). There was a significant difference of -2 mmHg between extreme quintiles of the fully adjusted model for DBP (P≤0.01). In addition there was a difference of -2 mmHg between extreme quintiles for SBP, however, this did not reach significance.

Table 5.17 Association of quintiles of ratio plant:animal protein intake (range and mean quintile intake) and blood pressure (mean and SE) in 2,000 men, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary plant:animal protein Intake					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		0.07-0.33	**0.34-0.40**	**0.41-0.48**	**0.49-0.59**	**0.60-2.71**	**P trend**
		0.28	**0.38**	**0.45**	**0.54**	**0.83**	
		n=400	**n=400**	**n=400**	**n=400**	**n=400**	
SBP	Unadjusted	142 (±0.96)	140 (±1.00)	140 (±0.86)	139 (±0.89)	137 (±0.90)	<0.001
	Model 1[1]	141 (±0.86)	139 (±0.86)	140 (±0.85)	139 (±0.86)	139 (±0.86)	0.13
	Model 2[2]	141 (±0.85)	139 (±0.85)	140 (±0.85)	139 (±0.85)	139 (±0.85)	0.17
DBP	Unadjusted	86.3 (±0.56)	85.6 (±0.66)	85.3 (±0.52)	85.1 (±0.56)	84.4 (±0.57)	0.02
	Model 1[1]	85.9 (±0.56)	85.4 (±0.56)	85.2 (±0.56)	84.3 (±0.56)	84.9 (±0.56)	0.21
	Model 2[2]	85.9 (±0.56)	85.3 (±0.55)	85.2 (±0.55)	85.3 (±0.55)	84.9 (±0.56)	0.23

[1]Model 1: age, BMI, smoking status, physical activity, education level, antihypertensive medication use

[2]Model 2: model 1 + baseline MI or diabetes, family history stroke, family history MI, alcohol intake, dietary sodium, total energy

Table 5.18 Association of quintiles of ratio plant:animal protein intake (range and mean quintile intake) and blood pressure (mean and SE) in 2,443 women, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary plant:animal protein intake					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		0.00-0.32	**0.33-0.38**	**0.39-0.46**	**0.47-0.57**	**0.58-22.4**	**P trend**
		0.27	**0.36**	**0.43**	**0.52**	**0.90**	
		n=489	**n=489**	**n=488**	**n=489**	**n=488**	
SBP	Unadjusted	137 (±0.89)	136 (±0.85)	138 (±0.91)	136 (±0.88)	133 (±0.87)	0.01
	Model 1[1]	135 (±0.77)	135 (±0.77)	137 (±0.77)	137 (±0.77)	136 (±0.77)	0.44
	Model 2[2]	135 (±0.77)	135 (±0.77)	137 (±0.77)	136 (±0.77)	135 (±0.77)	0.54
DBP	Unadjusted	82.1 (±0.52)	81.6 (±0.49)	82.6 (±0.52)	82.1 (±0.55)	80.5 (±0.49)	0.09
	Model 1[1]	81.4 (±0.48)	81.2 (±0.48)	82.3 (±0.48)	82.6 (±0.48)	81.5 (±0.48)	0.33
	Model 2[2]	81.4 (±0.48)	81.2 (±0.48)	82.3 (±0.48)	81.6 (±0.48)	81.5 (±0.48)	0.33

[1]Model 1: age, BMI, smoking status, physical activity, education level, antihypertensive medication use

[2]Model 2: model 1 + baseline MI or diabetes, family history stroke, family history MI, alcohol intake, dietary sodium, total energy

Ratio of plant:animal protein

The ratio of plant:animal protein intake was not significantly associated with blood pressure in men (**Table 5.17)** or women **(Table 5.18)** after adjustment for confounding variables including age, BMI, smoking status physical activity, education level, use of antihypertensive medication, MI or diabetes at baseline, family history of stroke or MI, alcohol intake, and dietary sodium and total energy intakes.

Table 5.19 Association of quintiles of percentage energy from protein (range and mean quintile intake) and cholesterol and sub fractions (means and SE) in 1,888 men, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary total protein as %en					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		7.18-12.93%	**12.94-14.16%**	**14.17-15.26%**	**15.27-16.95%**	**16.96-32.16%**	**P trend**
		11.7%	**13.6%**	**14.7%**	**16.0%**	**18.7%**	
		n=373	**n=383**	**n=375**	**n=379**	**n=378**	
Total Cholesterol	Unadjusted	5.98 (±0.06)	6.03 (±0.05)	6.00 (±0.06)	6.06 (±0.05)	6.02 (±0.05)	0.52
	Model 1[1]	5.97 (±0.06)	6.04 (±0.06)	6.00 (±0.06)	6.06 (±0.06)	6.01 (±0.06)	0.64
	Model 2[2]	5.94 (±0.06)	6.04 (±0.06)	6.02 (±0.05)	6.06 (±0.05)	6.03 (±0.06)	0.32
Triglycerides	Unadjusted	1.89 (±0.05)	1.87 (±0.05)	1.86 (±0.04)	1.92 (±0.05)	1.91 (±0.05)	0.55
	Model 1[1]	1.91 (±0.05)	1.94 (±0.05)	1.85 (±0.05)	1.91 (±0.05)	1.84 (±0.05)	0.25
	Model 2[2]	1.91 (±0.05)	1.93 (±0.05)	1.85 (±0.05)	1.92 (±0.05)	1.84 (±0.05)	0.36
HDL	Unadjusted	1.25 (±0.02)	1.25 (±0.02)	1.21 (±0.02)	1.24 (±0.02)	1.18 (±0.02)	<0.01
	Model 1[1]	1.25 (±0.02)	1.24 (±0.02)	1.22 (±0.02)	1.24 (±0.02)	1.20 (±0.02)* [3]	0.09
	Model 2[2]	1.20 (±0.02)	1.22 (±0.02)	1.23 0.02)	1.25 (±0.02)*	1.24 (±0.02)	0.03
LDL	Unadjusted	3.87 (±0.05)	3.93 (±0.05)	3.95 (±0.05)	3.96 (±0.05)	3.97 (±0.05)	0.14
	Model 1[1]	3.86 (±0.05)	3.93 (±0.05)	3.95 (±0.05)	3.95 (±0.05)	3.98 (±0.05)	0.12
	Model 2[2]	3.88 (±0.05)	3.94 (±0.05)	3.96 (±0.05)	3.94 (±0.05)	3.95 (±0.05)	0.45
HDL:LDL	Unadjusted	0.36 (±0.01)	0.34 (±0.01)	0.32 (±0.01)	0.33 (±0.01)	0.32 (±0.01)	0.001
	Model 1[1]	0.36 (±0.01)	0.33 (±0.01)	0.33 (±0.01)**	0.33 (±0.01)	0.32 (±0.01)**	0.01
	Model 2[2]	0.34 (±0.01)	0.33 (±0.01)	0.33 (±0.01)	0.34 (±0.01)	0.34 (±0.01)	0.51

[1]Model 1: age, BMI, smoking status, physical activity, education level, baseline MI, baseline DM, family history stroke or MI and statin medication use

[2]Model 2: model 1 + alcohol intake, total fat intake, total energy

[3]P value *≤0.05, **≤0.01 compared with Q1

Table 5.20 Association of quintiles of protein as percentage of energy intake (range and mean quintile intake) and cholesterol and sub fractions (means and SE) in 2,380 women, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary total protein as %en					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		8.13-13.34% 12.1% n=484	**13.35-14.82% 14.2% n=478**	**14.83-16.16% 15.5% n=475**	**16.17-17.91% 16.9% n=474**	**17.92-30.79% 20.0% n=469**	**P trend**
Total Cholesterol	Unadjusted	6.32 (±0.06)	6.26 (±0.05)	6.35 (±0.05)	6.24 (±0.05)	6.40 (±0.05)	0.45
	Model 1[1]	6.41 (±0.05)	6.28 (±0.05)	6.35 (±0.05)	6.20 (±0.05)** [3]	6.32 (±0.05)	0.09
	Model 2[2]	6.42 (±0.05)	6.29 (±0.05)	6.35 (±0.05)	6.20 (±0.05)**	6.31 (±0.05)	0.07
Triglycerides	Unadjusted	1.56 (±0.04)	1.53 (±0.04)	1.59 (±0.03)	1.58 (±0.04)	1.64 (±0.04)	0.08
	Model 1[1]	1.65 (±0.03)	1.56 (±0.03)	1.60 (±0.03)	1.54 (±0.03)*	1.54 (±0.03)*	0.02
	Model 2[2]	1.67 (±0.03)	1.56 (±0.03)*	1.60 (±0.03)	1.53 (±0.03)**	1.54 (±0.04)*	0.02
HDL	Unadjusted	1.60 (±0.02)	1.54 (±0.02)	1.61 (±0.02)	1.54 (±0.02)	1.53 (±0.02)	0.03
	Model 1[1]	1.57 (±0.02)	1.53 (±0.02)	1.60 (±0.02)	1.55 (±0.02)	1.56 (±0.02)	0.89
	Model 2[2]	1.53 (±0.02)	1.52 (±0.02)	1.60 (±0.02)**	1.57 (±0.02)	1.60 (±0.02)*	0.01
LDL	Unadjusted	4.02 (±0.05)	4.03 (±0.05)	4.03 (±0.05	3.98 (±0.05)	4.12 (±0.05)	0.31
	Model 1[1]	4.10 (±0.05)	4.05 (±0.05)	4.03 (±0.05)	3.95 (±0.05)*	4.06 (±0.05)	0.27
	Model 2[2]	4.14 (±0.05)	4.07 (±0.05)	4.02 (±0.05)	3.94 (±0.05)**	4.02 (±0.05)	0.03
HDL:LDL	Unadjusted	0.44 (±0.01)	0.42 (±0.01)	0.44 (±0.01)	0.42 (±0.01)	0.42 (±0.02)	0.18
	Model 1[1]	0.43 (±0.01)	0.42 (±0.01)	0.44 (±0.01)	0.43 (±0.01)	0.43 (±0.01)	0.39
	Model 2[2]	0.41 (±0.01)	0.41 (±0.01)	0.44 (±0.01)*	0.44 (±0.01)	0.45 (±0.01)**	<0.01

[1]Model 1: age, BMI, smoking status, physical activity, education level, baseline MI, baseline DM, family history stroke or MI and statin medication use

[2]Model 2: model 1 + alcohol intake, total fat intake, total energy

[3]P value *≤0.05, **≤0.01 compared with Q1

Lipid Levels

Analyses for associations between dietary protein intake and serum lipid levels were conducted on a sub-sample of the cohort, additionally excluding individuals with missing data for serum lipid levels. A total of 4,268 participants were included, 1,888 men and 2,380 women.

Total protein %en

Table 5.19 illustrates the findings of analyses assessing associations between quintiles of percentage of energy from protein in men. No significant associations were identified between total cholesterol, triglyceride, LDL levels or ratio of HDL:LDL levels. Although for triglycerides there was a non-significant trend (P trend=0.36) towards lower levels across quintiles and a non-significant difference of -0.05 mmol/L between extreme quintiles of the fully adjusted model. There was a significant positive trend identified between percentage energy from protein and HDL levels (P=0.03) and non-significant difference of +0.04 mmol/L between extreme quintiles in the fully adjusted model which included covariates such as age, BMI, use of statin medication, and dietary factors such as alcohol intake, total fat and energy intake. A significant inverse trend was identified for the ratio of HDL:LDL cholesterol and percentage of energy from protein in model 1 adjusting for anthropometric and lifestyle factors such as BMI, and smoking status. However, this was attenuated with the addition of dietary variables to the model. In women **(Table 5.20)** no significant association was reported for total cholesterol, but a significant difference of -0.22 mmol/L (P≤0.01) was seen between quintile 1 and 4. A non-significant difference of -0.09 mmol/L was present between extreme quintiles. Significant inverse associations were identified with increasing percentage of energy from dietary protein and triglyceride and LDL levels (P trend = 0.02 and 0.03 respectively). A significant difference between the extreme quintiles of 0.13 mmol/L was also identified in the fully adjusted model (P≤0.05). In addition significant positive associations were present between HDL and the ratio of HDL:LDL and quintiles of percentage energy from protein (P=0.01 and <0.01 respectively) after full adjustment for confounding variables. Significant differences of +0.07 mmol/L and +0.04 mmol/L were also identified between extreme quintiles for HDL and ratio HDL:LDL respectively (P≤0.05 for HDL and P≤0.01 for HDL:LDL).

Table 5.21 Association of quintiles of dietary plant protein intake (range and mean quintile intake) and cholesterol and sub fractions (means and SE) in 1,888 men, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary plant protein intake as %en					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		1.44-3.67%	**3.68-4.23%**	**4.24-4.75%**	**4.76-5.51%**	**5.52-11.0%**	**P trend**
		3.21%	**3.98%**	**4.49%**	**5.11%**	**6.42%**	
		n=377	**n=372**	**n=378**	**n=379**	**n=382**	
Total Cholesterol	Unadjusted	6.20 (±0.06)	6.07 (±0.05)	6.05 (±0.05)	5.90 (±0.06)	5.87 (±0.05)	<0.001
	Model 1[1]	6.19 (±0.06)	6.06 (±0.06)	6.05 (±0.05)	5.91 (±0.05)***[3]	5.87 (±0.05)***	<0.001
	Model 2[2]	6.13 (±0.06)	6.04 (±0.06)	6.05 (±0.05)	5.93 (±0.06)*	5.92 (±0.06)*	0.01
Triglycerides	Unadjusted	1.91 (±0.05)	1.83 (±0.04)	1.94 (±0.05)	1.90 (±0.05)	1.87 (±0.05)	0.98
	Model 1[1]	1.96 (±0.05)	1.82 (±0.05)*	1.93 (±0.05)	1.87 (±0.05)	1.88 (±0.05)	0.61
	Model 2[2]	1.97 (±0.05)	1.82 (±0.05)*	1.93 (±0.05)	1.87 (±0.05)	1.86 (±0.05)	0.34
HDL	Unadjusted	1.30 (±0.02)	1.24 (±0.02)	1.20 (±0.02)	1.19 (±0.02)	1.21 (±0.02)	<0.001
	Model 1[1]	1.29 (±0.02)	1.25 (±0.02)	1.21 (±0.02)***	1.19 (±0.02)***	1.21 (±0.02)***	<0.001
	Model 2[2]	1.23 (±0.02)	1.22 (±0.02)	1.21 (±0.02)	1.22 (±0.02)	1.26 (±0.02)	0.37
LDL	Unadjusted	4.04 (±0.05)	4.00 (±0.05)	3.96 (±0.05)	3.86 (±0.05)	3.81 (±0.04)	<0.001
	Model 1[1]	4.03 (±0.06)	4.00 (±0.05)	3.97 (±0.05)	3.88 (±0.05)*	3.82 (±0.05)**	0.001
	Model 2[2]	4.02 (±0.05)	4.00 (±0.05)	3.97 (±0.05)	3.87 (±0.05)*	3.82 (±0.05)*	0.01
HDL:LDL	Unadjusted	0.35 (±0.01)	0.33 (±0.01)	0.33 (±0.01)	0.33 (±0.01)	0.34 (±0.01)	0.43
	Model 1[1]	0.35 (±0.01)	0.33 (±0.01)	0.33 (±0.01)	0.33 (±0.01)	0.34 (±0.01)	0.41
	Model 2[2]	0.33 (±0.01)	0.32 (±0.01)	0.33 (±0.01)	0.34 (±0.01)	0.36 (±0.01)*	0.02

[1]Model 1: age, BMI, smoking status, physical activity, education level, baseline MI, baseline DM, family history stroke or MI and statin medication use

[2]Model 2: model 1 + alcohol intake, total fat intake, total energy, animal protein

[3]P value *≤0.05, **≤0.01, ***≤0.001 compared with Q1

Table 5.22 Association of quintiles of dietary plant protein intake (range and mean quintile intake) and cholesterol and sub fractions (means and SE) in 2,380 women, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary plant protein as %en					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		0.00-3.76%	**3.77-4.34%**	**4.35-4.91%**	**4.92-5.58%**	**5.59-13.2%**	**P trend**
		3.22%	**4.07%**	**4.63%**	**5.23%**	**6.51%**	
		n=478	**n=473**	**n=478**	**n=474**	**n=477**	
Total Cholesterol	Unadjusted	6.49 (±0.06)	6.33 (±0.05)	6.26 (±0.05)	6.31 (±0.05)	6.18 (±0.05)	<0.001
	Model 1[1]	6.44 (±0.05)	6.32 (±0.05)	6.27 (±0.05)*[3]	6.30 (±0.05)*	6.23 (±0.05)**	0.01
	Model 2[2]	6.45 (±0.06)	6.32 (±0.05)	6.28 (±0.05)*	6.39 (±0.05)	6.21 (±0.06)**	0.01
Triglycerides	Unadjusted	1.57 (±0.03)	1.58 (±0.04)	1.60 (±0.04)	1.56 (±0.03)	1.59 (±0.04)	0.81
	Model 1[1]	1.55 (±0.03)	1.59 (±0.03)	1.61 (±0.03)	1.54 (±0.03)	1.60 (±0.03)*	0.60
	Model 2[2]	1.58 (±0.04)	1.60 (±0.03)	1.61 (±0.03)	1.53 (±0.03)	1.57 (±0.04)	0.45
HDL	Unadjusted	1.60 (±0.02)	1.59 (±0.02)	1.56 (±0.02)	1.56 (±0.02)	1.51 (±0.02)	<0.001
	Model 1[1]	1.59 (±0.02)	1.59 (±0.02)	1.56 (±0.02)	1.57 (±0.02)	1.52 (±0.02)**	0.01
	Model 2[2]	1.54 (±0.02)	1.56 (±0.02)	1.56 (±0.02)	1.59 (±0.02)	1.58 (±0.02)	0.14
LDL	Unadjusted	4.18 (±0.05)	4.02 (±0.05)	3.98 (±0.05)	4.05 (±0.05)	3.95 (±0.05)	0.01
	Model 1[1]	4.15 (±0.05)	4.02 (±0.05)*	3.99 (±0.05)*	4.04 (±0.05)	3.98 (±0.05)*	0.04
	Model 2[2]	4.20 (±0.06)	4.04 (±0.05)*	3.99 (±0.05)**	4.03 (±0.05)*	3.92 (±0.06)***	<0.01
HDL:LDL	Unadjusted	0.43 (±0.02)	0.44 (±0.02)	0.43 (±0.01)	0.42 (±0.01)	0.42 (±0.01	0.25
	Model 1[1]	0.43 (±0.01)	0.44 (±0.01)	0.43 (±0.01)	0.42 (±0.01)	0.42 (±0.01)	0.21
	Model 2[2]	0.41 (±0.01)	0.43 (±0.01)	0.43 (±0.01)	0.43 (±0.01)	0.45 (±0.01)	0.14

[1]Model 1: age, BMI, smoking status, physical activity, education level, baseline MI, baseline DM, family history stroke or MI and statin medication use

[2]Model 2: model 1 + alcohol intake, total fat intake, total energy

[3]P value *≤0.05, **≤0.01 ***≤0.001 compared with Q1

Plant protein %en

For plant protein %en and lipid levels significant inverse associations were identified with total cholesterol and LDL levels in men **(Table 5.21)** (P=0.01 for both). Significant differences of -0.21 mmol/L (P≤ 0.05) and -0.20 mmol/L (P≤0.05) were also identified between extreme quintiles for total cholesterol and LDL respectively. A significant positive trend was identified for the ratio of HDL:LDL (P=0.02) and a significant difference of 0.03 mmol/L (P≤0.05) was also noted between extreme quintiles. There was a strongly significant positive association between plant protein %en and HDL levels after adjusting for age, BMI, smoking status, education, baseline MI or DM, family history of stroke or MI and statin medication use (P trend <0.001). However, this association was attenuated with the addition of dietary factors including alcohol, total fat, total energy and animal protein intakes. There was no significant association between dietary plant protein %en and triglyceride levels in men, there was however, a non-significant difference of -0.11 mmol/L between the extreme quintiles.

For women **(Table 5.22)** there was a significant inverse association between plant protein %en and total cholesterol (P trend = 0.01) and LDL (P trend <0.01) after full adjustment for confounding factors including age, BMI and dietary intakes such as total fat and protein from animal sources. There was also a strongly significant difference in LDL levels of -0.28 mmol/L between the extreme quintiles of plant protein %en in women (P≤0.001) in the fully adjusted model. There were no significant associations between triglyceride levels or ratio HDL:LDL and plant protein intakes as a percentage of energy in women. In model 1 after adjustment for anthropometric, lifestyle and medical history factors there was a significant inverse association between increasing plant protein intakes as a percentage of energy and HDL levels (P trend 0.01). However, this association was attenuated with the addition of dietary factors in model 2 (P trend 0.14). In addition the direction of effect changed towards a non-significant positive trend between HDL and plant protein %en in women.

Table 5.23 Association of quintiles of dietary animal protein intake (range and mean quintile intake) and cholesterol and sub fractions (means and SE) in 1,888 men, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary animal protein intake as %en					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		2.89-8.23%	**8.24-9.41%**	**9.42-10.4%**	**10.5-11.8%**	**11.9-27.4%**	**P trend**
		6.95%	**8.86%**	**9.93%**	**11.2%**	**13.7%**	
		n=378	**n=378**	**n=377**	**n=378**	**n=377**	
Total Cholesterol	Unadjusted	5.96 (±0.06)	5.95 (±0.05)	5.99 (±0.05)	6.09 (±0.06)	6.09 (±0.05)	0.02
	Model 1[1]	5.96 (±0.06)	5.96 (±0.06)	5.99 (±0.05)	6.09 (±0.06)	6.09 (±0.06)	0.03
	Model 2[2]	6.00 (±0.06)	5.97 (±0.06)	6.00 (±0.05)	6.07 (±0.06)	6.05 (±0.06)	0.30
Triglycerides	Unadjusted	1.91 (±0.05)	1.84 (±0.05)	1.84 (±0.04)	1.97 (±0.04)	1.88 (±0.05)	0.70
	Model 1[1]	1.96 (±0.05)	1.88 (±0.05)	1.84 (±0.05)	1.97 (±0.05)	1.81 (±0.05)*[3]	0.16
	Model 2[2]	1.97 (±0.05)	1.87 (±0.05)	1.83 (±0.05)*	1.97 (±0.05)	1.81 (±0.05)*	0.21
HDL	Unadjusted	1.25 (±0.02)	1.23 (±0.02)	1.23 (±0.02)	1.22 (±0.02)	1.21 (±0.02)	0.05
	Model 1[1]	1.24 (±0.02)	1.22 (±0.02)	1.23 (±0.02)	1.22 (±0.02)	1.23 (±0.02)	0.66
	Model 2[2]	1.21 (±0.02)	1.22 (±0.02)	1.24 (±0.02)	1.22 (±0.02)	1.25 (±0.02)	0.16
LDL	Unadjusted	3.84 (±0.05)	3.89 (±0.05)	3.93 (±0.04)	3.98 (±0.05)	4.04 (±0.05)	<0.01
	Model 1[1]	3.84 (±0.05)	3.89 (±0.05)	3.93 (±0.05)	3.98 (±0.05)*	4.04 (±0.05)**	<0.01
	Model 2[2]	3.90 (±0.05)	3.90 (±0.05)	3.93 (±0.05)	3.95 (±0.05)	3.98 (±0.06)	0.23
HDL:LDL	Unadjusted	0.35 (±0.01)	0.34 (±0.01)	0.33 (±0.01)	0.33 (±0.01)	0.32 (±0.01)	0.001
	Model 1[1]	0.35 (±0.01)	0.34 (±0.01)	0.33 (±0.01)	0.33 (±0.01)	0.33 (±0.01)*	0.02
	Model 2[2]	0.34 (±0.01)	0.34 (±0.01)	0.33 (±0.01)	0.33 (±0.01)	0.34 (±0.01)	0.98

[1]Model 1: age, BMI, smoking status, physical activity, education level, baseline MI, baseline DM, family history stroke or MI and statin medication use

[2]Model 2: model 1 + alcohol intake, total fat intake, total energy, plant protein

[3]P value *≤0.05, **≤0.01 compared with Q1

Table 5.24 Association of quintiles of dietary animal protein intake (range and mean quintile intake) and cholesterol and sub fractions (means and SE) in 2,380 women, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary animal protein intake as %en					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		0.43-8.56%	**8.57-9.97%**	**9.98-11.2%**	**11.3-12.7%**	**12.8-26.0%**	**P trend**
		7.11%	**9.35%**	**10.6%**	**1200%**	**14.8%**	
		n=476	**n=476**	**n=476**	**n=476**	**n=476**	
Total Cholesterol	Unadjusted	6.20 (±0.06)	6.38 (±0.05)	6.25 (±0.05)	6.34 (±0.06)	6.40 (±0.05)	0.03
	Model 1[1]	6.31 (±0.05)	6.41 (±0.05)	6.25 (±0.05)	6.28 (±0.05)	6.32 (±0.05)	0.49
	Model 2[2]	6.34 (±0.05)	6.42 (±0.05)	6.25 (±0.05)	6.27 (±0.05)	6.29 (±0.06)	0.13
Triglycerides	Unadjusted	1.57 (±0.04)	1.55 (±0.03)	1.50 (±0.03)	1.62 (±0.03)	1.64 (±0.04)	0.07
	Model 1[1]	1.67 (±0.03)	1.59 (±0.03)	1.53 (±0.03)**[3]	1.56 (±0.03)*	1.55 (±0.03)*	0.01
	Model 2[2]	1.67 (±0.03)	1.59 (±0.03)	1.52 (±0.03)**	1.57 (±0.03)*	1.55 (±0.04)*	0.03
HDL	Unadjusted	1.57 (±0.02)	1.57 (±0.02)	1.58 (±0.02)	1.56 (±0.02)	1.54 (±0.02)	0.32
	Model 1[1]	1.54 (±0.02)	1.56 (±0.02)	1.57 (±0.02)	1.57 (±0.02)	1.57 (±0.02)	0.31
	Model 2[2]	1.53 (±0.02)	1.55 (±0.02)	1.58 (±0.02)	1.58 (±0.02)	1.59 (±0.02)*	0.02
LDL	Unadjusted	3.92 (±0.05)	4.11 (±0.05)	3.99 (±0.05)	4.05 (±0.05)	4.12 (±0.05)	0.04
	Model 1[1]	4.01 (±0.05)	4.01 (±0.05)	4.00 (±0.05)	4.00 (±0.05)	4.05 (±0.05)	0.72
	Model 2[2]	4.06 (±0.05)	4.15 (±0.05)	3.99 (±0.05)	3.98 (±0.05)	4.00 (±0.05)	0.07
HDL:LDL	Unadjusted	0.45 (±0.01)	0.42 (±0.01)	0.44 (±0.01)	0.42 (±0.01)	0.42 (±0.02)	0.15
	Model 1[1]	0.43 (±0.01)	0.42 (±0.01)	0.43 (±0.01)	0.43 (±0.01)	0.44 (±0.01)	0.44
	Model 2[2]	0.42 (±0.01)	0.41 (±0.01)	0.43 (±0.01)	0.43 (±0.01)	0.45 (±0.01)*	0.02

[1]Model 1: age, BMI, smoking status, physical activity, education level, baseline MI, baseline DM, family history stroke or MI and statin medication use

[2]Model 2: model 1 + alcohol intake, total fat intake, total energy, plant protein

[3]P value *≤0.05, **≤0.01 compared with Q1

Animal protein %en

There were no significant associations reported between dietary animal protein intake and total cholesterol or sub fractions in men after full adjustment for confounding variables **(Table 5.23)**. A significant positive trend was identified in model 1 for total cholesterol and LDL (P trend 0.03 and <0.01 respectively) and an inverse trend with HDL:LDL levels (P trend 0.02) and animal protein %en in men. However, these associations were attenuated with the addition of dietary factors to the model; alcohol, total fat, total energy and plant protein intake, but there remained a non-significant trend towards higher total cholesterol levels and increasing animal protein %en.

In women there was no significant association between total cholesterol or LDL levels and animal protein %en **(Table 5.24)**. A significant inverse association was seen between animal protein %en and triglyceride levels in women after adjusting for model 1 which included anthropometric, lifestyle and medical history factors (P trend 0.01). This relationship remained after additional adjustment for dietary alcohol, total fat, total energy and plant protein intakes (P trend = 0.03). Additionally there was a significant difference of -0.12 mmol/L between the extreme quintiles (P≤0.05). A significant positive trend was identified between animal protein %en and HDL and ratio HDL:LDL levels in women after full adjustment for confounding variables (P trend 0.02 for both). There were also significant differences of 0.06 mmol/L (P≤0.05) and 0.03 mmol/L (P≤0.05) for HDL and ratio HDL:LDL between the extreme quintiles of animal protein %en in women.

Table 5.25 Association of quintiles of ratio plant:animal protein intake (range and mean quintile intake) and cholesterol and sub fractions (means and SE) in 1,888 men, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary ratio plant:animal protein intake					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		0.07-0.33	**0.34-0.40**	**0.41-0.48**	**0.49-0.59**	**0.60-2.71**	**P trend**
		0.28	**0.38**	**0.45**	**0.54**	**0.83**	
		n=376	**n=377**	**n=378**	**n=374**	**n=383**	
Total Cholesterol	Unadjusted	6.25 (±0.06)	6.00 (±0.06)	5.99 (±0.05)	6.01 (±0.05)	5.84 (±0.06)	<0.001
	Model 1[1]	6.23 (±0.05)	5.99 (±0.05)**[3]	5.99 (±0.06)**	6.02 (±0.06)**	5.85 (±0.05)***	<0.001
	Model 2[2]	6.19 (±0.06)	5.98 (±0.05)**	6.00 (±0.05)*	6.03 (±0.05)*	5.89 (±0.06)***	<0.01
Triglycerides	Unadjusted	1.92 (±0.05)	1.86 (±0.05)	1.89 (±0.05)	1.92 (±0.05)	1.86 (±0.05)	0.61
	Model 1[1]	1.91 (±0.05)	1.85 (±0.05)	1.87 (±0.05)*	1.93 (±0.05)	1.89 (±0.05)	0.86
	Model 2[2]	1.93 (±0.05)	1.86 (±0.05)	1.86 (±0.05)	1.93 (±0.05)	1.88 (±0.05)	0.84
HDL	Unadjusted	1.26 (±0.02)	1.22 (±0.02)	1.21 (±0.02)	1.21 (±0.02)	1.24 (±0.02)	0.19
	Model 1[1]	1.27 (±0.02)	1.23 (±0.02)	1.22 (±0.02)*	1.20 (±0.02)**	1.22 (±0.02)	0.04
	Model 2[2]	1.25 (±0.02)	1.22 (±0.02)	1.22 (±0.02)	1.21 (±0.02)	1.25 (±0.02)	0.77
LDL	Unadjusted	4.11 (±0.05)	3.93 (±0.05)	3.93 (±0.05)	3.94 (±0.05)	3.77 (±0.05)	<0.001
	Model 1[1]	4.10 (±0.05)	3.93 (±0.05)*	3.94 (±0.05)*	3.94 (±0.05)*	3.77 (±0.05)***	<0.001
	Model 2[2]	4.06 (±0.05)	3.92 (±0.05)*	3.94 (±0.05)	3.96 (±0.05)	3.80 (±0.05)***	<0.01
HDL:LDL	Unadjusted	0.33 (±0.01)	0.33 (±0.01)	0.33 (±0.01)	0.33 (±0.01)	0.36 (±0.01)	0.12
	Model 1[1]	0.33 (±0.01)	0.34 (±0.01)	0.33 (±0.01)	0.32 (±0.01)	0.35 (±0.01)	0.37
	Model 2[2]	0.33 (±0.01)	0.33 (±0.01)	0.33 (±0.01)	0.32 (±0.01)	0.36 (±0.01)*	0.08

[1]Model 1: age, BMI, smoking status, physical activity, education level, baseline MI, baseline DM, family history stroke or MI and statin medication use

[2]Model 2: model 1 + alcohol intake, total fat intake, total energy

[3]P value *≤0.05, **≤0.01 ***≤0.001 compared with Q1

Table 5.26 Association of quintiles of ratio dietary plant:animal protein intake (range and mean quintile intake) and cholesterol and sub fractions (means and SE) in 2,380 women, aged 40-75 years in EPIC-Norfolk cohort (1993-1997).

		Dietary ratio of plant:animal protein intake					
		Q1	**Q2**	**Q3**	**Q4**	**Q5**	
		0.00-0.32	**0.33-0.38**	**0.39-0.46**	**0.47-0.57**	**0.58-22.4**	**P trend**
		0.27	**0.36**	**0.43**	**0.52**	**0.90**	
		n=472	**n=474**	**n=478**	**n=478**	**n=478**	
Total Cholesterol	Unadjusted	6.46 (±0.06)	6.39 (±0.06)	6.30 (±0.05)	6.25 (±0.05)	6.17 (±0.06)	<0.001
	Model 1[1]	6.38 (±0.05)	6.35 (±0.05)	6.25 (±0.05)	6.30 (±0.05)	6.28 (±0.05)	0.12
	Model 2[2]	6.35 (±0.05)	6.34 (±0.05)	6.25 (±0.05)	6.30 (±0.05)	6.31 (±0.05)	0.44
Triglycerides	Unadjusted	1.62 (±0.04)	1.61 (±0.04)	1.57 (±0.04)	1.55 (±0.04)	1.54 (±0.03)	0.05
	Model 1[1]	1.57 (±0.03)	1.56 (±0.03)	1.55 (±0.03)	1.59 (±0.03)	1.62 (±0.03)	0.24
	Model 2[2]	1.59 (±0.03)	1.57 (±0.03)	1.55 (±0.03)	1.58 (±0.03)	1.60 (±0.03)	0.67
HDL	Unadjusted	1.57 (±0.02)	1.57 (±0.02)	1.59 (±0.02)	1.57 (±0.02)	1.53 (±0.02)	0.23
	Model 1[1]	1.58 (±0.02)	1.58 (±0.02)	1.59 (±0.02)	1.56 (±0.02)	1.52 (±0.02)*[3]	0.03
	Model 2[2]	1.56 (±0.02)	1.57 (±0.02)	1.58 (±0.02)	1.57 (±0.02)	1.54 (±0.02)	0.37
LDL	Unadjusted	4.15 (±0.05)	4.10 (±0.05)	4.00 (±0.05)	3.99 (±0.05)	3.94 (±0.05)	0.001
	Model 1[1]	4.09 (±0.05)	4.07 (±0.05)	3.97 (±0.05)	4.02 (±0.05)	4.03 (±0.05)	0.25
	Model 2[2]	4.08 (±0.05)	4.06 (±0.05)	3.97 (±0.05)	4.03 (±0.05)	4.05 (±0.05)	0.56
HDL:LDL	Unadjusted	0.43 (±0.02)	0.42 (±0.01)	0.43 (±0.01)	0.43 (±0.01)	0.43 (±0.01)	0.53
	Model 1[1]	0.44 (±0.01)	0.43 (±0.01)	0.44 (±0.01)	0.43 (±0.01)	0.42 (±0.01)	0.32
	Model 2[2]	0.43 (±0.01)	0.43 (±0.01)	0.43 (±0.01)	0.43 (±0.01)	0.42 (±0.01)	0.57

[1]Model 1: age, BMI, smoking status, physical activity, education level, baseline MI, baseline DM, family history stroke or MI and statin medication use

[2]Model 2: model 1 + alcohol intake, total fat intake, total energy

[3]P value *≤0.05, **≤0.01 compared with Q1

Ratio of plant:animal protein

For lipid profiles in relation to ratio of plant:animal protein intakes in men, a significant inverse trend was identified with total cholesterol (P<0.01) and LDL (P<0.01) after full adjustment for confounding variables including age, BMI, smoking status, physical activity, education, MI or diabetes at baseline, family history of stroke or MI, use of statin medication, alcohol intake dietary total fat and total energy intake **(Table 5.25)**. In addition significant differences of -0.30 mmol/L (P≤0.001) and -0.26 mmol/L (P≤0.001) between the extreme quintiles for total cholesterol and LDL were also seen. There were no significant associations between the ratio of plant:animal protein and triglyceride and ratio HDL:LDL after adjustment for relevant confounding variables. There was however, a significant inverse association between increasing ratio of plant:animal protein and HDL levels in men after adjustment for model 1 (P trend = 0.04). However, this association was attenuated with the addition of dietary factors including alcohol, total fat and total energy intakes.

No significant associations between cholesterol or sub fractions and ratio of plant:animal protein was identified in women **(Table 5.26)** after fully adjusting for relevant confounding variables. A significant inverse trend (P trend 0.03) between ratio of plant:animal protein and HDL levels was identified in women after adjusting for model 1, including anthropometric and lifestyle factors, but this association was attenuated after adjusting for dietary factors (P trend 0.37). A significant difference of -0.06 mmol/L between the extreme quintiles was also noted in model 1 (P≤0.05).

Table 5.27 Quintiles of dietary protein as percentage of energy (range and mean quintile intake) at baseline (1993-1997) and stroke risk (HR and 95%CI), follow-up March 2008, in 4443 men and women, aged 40-75 in EPIC-Norfolk cohort.

	Dietary total protein as %en					
Men	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
	7.18-12.93% 11.8% n=400	**12.94-14.16% 13.7% n=400**	**14.17-15.26% 14.7% n=400**	**15.27-16.95% 16.0% n=400**	**16.96-32.16% 18.8% n=400**	**P trend**
Stroke Events	77 (19.3%)	83 (20.8%)	92 (23.0%)	100 (25.0%)	97 (24.3%)	0.03
Model 1[1]	1.00	1.01 (0.69-1.49)	1.11 (0.75-1.64)	1.24 (0.85-1.83)	1.25 (0.85-1.86)	0.14
Model 2[2]	1.00	1.01 (0.69-1.49)	1.03 (0.69-1.53)	1.15 (0.77-1.72)	1.04 (0.69-1.56)	0.65
Model 3[3]	1.00	1.04 (0.70-1.55)	1.01 (0.67-1.51)	1.03 (0.67-1.56)	0.91 (0.59-1.41)	0.67
Women	**8.13-13.33% 12.1% n=489**	**13.34-14.82% 14.2% n=489**	**14.83-16.16% 15.5% n=488**	**16.17-17.91% 16.9% n=489**	**17.92-30.79% 20.0% n=488**	
Stroke Events	92 (18.8%)	90 (18.4%)	93 (19.1%)	105 (21.5%)	99 (20.3%)	0.29
Model 1[1]	1.00	0.94 (0.65-1.37)	0.90 (0.62-1.31)	0.95 (0.66-1.36)	0.78 (0.53-1.14)	0.26
Model 2[2]	1.00	0.94 (0.64-1.37)	0.90 (0.62-1.31)	0.89 (0.62-1.30)	0.77 (0.53-1.13	0.19
Model 3[3]	1.00	0.89 (0.60-1.33)	0.87 (0.59-1.28)	0.91 (0.62-1.36)	0.74 (0.48-1.13)	0.25

[1]model 1: age, BMI, education status, physical activity, smoking status, alcohol intake

[2]model 2: model 1 + serum total cholesterol, baseline MI or diabetes, family history stroke, or MI

[3]model 3: model 2 + SBP, DBP, aspirin use >3 months, antihypertensive medication, total energy

Total protein %en

In men there was a significant trend towards a higher number of stroke events with increasing total protein %en (P trend 0.03) **(Table 5.27)**. There was no significant association between dietary protein %en and stroke risk in men. In the fully adjusted model which included; age, BMI, smoking, education, physical activity, alcohol intake, serum total cholesterol, baseline MI or DM, family history stroke or MI, SBP, DBP, aspirin use, antihypertensive medication and total energy the HR in the highest quintile of protein %en compared with lowest quintile of intake was HR 0.91 (95%CI 0.59-1.41). There were similar results for women (Table 5.26), in that no significant association was reported between total protein %en and stroke risk. The HR for highest protein intakes compared with lowest quintile was 0.74 (95% CI 0.48-1.13).

Table 5.28 Quintiles of dietary plant protein intake as percentage of energy (range and mean quintile intake) at baseline (1993-1997) and stroke risk (HR and 95%CI), follow-up March 2008, in 4443 men and women, aged 40-75 in EPIC-Norfolk cohort.

	Dietary plant protein as %en					
Men	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
	1.44-3.67% 3.20% n=400	**3.68-4.23% 3.98% n=400**	**4.24-4.75% 4.49% n=400**	**4.76-5.51% 5.11% n=400**	**5.52-11.0% 6.43% n=400**	**P trend**
Stroke Events	113 (28.3%)	80 (20.0%)	75 (18.8%)	88 (22.0%)	93 (23.3%)	
Model 1[1]	1.00	0.80 (0.55-1.15)	0.79 (0.55-1.13)	0.94 (0.65-1.36)	1.08 (0.75-1.55)	0.53
Model 2[2]	1.00	0.83 (0.57-1.20)	0.79 (0.54-1.14)	0.88 (0.60-1.29)	0.93 (0.63-1.37)	0.79
Model 3[3]	1.00	0.85 (0.58-1.24)	0.77 (0.52-1.12)	0.84 (0.56-1.25)	0.89 (0.60-1.33)	0.55
Women	**8.13-13.33% 12.1% n=489**	**13.34-14.82% 14.2% n=489**	**14.83-16.16% 15.5% n=488**	**16.17-17.91% 16.9% n=489**	**17.92-30.79% 20.0% n=488**	
Stroke Events	103 (21.1%)	110 (22.5%)	97 (19.9%)	81 (16.6%)	88 (18.0%)	
Model 1[1]	1.00	1.17 (0.82-1.68)	1.21 (0.83-1.75)	0.73 (0.51-1.07)	0.99 (0.67-1.46)	0.25
Model 2[2]	1.00	1.17 (0.82-1.68)	1.21 (0.83-1.75)	0.73 (0.50-1.07)	0.94 (0.63-1.39)	0.16
Model 3[3]	1.00	1.08 (0.73-1.60)	1.10 (0.74-1.65)	0.68 (0.45-1.03)	0.91 (0.59-1.40)	0.15

[1]model 1: age, BMI, education status, physical activity, smoking status, alcohol intake

[2]model 2: model 1 + serum total cholesterol, baseline MI or diabetes, family history stroke, or MI

[3]model 3: model 2 + SBP, DBP, aspirin use >3 months, antihypertensive medication, total energy

Plant protein %en

For both men and women **(Table 5.28)** plant protein %en was not significantly associated with stroke risk. The lowest, non-significant, risk of stroke for men was in those with mean plant protein %en of 4.49% (quintile 3). The HR in the fully adjusted model was 0.77 (95% CI 0.52-1.12) compared with the lowest intakes, mean 3.20% of energy from plant protein. In women the lowest, non-significant, risk of stroke was in those with mean intakes of 16.9% of energy from plant protein (quintile 4). HR for this intake of the fully adjusted model was 0.68 (95% CI 0.45-1.03) compared with the lowest intakes of plant protein %en (mean 12.1%).

Table 5.29 Quintiles of dietary animal protein intake as percentage of energy (range and mean quintile intake) at baseline (1993-1997) and stroke risk (HR and 95%CI), follow-up March 2008, in 4443 men and women, aged 40-75 in EPIC-Norfolk cohort.

	Dietary animal protein as %en					
Men	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
	2.71-8.23% 6.96% n=400	**8.24-9.41% 8.87% n=400**	**9.42-10.4% 9.94% n=400**	**10.5-11.8% 9.94% n=400**	**11.9-27.4% 13.7% n=400**	**P trend**
Stroke Events	81 (20.3%)	78 (19.5%)	88 (22.0%)	102 (25.5%)	100 (25.0%)	
Model 1[1]	1.00	0.70 (0.47-1.05)	0.88 (0.60-1.31)	0.93 (0.64-1.36)	1.04 (0.71-1.54)	0.32
Model 2[2]	1.00	0.70 (0.45-1.05)	0.92 (0.54-1.22)	0.90 (0.61-1.32)	0.96 (0.65-1.43)	0.58
Model 3[3]	1.00	0.66 (0.43-1.01)	0.81 (0.53-1.24)	0.80 (0.53-1.20)	0.84 (0.54-1.29)	0.90
Women	**0.43-8.58% 7.14% n=489**	**8.56-9.99% 9.37% n=489**	**10.0-11.2% 10.6% n=488**	**11.3-12.8% 12.0% n=489**	**12.9-26.0% 14.9% n=488**	
Stroke Events	86 (17.6 %)	88 (18.0%)	105 (21.5%)	97 (19.8%)	103 (21.1%)	
Model 1[1]	1.00	0.83 (0.54-1.17)	1.11 (0.77-1.61)	0.85 (0.58-1.23)	0.78 (0.54-1.14)	0.36
Model 2[2]	1.00	0.80 (0.68-1.40)	1.10 (0.76-1.59)	0.75 (0.51-1.09)	0.99 (0.68-1.45)	0.33
Model 3[3]	1.00	0.70 (0.47-1.06)	1.07 (0.72-1.58)	0.78 (0.52-1.16)	0.70 (0.45-1.09)	0.29

[1]model 1: age, BMI, education status, physical activity, smoking status, alcohol intake

[2]model 2: model 1 + serum total cholesterol, baseline MI or diabetes, family history stroke, or MI

[3]model 3: model 2 + SBP, DBP, aspirin use >3 months, antihypertensive medication, total energy

Animal protein %en

As with total protein and plant protein %en, there was no significant association between animal protein %en and stroke risk in men or women **(Table 5.29)**. In men, after adjustment for model 1 including age, BMI, education, physical activity, smoking, and alcohol intake the highest percentage of energy from animal protein (mean 13.7%) had a HR of 1.04 (95% CI 0.71-1.54) compared with those with the lowest mean intakes (6.96% energy from animal protein). The lowest risk of stroke in the fully adjusted model was in those with a mean of 8.87% of energy from animal protein (quintile 2) where the HR was 0.66 (95% CI 0.43-1.01). In women (Table 5.28) intakes ranged from 0.43-26.0% of energy from animal protein sources. The HR for stroke risk in the highest quintile of animal protein %en in the fully adjusted model was 0.70 (95% CI 0.45-1.09) for women. This HR was similar to that of quintile 2 (mean intake 9.37% of energy from animal protein), where the HR was 0.70 (95% CI 0.47-1.06).

Table 5.30 Quintiles of ratio of plant:animal protein intake (range and mean quintile intake) at baseline (1993-1997) and stroke risk (HR and 95%CI), follow-up March 2008, in 4443 men and women, aged 40-75 in EPIC-Norfolk cohort.

	Dietary ratio of plant:animal protein intake					
Men	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
	0.07-0.33	**0.34-0.40**	**0.41-0.48**	**0.49-0.59**	**0.60-2.71**	**P trend**
	0.28	**0.38**	**0.45**	**0.54**	**0.83**	
	n=400	**n=400**	**n=400**	**n=400**	**n=400**	
Stroke Events	108 (27.0%)	95 (23.8%)	80 (20.0%)	76 (19.0%)	90 (22.5%)	
Model 1[1]	1.00	0.94 (0.66-1.33)	0.78 (0.54-1.11)	0.78 (0.54-1.12)	1.11 (0.77-1.59)	0.93
Model 2[2]	1.00	0.98 (0.69-1.39)	0.80 (0.55-1.15)	0.74 (0.50-1.08)	1.07 (0.73-1.56)	0.66
Model 3[3]	1.00	1.00 (0.70-1.44)	0.81 (0.55-1.17)	0.76 (0.52-1.13)	1.10 (0.74-1.63)	0.77
Women	**0.00-0.32**	**0.33-0.38**	**0.39-0.46**	**0.47-0.57**	**0.58-22.4**	
	0.27	**0.36**	**0.43**	**0.52**	**0.90**	
	n=489	**n=489**	**n=488**	**n=489**	**n=488**	
Stroke Events	100 (20.5%)	114 (23.3%)	93 (19.1%)	89 (18.2%)	83 (17.0%)	
Model 1[1]	1.00	1.29 (0.90-1.84)	1.01 (0.70-1.46)	1.12 (0.77-1.62)	1.17 (0.80-1.71)	0.72
Model 2[2]	1.00	1.31 (0.91-1.88)	1.04 (0.72-1.50)	1.09 (0.75-1.59)	1.13 (0.77-1.67)	0.89
Model 3[3]	1.00	1.37 (0.94-1.99)	1.01 (0.69-1.49)	1.09 (0.73-1.62)	1.17 (0.79-1.75)	0.85

[1]model 1: age, BMI, education status, physical activity, smoking status, alcohol intake

[2]model 2: model 1 + serum total cholesterol, baseline MI or diabetes, family history stroke, or MI

[3]model 3: model 2 + SBP, DBP, aspirin use >3 months, antihypertensive medication, total energy

Ratio of plant:animal protein

There was no significant association between the ratio of plant:animal protein and stroke risk in men or women **(Table 5.30)**. In the fully adjusted model the HR for highest ratio of plant:animal protein intake (mean 0.83) was 1.10 (95% CI 0.74-1.63) compared with lowest intakes (mean 0.28) for men. In women the HR was 1.17 (95% CI 0.79-1.75) for the highest intakes (mean 0.90) compared with lowest ratio of plant:animal protein (mean 0.27).

5.4 Discussion

The main findings of this chapter, summarised in **Tables 5.31** and **5.32**, involving 2,000 men and 2,443 women aged 39-80 years, at the start of the study, indicated that dietary protein intake is more strongly associated with blood pressure and lipid profile in women than men. There was no significant association with protein intake or sources of protein and stroke risk in either men or women, but for women the beneficial associations identified with stroke risk factors may indicate a potentially protective effect.

Table 5.31 Summary of the results of associations between dietary protein intake and sources of dietary protein %en and stroke risk factors, blood pressure and cholesterol and stroke risk in men

	Protein source as percentage of energy intake			
	Total protein	**Plant protein**	**Animal protein**	**Ratio plant:animal**
Blood Pressure				
SBP	↔	↔	↔	↔
DBP	↔	↓	↔	↔
Cholesterol				
Total cholesterol	↔	↓	↔	↓
Triglycerides	↔	↔	↔	↔
HDL	↑	↔	↔	↔
LDL	↔	↓	↔	↓
HDL:LDL	↔	↑	↔	↔
Stroke risk				
Stroke risk	↔	↔	↔	↔

↔ non-significant association
↓ significant inverse association
↑ significant positive association

Table 5.32 Summary of the results of associations between dietary protein intake and sources of dietary protein %en and stroke risk factors, blood pressure and cholesterol and stroke risk in women

	Protein source as percentage of energy intake			
	Total protein	**Plant protein**	**Animal protein**	**Ratio plant:animal**
Blood Pressure				
SBP	↓	↔	↓	↔
DBP	↓	↓	↓	↔
Cholesterol				
Total cholesterol	↔	↓	↔	↔
Triglycerides	↓	↔	↓	↔
HDL	↑	↔	↑	↔
LDL	↓	↓	↔	↔
HDL:LDL	↑	↔	↑	↔
Stroke risk				
Stroke risk	↔	↔	↔	↔

↔ non-significant association
↓ significant inverse association
↑ significant positive association

Blood pressure and protein intake

Total protein

The current null findings in relation to total protein %en and blood pressure in men (Table 5.11), is similar to the results of the INTERMAP study (141). Two other observational studies also reported no significant association between total protein intake and blood pressure, however, the analyses were not sex stratified (123, 124). Elliot et al (141) did however, report no significant association in women, whereas our results (Table 5.12) indicated an inverse association between protein %en and SBP and DBP in women (P=0.02 and 0.01 respectively). This difference in effects between sexes may be explained by differences in the source of protein consumed, as was identified in the previous chapter (Chapter Four). In these same observational studies the influence of different sources of protein was also assessed. Significant associations between plant protein and blood pressure were identified (123, 124, 141). Additionally a recent meta-analysis by Tielemans et al reported a non-significant inverse trend between SBP and plant protein intake (174).

Animal studies including the work of Endoh et al (354) and a number of human observational and intervention trials indicate the potential for the total amount of protein and different sources of dietary protein to modify blood pressure (123, 124, 141, 251, 364, 391). Endoh et al (2001) reported that in male Sprague-Dawley rats fed diets deficient in protein, in which 6% of energy came from casein protein, compared with high protein diet, with 23% energy from casein, SBP was significantly increased by 10-15 mmHg (P<0.05). They however, also reported a significant increase in SBP in rats fed the low protein diet with high sucrose content versus those on low protein and low sucrose diet suggesting that the CHO content or CHO:protein ratio of the diet is also an important and potentially contributory factor. This effect may be explained by the high sucrose content of the diet which has previously been associated with significant increases in blood pressure in animal models and humans (397, 398).

Plant protein

In this cohort there was no significant association between plant protein intake and SBP blood pressure in men or women (Table 5.13 and 5.14). However, a significant inverse trend

was identified in association with DBP for both men and women (Table 5.13 and 5.14), and a non-significant difference of -1.1 mmHg was seen between the extreme quintiles for DBP. The finding in relation to SBP is in contrast to a study by Altorf-van der Kuil (124), but the association with DBP is similar. They showed that higher intakes of plant protein were associated with modest reductions of -1.8 and -1.0 mmHg in SBP and DBP respectively in the highest quintiles of intake compared to people in the lowest quintile of intake (P trend <0.01).

The range of mean intakes of plant protein intake in grams/d in our study was wider 15.7-37.4 g/d and 12.4-28.3 g/d for men and women respectively than the previous study, 25-39 g/d. Therefore it may be that the beneficial effect for SBP is seen only in the highest range of intakes as the participants were already consuming relatively high plant protein intakes. It may also be that due to this increase in plant protein intakes the proportion of animal protein intake decreases which may also translate to lower intakes of other nutritional components associated with animal products such as saturated fat intakes.

Animal protein

In women there was an inverse association between animal protein and blood pressure. A study by Masala et al (360) in 7,601 Italian women aged 35-64 reported no significant association between animal protein intake and SBP or DBP in women (360). Regression coefficients, although non-significant, indicated a positive association between animal protein intake and blood pressure; 0.99 mmHg for SBP and 0.58 mmHg for DBP (360). This difference in findings may be due to differences in the food sources contributing to animal protein intake. In the study by Masala et al (360) they reported participants as having high processed meat intake, although the mean processed meat intakes of women in the Italian study and this EPIC-Norfolk sub-cohort were similar (22.4 g/d vs 21.8 g/d respectively). However, the authors of the Italian study report that processed meat is especially high in salt in this particular Italian region which may have an influence on blood pressure (360). They did however, have higher red meat intake, mean intake of 72.7 g/d compared with 64.4 g/d in the EPIC-Norfolk sub-cohort (which includes red meat, processed red meat and red meat in dishes). In men there was no significant association between animal protein %en and blood pressure. This finding, in men, concurs with that of a recent meta-analysis

(174) and also the individual results of several studies including the INTERMAP study which included UK population (141). Similarly in a Dutch population animal protein intake was not significantly associated with blood pressure or the risk of developing hypertension (123, 124). This discrepancy between men and women may be due in part to differences in the composition of diets. Similarly the Dutch diet and the INTERMAP cohort which additionally includes populations from China, Japan and US may have different dietary compositions which could in part explain the differences in findings.

The observed differences in associations between men and women may in part be attributable to differences in the contributing sources to protein intake as was highlighted in the previous chapter, Chapter Four – Dietary contributions to protein intake. There were small but significant differences in the mean intakes of all types of protein %en for men and women, P<0.001 for total protein, animal protein, animal-marine and animal-derived and P=0.01 for plant protein (Chapter Four; Table 4.0). There was no significant difference in mean intakes of animal-land %en (P0.11) and ratio of plant:animal protein (P=0.82). Despite this initial difference in reported mean intakes, on further inspection of contributing sources there were small but perhaps significant differences in patterns of consumption. For example men tended to have a higher contribution to protein intake from red meat than women, and when looking at specific nutrients and foods associated with risk of stroke and risk factors they also tended to have higher intakes of dietary sodium and fat.

Lipid profile and protein intake

Previous research has indicated the potential for dietary protein intake to beneficially influence lipid profile, a strong risk factor for stroke incidence. A large proportion of these studies have investigated the effect of soy protein sources on lipid profile in both humans and animals (27, 28, 347, 348).

The present results report inconsistent effects of dietary protein and sources of protein on total cholesterol and cholesterol sub-fractions. Additionally associations between dietary protein intakes and serum lipid levels differed between sexes. In men increasing total protein was associated with higher HDL levels (P trend=0.03) whereas in women total

protein was inversely associated with triglycerides and LDL, and higher HDL levels and ratio of HDL:LDL. Plant protein was inversely associated with total cholesterol and LDL levels for both men (P trend=0.01 for both) and women (P trend=0.01 and <0.01 respectively) and a higher ratio of HDL:LDL in men only (P trend=0.02). Animal protein intake was not significantly associated with serum lipid levels in men, but an inverse association with triglyceride levels was seen for women (P trend=0.03) and higher HDL level and ratio of HDL:LDL were also noted (P trend=0.2 for both). In men the ratio of plant:animal protein intake was inversely associated with total cholesterol and LDL levels (P trend <0.01 for both), but there were no significant associations in women. Previous research has also presented differing effects of protein on the individual serum lipid levels (27-29, 173). Similar inconsistent effects on cholesterol sub-fractions have also been reported in randomised trials (127, 128).

Plant protein

Jenkins et al (396) investigated the effects of a high plant protein diet compared with a lacto-ovo vegetarian diet similar to a traditional cholesterol lowering. The intervention diet substituted 93% of animal protein for plant protein sources; legumes (including soy), and cereals. The test diet was also considerably higher in fibre than the control diet, however, fat and cholesterol content were similar between the two diets. They identified a significant decrease in total cholesterol and LDL levels for the test diet compared with control (P<0.001 for both). Additionally significant decreases in ratio of TC:HDL and LDL:HDL were also reported (P=0.002 and 0.004 respectively). There was also a small but non-significant decrease in HDL and triglyceride levels. I had some similar findings in relation to associations between plant protein intake and serum lipid levels, particularly in men. In men significant inverse associations were seen in relation to total cholesterol and LDL, and a direct relationship between higher percentage of energy from plant protein and ratio of HDL:LDL. I also noted no significant association with triglyceride or HDL levels. However, the Jenkins study had a very small sample size n=19 men and n=12 women and did not stratify their analyses by sex.

In animal models there tended to be a trend towards lower lipid levels with intakes of plant based proteins such as buckwheat protein and soy protein isolate when compared with an

animal based protein such as casein (346, 347). However this lowering of lipid levels, in some instances, also included reductions in HDL levels suggesting a negative effect.

Animal protein

In men animal protein %en was not associated with total cholesterol or sub-fractions, whereas in women there were significant inverse associations with triglyceride levels and direct relationship with HDL and ratio HDL:LDL. In men there was a range of 2.89-27.4% of energy from animal protein sources compared with a range of 0.00-13.2% of energy from animal protein sources for women. As detailed in Chapter Four – Dietary contribution to protein intake (Table 4.0) there were small but statistically significant differences in the animal protein intakes of men and women, with women tending to have a slightly greater percentage of energy from animal protein than men, despite crude protein intakes in g/d being lower. The differences in the type of animal protein consumed by men and women may explain the differences in associations presented. For example men consumed a higher percentage of protein energy from land based animal protein compared with women (5.08% compared with 4.96%) although this difference was not statistically significant (p=0.11). This category includes meat, poultry and processed products. Whereas women had a statistically higher percentage of energy and protein energy from marine based animal protein than men (P <0.001 for total energy and 0.001 for energy from protein) 1.36 %en men and 1.54 %en women and 8.79 %protein for men and 9.56 %protein for women. Fish and other seafood that are included in the marine category are leaner sources of protein than land based animal protein, and may also contribute other beneficial nutrients such as omega-3 and -6 which increased intakes may be associated with beneficial effects on lipid levels.

Despite the differences being modest they may still be clinically relevant as even slight increases in lipid levels can negatively influence stroke risk. For example increases of 0.55 mmol/l of LDL or TG can increase stroke risk by approximately 11% and 5.5% respectively (284, 399).

The mechanisms of action of dietary protein on lipid profile are not well established. Particular protein sources, such as soy, may exert beneficial effects such as reducing the

synthesis of cholesterol and an increase in activation of LDL receptors leading to increased clearance of LDL cholesterol from circulation (400). It also must not be ruled out that the potential beneficial effect is due to substitution of other macronutrients. For example a diet higher in protein but lower in fat or carbohydrates.

Stroke risk and protein intake

There were no significant associations between dietary protein intakes and risk of stroke in both men and women after adjusting for relevant confounding factors. This is in contrast to some previous work in animal models and human observational studies where significant effects were noted (176, 177, 324, 352, 356, 383). However, not all studies have provided conclusive findings and some like the current findings report no significant association (177, 382).

Bernstein et al (175) indicated a benefit of substituting different meat protein sources during cross-sectional analyses. For example, swapping one serving of red meat for poultry or nuts may reduce stroke risk by 27% (95% CI, 12-39%) and 17% (95% CI 4-27%) respectively compared with red meat consumption. These sources of protein have very different fatty acid profiles. This may contribute to the beneficial effects reported for poultry and nut consumption compared with red meat consumption. Additionally there may be a difference in amino acid composition of these food products which could be influencing stroke risk. Future work could seek to elucidate whether the mechanism is due to macronutrient content, specific amino acid components or other factors.

5.4.1 Strengths and limitations

The limitations of the present analyses include those previously detailed earlier in Chapter Three, section: 3.4.1 Strengths and limitations. As with any epidemiological study it is possible that despite adjusting for a number of relevant confounding factors including lifestyle and dietary intakes residual confounding may have occurred. Self-reported dietary intakes are prone to bias in reporting by the individual, however the dietary data analysed in this thesis was derived from 7DD which are a more reliable and accurate method of determining dietary intakes, including protein as has been shown in validation studies (215,

362) and in particular FFQs may underestimate total energy and protein intake and over report percentage energy from protein (401). In addition due to the high detail of information that could be entered into the specifically designed DINER programme it was possible to disaggregate total protein intake into sub-fractions of constituent sources (animal, plant and ratio plant:animal). The study cohort includes a large number of men and women, who are representative of the whole EPIC-Norfolk cohort which is in itself representative of the general UK population. Despite the limitations noted the strengths of the study provide confidence in the results presented.

The analysis presented in this chapter could be further extended, future work could also investigate the potential role of specific amino acids in relation to stroke risk and risk factors. In addition it would be of interest to determine if the different sources of protein influenced sub-types of stroke differently. In the current analysis this was not possible due to the size of cohort and number of stroke cases.

5.5 Summary

The current analyses indicate that in this particular cohort a higher percentage of energy from total protein may beneficially influence blood pressure in women. In part this may be mediated by percentage of energy from animal protein which was also inversely associated with blood pressure in women with approximate 2 mmHg difference of both SBP and DBP between those in the lowest vs highest quintiles of protein from animal sources as %en. In addition percentage energy from plant protein was inversely associated with DBP but not SBP in women. The effect of animal protein may be due to the composition of diet contributing to animal protein intakes. For example investigations detailed in Chapter Four – Dietary contributions to protein intake, showed women had higher intakes of marine protein sources compared with men, and slightly lower land based animal protein intakes. It therefore cannot be ruled out that the differences in the nutrient composition of these food groups may also influence blood pressure.

There were inconsistent findings in relation to serum lipid levels, however, in both men and women a higher percentage of energy from plant protein was inversely associated with total cholesterol and LDL levels. No significance was seen in relation to percentage of energy from animal protein, therefore it may be postulated that higher intakes of plant protein may beneficially influence serum lipid levels and thus stroke risk. Despite this there was no significant association between total protein intake or subtypes of protein and stroke risk. However, the risk of stroke in highest quintile of protein %en was 0.91 (95%CI 0.59-1.40) for men and 0.74 (95%CI 0.48-1.13) for women and therefore although not statistically significant higher protein intakes may beneficially influence stroke risk.

To date there has been limited research investigating the influences of different protein sources on stroke risk and risk factors simultaneously in the same cohort. Therefore this is an area which required development and further research, to identify potential beneficial associations between dietary protein, including sources of protein, in relation to stroke risk, which this chapter sought to address.

Chapter Six

ASSOCIATIONS BETWEEN DIETARY INTAKES AND BIOMARKERS OF MAGNESIUM AND PROTEIN INTAKE ON BLOOD PRESSURE IN THE NU-AGE STUDY

6.0 Introduction

The inherently subjective nature of reporting dietary intakes can lead to inaccuracies, for example if a 24hr recall were used, some participants, particularly the elderly, may have difficulties in recalling food and drink that was consumed during the previous day (37). The one-off nature of the recall also does not account for day-to-day variability in consumption and thus foods that are eaten frequently but not daily may be missed, this can be somewhat overcome by conducting repeated 24hr recalls at varied intervals including different seasons, weekdays and weekends (37). The use of FFQ may more accurately represent habitual intake, but FFQs tend to lack specific details of food items and cooking methods for example, and they also typically rely on standard portion sizes. FFQs are however, less burdensome to the participant and researcher, particularly in large studies, and can be the most cost effective method of determining habitual intake (179).

In the preceding chapters, the associations between dietary intakes of magnesium and protein with blood pressure, a risk factor for stroke, were explored. As has been indicated above, and in Chapter One – Introduction, self-reported dietary intakes may be prone to bias, and it would be of interest to understand if biomarkers, namely serum magnesium and urinary nitrogen, which are objective measures of intake, were also related to blood pressure.

The potential benefit of using recovery biomarkers of dietary intake is that they are an objective measure and are thus less prone to intentional or unintentional bias. A recovery biomarker correlates directly with total intake over a given period, usually 24 hours (37). There are several recovery biomarkers including urinary nitrogen (for protein intake), urinary sodium and total energy using doubly labelled water which are well correlated with dietary intakes in the general population (179). Urinary nitrogen is an established and valid measure of dietary protein intake, in the general population (215) as approximately 80% of the nitrogen from protein consumed daily will be present in a 24 hour urine sample (402). Urinary urea concentrations can be used to determine urinary total nitrogen (359). Some more recent evidence of potential biomarkers for specific types of protein have also been

investigated (403) for example biomarkers of animal protein intake, using isotopes from hair sample and compounds specific to red meat, such as creatinine, taurine 1-methylhistidine and 3-methylhistidine, have been suggested as biomarkers for red meat intake (403). There is less evidence relating biomarkers of magnesium to dietary intake (404) this may in part be due to the way in which magnesium is stored. Approximately 60-65% of magnesium is present in bones and a further 27% in muscle tissue (405). As a result extracellular levels only account for approximately 1% of total magnesium (406). Magnesium absorption occurs mainly in the intestine, and reabsorption in the long distal tube of the Loop of Henle in the kidneys. An increase in dietary intakes of magnesium would be reflected in increased concentrations in 24hr urine output.

There is conflicting evidence whether serum magnesium levels are substantially influenced by dietary intakes (407). Arnaud (407) reports that serum magnesium levels are in part dependent on dietary intakes as well as absorption of magnesium. Whereas Witkowski et al (406) indicates that increased dietary magnesium intakes, have been reported to have little effect on serum magnesium levels due to the tight homeostatic regulation (406). In two independent studies by Czernichow et al (404) and Galan et al (408), in the SUVIMAX cohort, no significant association between dietary magnesium intake and serum magnesium levels (mmol/L) was reported (404, 408). They indicated that few studies exist investigating the relationship of dietary magnesium intake with magnesium status (404). A recent systematic review of magnesium biomarkers indicated that in studies looking at supplementation or depletion of magnesium, plasma and serum magnesium may be used as a biomarker of status. Analysis was pooled for plasma and serum magnesium and for supplementation and depletion studies and comprised 22 studies and 322 participants (406). A pooled effect size of 0.03 (95% CI 0.01-0.06) was reported for the relationship between plasma and serum magnesium, but a high level of heterogeneity I^2 96% was also shown.

Although biomarkers are an objective measure of diet they are not without disadvantages. For example not all dietary intakes correlate well with a biomarker and in this instance it may not be accurate to rely on a biomarker to validate self-reported intakes. Other factors that may influence the efficacy of biomarkers include between-person variation in

absorption of nutrients (409), as some nutrients are absorbed at a dose-dependent rate which is strongly influenced by body status. There may also be differences in the storage and excretion of nutrients, although the specific mechanisms related to variation are unclear. In terms of excretion renal function is likely to play a key role and a number of medications and medical conditions can also influence renal function and excretion rate (409). It is worth being aware of these factors when utilising biomarkers of dietary intakes.

There have been limited studies undertaken with the aim to ascertain the correlation between dietary intake and biomarkers of magnesium and protein in older populations. As the UK population becomes increasingly aged it is important to determine if biomarkers are still appropriate for use in older people. Differences in the reliability of these methods in older people compared with a younger general population may be due to physiological changes occurring as a natural part of the ageing process. For example a decline in kidney function may influence the absorption and excretion of some nutrients including both magnesium and protein/nitrogen as these are handled by the kidneys (407).

In addition there is limited evidence on whether dietary intakes of magnesium and protein or their biomarkers serum magnesium and urinary nitrogen respectively are more highly correlated with blood pressure, a significant risk factor for stroke, in older populations. Understanding the difference in relationships of dietary derived intakes and biomarker measures may be important in determining a more appropriate method to use as a predictor of difference in blood pressure. A large study (INTERSALT), n=10,020, of 20-59 year old men and women previously indicated an inverse association between urinary urea, and total nitrogen, from a 24-hour urine collection, with blood pressure (359). After adjustment for confounding factors; age, sex, BMI, alcohol intake, urinary sodium, potassium, magnesium and calcium, significant inverse associations were seen between SBP and DBP and urea nitrogen as well as total nitrogen. A pooled regression coefficient, after adjustment for regression dilution bias, of -0.570 mmHg SBP/g urea nitrogen and -0.494 mmHg DBP/g urea nitrogen was reported (359). However, they did not concurrently assess the similarities/differences of biomarker and dietary intake with blood pressure. Although, dietary protein has in some instances been independently inversely associated with blood pressure (140) in analyses looking at the older age group in the INTERSALT

cohort, aged 40-50 years, stronger inverse associations were reported for SBP and urea nitrogen and total nitrogen thus indicating a potential importance of this measure in the older population (359). Research is also lacking specifically looking at the relationships in the older population.

Therefore this chapter discusses analyses on data from the NU-AGE study, which assessed associations between dietary intakes and biomarkers of magnesium and protein intake with blood pressure. The NU-AGE study was chosen for several reasons including that the study participants had a smaller age range, of older participants, than the EPIC-Norfolk cohort. For urinary markers, such as urinary nitrogen, this may have importance as renal function decreases with increasing age. Thus establishing associations between biomarker and blood pressure in this older population, specifically, is important.

This chapter will build upon previous findings in the EPIC-Norfolk cohort, with the premise that in the past two decades since the commencement of EPIC-Norfolk and the recording of food intakes that food consumption and dietary patterns have changed. There have been changes in the consumption of a number of foods including soft drinks, fruit juices and pre-packaged meals and processed foods which may have had an impact on health in terms of stroke risk such as blood pressure. One nutrient which may be more prevalent in processed foods is salt which is a significant cause of hypertension, which itself is strongly linearly correlated with increase in blood pressure and stroke risk (410). Assessing the modern diet of the older population may provide an insight into whether dietary habits are influencing stroke risk via mechanisms relating to risk factors such as blood pressure.

The aim of this chapter was to identify if dietary intakes of magnesium and protein were well correlated with biomarkers (serum and urine respectively) in an older population and to determine if one of these methods was more consistently and accurately associated with blood pressure than other measures in a cohort of 65-79 year old British men and women who were part of the NU-AGE study. The primary aim of the NU-AGE study is to improve the health and quality of life of the ageing European population by assessing the effects of a whole diet intervention. This chapter is cross-sectional in design and is concerned with

the habitual dietary intakes recorded at baseline prior to commencement of the intervention.

6.1 Aims and hypotheses

This chapter aims to address research question 4, outlined in the introductory Chapter, Chapter One.

4. How do biomarkers of magnesium and protein intake compare with dietary reported intakes in relation to blood pressure in older men and women?

4a. Firstly the aim was to confirm the correlation between biomarker and dietary intakes; serum magnesium with dietary magnesium and urinary urea with dietary protein intake. The hypothesis was that the biomarker would correlate well with dietary intake.

4b. Secondly the aim was to assess the relationship between each of the biomarkers and dietary intake with blood pressure. This was to determine whether the biomarker and dietary intake had a similar relationship with blood pressure and which of the measures was most significantly associated with blood pressure and so if one could be recommended for future use. The hypothesis was that the biomarker and dietary intakes would have similar relationships with blood pressure.

6.2 Methods

The methods of the NU-AGE study related to this thesis can be found in Chapter Two – Subjects and methods, section 2.2. Additionally more detailed information on the NU-AGE study can be found in the published study design (211).

Briefly, before attending for a study day at the CRTU participants recorded their dietary intake over the preceding 7 day period using a food diary. During the same 7 day period physical activity levels were also monitored using an accelerometer. After this, in the 24 hours prior to the study day, a 24hr urine collection was undertaken. At the study day a range of anthropometric, biological and cognitive assessments were undertaken including; height, weight, blood pressure and venous blood samples.

This cohort consisted of 272 men and women aged 65-79 years at the start of the study. Participants with missing data for one or more variables of interest were excluded from the analysis (n=38). After this 234 men and women remained for analysis.

The aim of this chapter was to determine the association between dietary intakes and biomarkers of magnesium and protein with blood pressure in older men and women.

Statistical methods

All analyses were conducted in Stata version 11.

A pairwise Pearson's correlation was used to assess correlations between dietary intake and each biomarker and each measurement independently with blood pressure.

Regression, with adjustment for confounding variables, detailed below, was used to assess the relationship between dietary intake and biomarkers of magnesium and protein intake and blood pressure. Model 1 included age, BMI, smoking status, moderate physical activity, school years, use of antihypertensive medication, total energy intake and dietary sodium intake. Model 2 (magnesium analysis only) additionally adjusted for dietary potassium intake and calcium supplement use.

In addition sensitivity analysis was conducted excluding those taking antihypertensive medication and adjusting for confounding factors (model 1 for protein and model 2 for magnesium intake and biomarkers respectively).

6.3 Results

In this cohort of 234 men and women aged 65-79 years at baseline 37% were men **(Table 6.0)**. The mean age was 70.1 years (70.6 for men and 69.7 for women). The mean BMI of 26.9 (±4.13) was in the overweight range. BMI for men was slightly higher than the cohort average at 27.1 (±3.70) and women was 26.8 (±4.38). The mean SBP of the whole cohort 152 (±19.3) mmHg and sex-specific 149 (±16.7) mmHg for men and 153 (±20.5) mmHg for women were in the hypertensive range (SBP>140) mmHg. The mean DBP and sex-specific means were in the prehypertensive range, 81.8 (±9.84) mmHg the whole cohort, 83.1 (±9.68) mmHg for men and 81.1 (±9.88) mmHg for women. Years in education was similar across the cohort, 11.8 (±1.83), 11.9 (±2.03) and 11.8 (±1.72) for the whole cohort, men and women respectively.

The cohort was sedentary for an average of 9.17 hours (9.72 for men and 8.83 hours for women). The average amount of time doing moderate physical activity was 27.4 (±21.3) minutes for the whole cohort, and 31.1 (±20.6) and 25.2 (±21.5) for men and women respectively. The majority of the cohort had never smoked 60.3%, 40.9% of men and 71.9% of women. There was similar distribution of use of antihypertensive medication between men and women 29.6% and 29.5% respectively and 29.5% for the whole cohort.

Urinary urea nitrogen was 8.74 (±2.76) g/d for the whole cohort, 9.98 (±2.67) and 7.99 (±2.37) g/d for men and women respectively. Serum magnesium values were all within the normal range of 0.7-1.0 mmol/L for the whole cohort, men and women 0.87 mmol/L for all.

The mean dietary energy intake was 1927 (±396) kcal/d. Mean energy intake was lower the than the recommended intake for both men 2198 (±406) and women 1763 (±286) kcal/d. Total protein intake was 77.4 (±16.2) g/d for the whole cohort and 86.5 (±17.6) g/d for men and 72.0 (±12.6) g/d for women. Magnesium intakes were within the RNI for both men 364 (±104) mg/d and women 307 (±72.5) mg/d. The RNI for men is 300 mg/d and 270 mg/d for women in the UK. Potassium intakes were higher in men with a mean of 3872 (±884) compared with women 3389 (±699) mg/d. Dietary sodium intakes were 2440 (±804) for the whole cohort and 2842 (±907) and 2198 (±623) for men and women respectively. Alcohol

intake was higher in men 12.9 (±11.2) g/d than women 8.38 (±8.60) g/d. Approximately 7% of the cohort were taking calcium supplements, a higher proportion of women (~10%) were taking calcium supplements than men (~2%).

Table 6.0 Baseline characteristics of 234 men and women aged 65-79 in the NU-AGE cohort

	Total n=234	**Men n=88**	**Women n=146**
Age	70.1 (±4.04)	70.6 (±4.20)	69.7 (±3.91)
BMI	26.9 (±4.13)	27.1 (±3.70)	26.8 (±4.38)
SBP (mmHg)	152 (±19.3)	149 (±16.7)	153 (±20.5)
DBP (mmHg)	81.8 (±9.84)	83.1 (±9.68)	81.1 (±9.88)
Antihypertensive medication	69 (29.5%)	26 (29.6%)	43 (29.5%)
School years	11.8 (±1.83)	11.9 (±2.03)	11.8 (±1.72)
Physical activity			
Sedentary (time)	550 (±102)	583 (±118)	530 (±85.1)
Moderate activity (time)	27.4 (±21.3)	31.1 (±20.6)	25.2 (±21.5)
Vigorous activity (time)	0.83 (±3.04)	1.75 (±4.36)	0.28 (±1.61)
Smoking			
Current	5 (2.14%)	3 (3.41%)	2 (1.37%)
Former	88 (37.6%)	49 (55.7%)	39 (26.7%)
Never	141 (60.3%)	36 (40.9%)	105 (71.9%)
Biomarkers			
Urinary Urea (g/d)	8.74 (±2.76)	9.98 (±2.67)	7.99 (±2.37)
Serum Magnesium (mmol/L)	0.87 (±0.06)	0.87 (±0.06)	0.87 (±0.05)
Dietary Intakes			
Total energy (kcal)	1927 (±396)	2198 (±406)	1763 (±286)
Protein (g)	77.4 (±16.2)	86.5 (±17.6)	72.0 (±12.6)
Magnesium (mg)	329 (±90.1)	364 (±104)	307 (±72.5)
Potassium (mg)	3570 (±806)	3872 (±884)	3389 (±699)
Sodium (mg)	2440 (±804)	2842 (±907)	2198 (±623)
Alcohol (g)	10.1 (±9.89)	12.9 (±11.2)	8.38 (±8.60)
Calcium supplement	16 (6.84%)	2 (2.27%)	14 (9.59%)

Correlations between dietary intakes and biomarkers

Correlations of dietary intake with biomarker indicated a weak correlation between dietary and serum magnesium *r*=0.14 (P value=0.03), *r*=0.18 (P value=0.09) and *r*=0.13 (P value=0.12) for the whole cohort, men and women respectively. Dietary protein intake was more strongly correlated with urinary urea; *r*=0.60 (P value≤0.001), *r*=0.65 (P value=≤0.001) and *r*=0.42 (P value=≤0.001) for the whole cohort, men and women respectively.

Table 6.1 Pearson's correlation coefficients for dietary magnesium and protein and the biomarkers serum magnesium and urinary urea with blood pressure in 234 men and women aged 65-79 years.

	Dietary Magnesium mg/d	**Serum magnesium mmol/L**	**Dietary protein g/d**	**Urinary urea g/d**
Whole cohort (n=234)				
SBP	-0.24**	-0.06	-0.15*	-0.08
DBP	-0.13*	-0.03	0.01	-0.13*
Men (n=88)				
SBP	-0.22*	0.14	-0.15	-0.07
DBP	-0.20	0.14	-0.07	-0.23*
Women (n=146)				
SBP	-0.22**	-0.17*	-0.09	-0.03
DBP	-0.15	-0.15	-0.02	-0.15

P values*≤0.05, **≤0.01, ***≤0.001

For the whole cohort, dietary magnesium intake was inversely correlated with both SBP -0.24 (P value≤0.001) and DBP (-0.13 (P value=0.04) **(Table 6.1)**. There was a weaker, and non-significant, inverse correlation between serum magnesium and blood pressure, -0.06 and -0.03 for SBP and DBP respectively in the whole cohort. Dietary protein intake was inversely correlated with SBP -0.15 (P value=0.02) and only minimally correlated with DBP 0.01 in the whole cohort and an inverse correlation with urinary urea of -0.08 for SBP and -0.13 (P value=0.05) for DBP was also present.

In 87 men dietary magnesium was inversely correlated with both SBP and DBP -0.22 and -0.20 respectively, this was significant for SBP P value=0.04. In 146 women there were similar inverse correlations -0.22 and -0.15 for SBP and DBP with dietary magnesium, the coefficient for SBP was significant, P value=0.01. There was a non-significant positive

correlation between serum magnesium and blood pressure in men 0.14 for both SBP and DBP, and an inverse correlation for women -0.17 and -0.15 for SBP and DBP which was significant for SBP (P value=0.04). Dietary protein intake was non-significantly inversely correlated with blood pressure in both men and women -0.15 and -0.09 for SBP and weaker inverse correlations of -0.07 and -0.02 for DBP for men and women respectively. Urinary urea was inversely correlated with both SBP and DBP in men and women -0.07 and -0.23 SBP and DBP in men -0.03 and -0.15 in women respectively. In men the coefficient for urinary urea and DBP was significant (P value=0.03).

Table 6.2 Regression coefficients for whole cohort, men and women in NU-AGE study aged 65-79 years for SBP and DBP with dietary intakes and biomarkers of magnesium and protein intake.

	Dietary Magnesium mg/d		Serum magnesium mmol/L		Dietary protein g/d		Urinary urea g/d	
	SBP	**DBP**	**SBP**	**DBP**	**SBP**	**DBP**	**SBP**	**DBP**
Whole cohort (n=234)								
Unadjusted	-0.05***	-0.02*	-20.5	-6.14	-0.17*	0.00	-0.59	-0.48*
Model 1	-0.05**	-0.03**	-4.75	-4.74	-0.10	-0.07	-0.20	-0.78**
Model 2	-0.07*	-0.02	-1.74	-1.39	-	-	-	-
Sensitivity analysis (n=164)	-0.08*	-0.01	-16.2	-12.6	-0.07	-0.08	0.11	-0.77*
Men (n=88)								
Unadjusted	-0.04*	-0.02	38.4	23.3	-0.15	-0.04	-0.45	-0.84*
Model 1	-0.06**	-0.04***	53.4	23.8	-0.18	-0.17*	-0.30	-1.54***
Model 2	-0.10*	-0.02	58.0	30.1	-	-	-	-
Sensitivity analysis (n=61)	-0.07	-0.02	34.2	30.3	-0.10	-0.22*	-0.16	-1.78***
Women (n=146)								
Unadjusted	-0.06*	-0.02	-66.5*	-28.3	-0.12	-0.03	-0.25	-0.61
Model 1	-0.05	-0.02	-53.1	-20.6	-0.08	-0.05	-0.15	-0.66
Model 2	-0.07	-0.01	-49.3	-17.6	-	-	-	-
Sensitivity analysis (n=103)	-0.11	-0.00	-44.8	-21.9	-0.03	-0.02	-0.25	-0.56

P values*≤0.05, **≤0.01, ***≤0.001

Model 1: age, BMI, smoking status, physical activity, school years, antihypertensive medication, total energy, dietary sodium

Model 2: model 1 + dietary potassium, calcium supplement use (Magnesium analysis only)

Sensitivity analysis: excluding those taking antihypertensive medication (model 1* for protein and urinary urea, model 2* for dietary and serum magnesium)*excluding use of antihypertensive medication.

Dietary magnesium

In the whole cohort after adjusting for age, BMI, smoking status, physical activity levels, years in education, use of antihypertensive medication, dietary intakes of total energy, sodium, and potassium and calcium supplement use there was a significant correlation between dietary magnesium intake and SBP, regression coefficient -0.05 (P=0.01). In men this association maintained significance -0.06 (P=0.01), but was non-significant for women -0.05 (P=0.07). Sensitivity analysis excluding those using antihypertensive medication provided similar results in the whole cohort (n=163) -0.08 (P=0.02). However, in men the association was no longer significant -0.07 (P=0.10) and was non-significant in women -0.11 (P=0.08). Similar associations were seen with DBP; -0.03 (P≤0.01) for whole cohort, -0.04 (P≤0.001) for men and -0.02 (P=0.11) for women after adjustment for the above factors. Sensitivity analysis provided similar coefficients and yielded no significant association.

Serum magnesium

There was no significant association between serum magnesium and SBP or DBP with the exception of unadjusted SBP in women, coefficient of -66.5 (P=0.04). However, sensitivity analysis excluding those taking antihypertensive medication and adjusting for age, BMI, smoking status, moderate physical activity levels, education, total energy, dietary sodium, potassium and use of calcium supplements produced coefficients of -16.2 (P=0.57), 34.2 (P=0.47) and -44.8 (P=0.26) for SBP in the whole cohort, men and women respectively. And -12.6 (P=0.39), 30.3 (P=0.21) and -21.9 (P=0.25) for DBP in the whole cohort, men and women respectively.

Dietary protein

After adjustment for age, BMI, smoking status, physical activity levels, years in education, use of antihypertensive medication, total energy and sodium intakes there was no significant relationship between SBP and dietary protein intake in the whole cohort, men or women. Regression coefficients were -0.10 (P=0.36), -0.18 (P=0.22) and -0.08 (P=0.63) for SBP in the whole cohort, men and women respectively. Sensitivity analysis (n=163) excluding those taking antihypertensive medication provided similar results -0.07 (P=0.54), -0.10 (P=0.60) and -0.03 (P=0.85) for whole cohort, men and women respectively for SBP. Coefficients of -0.07 (P=0.20), -0.17 (P=0.04) and -0.05 (P=0.52) were detected for DBP in

the whole cohort, men and women respectively after adjustment for the above confounding factors. Sensitivity analysis excluding those taking antihypertensive medication yielded similar results and the significance in association between DBP and dietary protein remained in men -0.22 (P=0.03).

Urinary urea

There was no significant relationship between urinary urea and SBP in the whole cohort, men or women. Regression coefficients of the adjusted model were -0.20 (P=0.69), -0.30 (P=0.69) and -0.15 (P=0.83) for SBP for whole cohort, men and women respectively. Sensitivity analysis was similar, yielded some difference in direction but not in significance 0.11 (P=0.86), -0.16 (P=0.87) and 0.25 (P=0.77) for SBP for whole cohort, men and women respectively.

A significant inverse association was seen between DBP and urinary urea in the whole cohort -0.78 (P=<0.01) and in men -1.54 (P≤0.001) but not women -0.66 (P=0.06) after adjustment for confounding factors. Similar results were seen for sensitivity analysis -0.77 (P=0.01) for whole cohort, -1.78 (P=0.001) for men and -0.56 (P=0.17) for women.

6.4 Discussion

The findings of this study on 234 men and women aged 65-79 years, summarised in **Table 6.3**, suggest that dietary magnesium intake was not well correlated with serum magnesium levels in this cohort of older men and women (*r*=0.14, *r*=0.18 and *r*=0.13 whole cohort, men and women respectively). This is however a slightly stronger relationship than has been previously reported in the ARIC study where a coefficient of *r*=0.053 was reported in a cohort of 7,731 men and women aged 45-64 (164) and ρ=0.04 in a larger sample of 13,560 men and women, 45-64 years (167). This difference may in part be due to demographic differences in study populations, for example the NU-AGE cohort is fairly homogenous in its ethnicity, white Caucasian, whereas the ARIC study includes respondents from a range of ethnicities. As serum magnesium is in part influenced by dietary intake (407) it is possible that cultural related differences in dietary composition may have been a factor. There was also a wide range of dietary magnesium intakes reported in the ARIC study, 31-864 mg/d, which may also be a factor (164).

Urinary urea was more strongly correlated with dietary protein intake in this cohort (*r=0.60, r=0.65 and r=0.41* whole cohort, men and women respectively). This finding is generally in line with previous studies which have been conducted in general populations, rather than older cohorts specifically (215). Partial 24hr urine collections have been reported to correlate well with dietary intake in the order of 0.50-0.81 (215, 411, 412). Therefore, the reported correlations for the whole cohort and men are within this range (*r*=0.60 and *r*=0.65 respectively). Urinary urea nitrogen is reported to account for ~85% of total urinary nitrogen excretion and there is little variation in this amount between people (215). The correlation for dietary intake and urinary urea was lower in women (*r*=0.41) which may indicate an element of under-reporting, which would be plausible given the relatively low mean total energy intakes of women (1770 kcal/d). In addition the mean BMI for women of 26.7 would be indicative of higher habitual intakes than those reported.

In the present study no measure of completeness, such as para-aminobenzoic acid (PABA), was used during the 24hr collection, thus it would be sensible to be cautious and estimate that not all urine collections would be complete, previous studies have reported 17% and

25% of urinary collections to be incomplete (362, 413). However, it is worth noting that a recent study investigating how urine samples without PABA provided estimates of intakes of protein and potassium (414). They reported that there was little difference in the mean levels of urinary nitrogen and potassium between those who reported taking and not taking PABA (414). This would suggest that even in the absence of a measure of completeness, as in the NU-AGE study, the 24hr urine samples may still provide a good representation of daily protein intake. In addition the ratio of protein intake estimated from total urinary nitrogen and reported dietary protein intakes for the whole population 0.85 (±0.23), men 0.86 (±0.18) and women 0.84 (±0.26) were within the range of the anticipated ratio of estimated protein to reported intake if values were valid; ratio 0.81 ±0.05 (215).

Table 6.3 Summary of the relationship (based on regression coefficients) of dietary intake and biomarkers of magnesium and protein with blood pressure in 234 men and women aged 65-79

	Dietary magnesium mg/d	**Serum magnesium mmol/L**	**Dietary protein g/d**	**Urinary urea g/d**
Whole cohort				
SBP	↓	↔	↔	↔
Sensitivity analysis	↓	↔	↔	↔
DBP	↔	↔	↔	↓
Sensitivity analysis	↔	↔	↔	↓
Men				
SBP	↓	↔	↔	↔
Sensitivity analysis	↔	↔	↔	↔
DBP	↔	↔	↓	↓
Sensitivity analysis	↔	↔	↓	↓
Women				
SBP	↔	↔	↔	↔
Sensitivity analysis	↔	↔	↔	↔
DBP	↔	↔	↔	↔
Sensitivity analysis	↔	↔	↔	↔

↓ - significant inverse association (fully adjusted model)
↔ - no significant association (fully adjusted model)

Correlations between dietary intakes and biomarkers of magnesium and protein with blood pressure were strongest in relation to dietary magnesium intake. Although the correlations were weak (mmHg/mg magnesium) at *r*= -0.23 SBP and *r*= -0.14 DBP for whole cohort, *r*= -0.22 and *r*= -0.20 for SBP and DBP in men and *r*= -0.22 and *r*= -0.16 in women it may suggest that higher dietary magnesium intakes could lead to reductions in blood pressure of older men and women.

A review of observational studies assessing the relationship between dietary magnesium intakes and blood pressure noted that other studies have also reported negative correlations between dietary magnesium intake and SBP and most also report negative correlations with DBP but not all (415). Studies were adjusted for classical covariates including age, BMI, total energy, sodium and potassium intakes. Only one paper included in the review was looking exclusively at an older population, aged 65-89. The study was small (n=40) and conducted in US men and women, dietary intake was recorded using 24hr recall and they did not adjust for confounding factors in analysis and reported a negative correlation with DBP only (416). The lack of adjustment in analysis makes it difficult to make any conclusions based on these results, as they may be influenced by a number of factors including total energy intake, BMI and smoking status.

Joffres et al (165) reported on correlations between dietary magnesium intake and blood pressure in Japanese men in Hawaii aged 46 years and older (165). They reported correlation coefficients of -0.12 and -0.09 for SBP and DBP respectively, but did not investigate circulating magnesium levels (165). In a study by Kesteloot and Joossens (417) in a Belgian population of men and women aged 25-74 years an inverse correlation was reported for dietary magnesium intake in g/d and SBP but not DBP in women (417). No data were reported for dietary magnesium and blood pressure in men. In women the regression coefficients for dietary magnesium and SBP were -14.43 and -0.07 for partial regression and standardised partial regression respectively. This was slightly weakened when those taking antihypertensive medication were excluded; -11.71 and -0.06 for partial regression and standardised partial regression respectively (417). In the NU-AGE analysis the relationship between dietary magnesium intake mg/d and SBP in women was -0.06 (P=0.01) for the unadjusted model, and after adjusting for confounding factors including

age, BMI, dietary total energy, sodium and potassium intakes the association was non-significant; -0.07 (0.12) but was slightly strengthened following exclusion of those taking antihypertensive medication -0.11 (P=0.08). This final finding is similar to that of Kesteloot and Joossens (1988) and suggests that dietary magnesium intake may be an important factor in reducing blood pressure in older women.

Serum magnesium was not correlated with blood pressure in the whole cohort -1.74 (P=0.94) and -1.39 (P=0.91) for SBP and DBP respectively. Sensitivity analysis, excluding those taking antihypertensive medication, was also non-significant -16.2 (P=0.57) and -12.6 (P=0.39). This is similar to previous findings, including a small study of 26 subjects that included untreated hypertensives (n=11), treated hypertensives (n=8) and normotensive subjects (n=7). The authors reported no significant correlation between serum magnesium and blood pressure (418). In addition in a study of elderly men and women with type II diabetes, there was no significant difference in the serum magnesium levels of participants with SBP <130 and DBP <80 compared with those with SBP ≥130 and DBP ≥80 (254).

In the present study there were differences in the relationship of serum magnesium and blood pressure in men and women. In men a non-significant positive relationship was seen between serum magnesium and SBP after full adjustment 58.0 (P=0.07) which was attenuated during sensitivity analysis, 34.2 (P=0.47). And a non-significant positive relationship with DBP 30.1 (P=0.08) and 30.3 (P=0.21) for fully adjusted and sensitivity analysis respectively. Whereas in women the relationship for SBP and DBP were negative, albeit non-significant after adjustment, -49.3 (P=0.14) and -44.8 (P=0.26) for SBP and -17.6 (P=0.28) and -21.9 (P=0.25) for DBP for the fully adjusted model and sensitivity analysis respectively. These findings are important as although dietary magnesium was only weakly correlated with serum magnesium, it may have relevance for those taking supplements or medications containing magnesium as supplementation with magnesium, including bound to other medications, may be sufficient to increase circulating levels (405). A non-significant association of higher blood pressure with higher serum magnesium levels was seen in men in this study, which warrants further investigation to determine whether high magnesium intakes in the form of supplements or in medication may lead to a detrimental increase in blood pressure in men which would require monitoring.

There was a strong correlation between dietary protein and urinary urea for the whole cohort *r=0.60*, men *r=0.65* and women *r=0.41*. In terms of their respective relationships with blood pressure, there were significant associations between dietary intake and DBP in men and urinary urea with DBP in whole cohort and men, but not women. The findings of other studies in younger populations have been somewhat inconsistent with some showing no significant association (141, 419), and others report a significant inverse association (359, 420).

An Italian cohort (n=3705) which included older people, 25-74 years, reported a significant inverse relationship between overnight urinary urea nitrogen and SBP -5.2 mmHg/log(urea) mmol/h (P<0.001) after adjustment for a number of confounding factors including age, sex, BMI, antihypertensive medication and urinary sodium, potassium and calcium (420). However, they did not conduct any age stratified analysis and as the age range of the participants is very wide, a different relationship may be present in the older adults. This could be due to age related decline in kidney function, as the kidneys have an important role in processing nitrogen. In the INTER-SALT study which includes men and women from 32 countries aged 20-59 years, they reported on differences in associations of total nitrogen and blood pressure between younger (20-39 years) and older (40-59 years) participants (359). They reported that inverse associations between total nitrogen and SBP and DBP blood pressure which were stronger in older participants -0.92 (P<0.001) and -0.48 (P<0.05) than younger participants -0.20 (P not reported) and -0.38 (P<0.05) for SBP and DBP respectively (359). In addition Cirillo et al (420) in the Italian cohort used only an overnight urine collection, which may not provide as accurate a measure as 24hr collection, as urinary urea excretion is increased about 2 hours postprandially (420). Thus if protein intake is unevenly distributed throughout the day with higher intakes at the evening meal, this may lead to higher levels of urinary urea suggesting higher total intake than might otherwise be seen if a 24hr collection was completed. Additionally a Japanese study reported that higher protein intakes estimated from urinary urea was associated with lower SBP and DBP, reaching significance in men but not women, compared with lower protein intakes (421). A study by Kihara et al (419) also reported different directions of effect for men and women in association with urinary urea nitrogen in Japanese men and

women aged 30 years. In that study women had slightly lower blood pressure 129/75.6 mmHg compared with 132/78.5 mmHg in men which was significantly different ($P<0.05$). This in conjunction with the women's slightly higher urinary nitrogen/creatinine ratio, a potential marker of protein intake, may partially explain this inconsistency in direction of effects (419).

6.4.1 Strengths and limitations

Due to the cross sectional design of the current analysis it is not possible to infer causality, and despite adjusting for several confounding factors it may be that other factors not accounted for in analysis would influence the outcome. Despite using a more robust method of dietary assessment, 7DD, it is possible that bias in reporting may have occurred. In addition to dietary reported intakes, serum magnesium and urinary urea were also measured. Urinary urea is a recovery biomarker which has been reported to accurately represent absolute intakes, of protein. However, it is only reflective of the time period in which the collection was taken, in this study one 24 hour period. Thus if protein intake was particularly high or low on this particular day then this would be reflected in urinary nitrogen levels. To more accurately ascertain habitual intake the 24hr urine collection could be repeated on several occasions, although this becomes burdensome to the participant and increases costs of the study.

6.5 Summary

In this study of older adults as has been found previously, dietary magnesium intake was not well correlated with serum magnesium levels. However, dietary protein was well correlated with urinary urea. The strongest relationship was seen between dietary magnesium intake and blood pressure, despite relatively weak correlation coefficients. This relationship was reflected in significant inverse associations between dietary magnesium intake and SBP in the whole cohort after adjustment and sensitivity analysis, and in men only after adjustment. There was also a similar strengthened correlation between urinary urea and DBP in men only. This was also reflected in significant inverse association with DBP in men only after adjustment and sensitivity analysis, and was also seen for dietary protein intake and DBP in men only, despite a much weaker correlation coefficient. These findings indicate that dietary magnesium intake may be correlated with blood pressure in older adults, however, as there was little significant correlation between serum magnesium and blood pressure in men, women or the whole population serum magnesium would not be recommended for predicting blood pressure in the older UK population. Dietary protein and urinary urea may be associated with DBP in older men and urinary urea in whole populations also. This finding would suggest a potential benefit of urinary urea in predicting DBP, although further research to confirm the association would be necessary, especially as the strongest correlations were seen between this biomarker and blood pressure outcome (in men only).

Chapter Seven

DISCUSSION

7.0 Summary of research

This thesis set out to answer five research questions which were:

1. What is the relationship between dietary magnesium intake and stroke risk factors, blood pressure and serum lipid levels and the risk of stroke in middle and older aged men and women?
2. What are the main contributing sources of dietary protein intake, and how do the types of protein (animal, plant and ratio of plant:animal) and food group sources of protein differ between men and women?
3. What are the relationships between dietary protein intake, including that from different types (animal and plant) and stroke risk factors, blood pressure and serum lipid levels and the risk of stroke in middle and older aged men and women?
4. How do biomarkers of magnesium and protein intake compare with dietary reported intakes in relation to blood pressure in older men and women?
5. What are the implications of the findings of this thesis for public health nutrition

In Chapter Three this thesis addressed research question 1 by determining the relationship between dietary magnesium intake and stroke risk and risk factors, through the use of linear regression analysis of 4,443 British men and women aged 39-80, part of the EPIC-Norfolk cohort. In doing so a significant inverse association was shown between dietary magnesium intake and blood pressure in men, but not women. Dietary magnesium intake was also significantly associated with circulating lipid levels. In men higher magnesium intakes were inversely associated with total cholesterol and positively associated with the ratio of HDL:LDL. In women a significant inverse association was seen with triglyceride levels and positive associations with HDL and the ratio of HDL:LDL cholesterol. Both blood pressure and cholesterol levels are risk factors for stroke. In men we identified that having lower dietary magnesium intakes was associated with higher risk of stroke but that there was no significant association for women.

In Chapter Four this thesis addressed research question 2 and identified that the primary contributing source to total protein intake was from animal and animal products in both

men and women. Women, compared to men, had small but significantly higher intakes of total protein as a percentage of energy and protein from animal-marine, animal-derived and plant sources as a percentage of energy than men. There was no significant difference, between men and women, for protein from total animal sources and the ratio of plant:animal protein intake. There were differences in the consumption of individual food items and food groups between men and women, with men consuming higher amounts of red and processed meat than women. Women tended to consume more fish and poultry. These differences in the makeup of protein intake may have implications on the effects of total protein intake on stroke risk and risk factors.

In Chapter Five research question 3 was addressed and the findings indicated that there were differences in the effects of protein and types of protein (plant and animal) on risk factors for stroke between men and women. However, one consistency across total protein intake and types of protein was no significant association with stroke risk directly in either men or women. In men only protein from plant sources was significantly inversely associated with DBP exclusively. In men total protein intake was positively associated with HDL levels and protein from plant sources were inversely associated with total cholesterol and LDL and positively associated with ratio HDL:LDL. The ratio of plant:animal protein intake was inversely associated with total cholesterol and LDL cholesterol. There were no significant associations between protein from animal sources and blood pressure, lipid levels or stroke risk in men. In women total protein and protein from animal sources were inversely associated with SBP and DBP and plant protein with DBP only. Total protein and protein from animal sources also had similar associations for cholesterol levels both indicating an inverse association with triglycerides and positive associations with HDL and ratio HDL:LDL, total protein was additionally inversely associated with LDL levels in women. There was no significant association between the ratio of plant:animal protein intake and blood pressure, cholesterol levels or stroke risk in women after adjustment for confounding variables.

In Chapter Six of this thesis research question 4 was addressed to determine if dietary intakes of magnesium and protein and biomarkers, serum magnesium and urinary urea, were correlated with blood pressure in an older population of men and women. In addition

to determine if one of the measures was more strongly associated with blood pressure than the others in an older population. In a cohort of 234 older men and women, aged 65-79 years, in the NU-AGE study, a strong correlation between dietary protein and urinary urea was reported for the whole cohort r=0.61, men r=0.65 and women r=0.44 respectively. Dietary magnesium was less well correlated with serum magnesium (r=0.14, r=0.18 and r=0.13 whole cohort, men and women respectively). However, dietary magnesium had the strongest relationship with blood pressure, specifically SBP in the whole cohort and men. Dietary protein and urinary urea were also significantly inversely associated with DBP in men only.

All together the chapters of this thesis combine to indicate that in middle aged and older adults dietary magnesium intake may be an important component in reducing the risk of stroke, particularly in men, whereby inverse associations were reported with both blood pressure and some lipid levels (total cholesterol and ratio HDL:LDL) in the EPIC-Norfolk cohort, and dietary magnesium intake was additionally associated with lower blood pressure in older population part of the NU-AGE study. Protein intake may have implications for stroke risk and risk factors for stroke, blood pressure and total cholesterol, and may be determined by the quality of protein rather than just quantity consumed.

Public health perspective

The findings of this study concur with the current UK guidelines in terms of increasing intakes of fruit and vegetables and wholegrains, as these are rich sources of magnesium. In addition other plant sources such as nuts and legumes also provide quality protein, as well as containing magnesium. In this study the difference in magnesium intake of the extreme quintiles was 250 mg/d for men and 198 mg/d for women. This is the equivalent to approximately 2 slices of wholemeal bread with peanut butter and 9 Brazil nuts (29) which could easily be incorporated as part of a healthy diet and could have significant effects on blood pressure.

A more plant-based diet may have health benefits on a number of levels including on blood pressure, lipid levels and stroke risk and may also be more environmentally sustainable in the long term for the expanding population. As well as increased plant protein intakes,

quality animal protein sources could also be included in the diet. I believe the beneficial associations shown between animal protein intake and blood pressure and lipid levels in women were driven by women's higher intakes of lean animal protein compared to men and their concurrently lower intake of red and processed meats, which negatively influence these outcomes (316-318). Therefore it would be recommended to provide education on improving the quality of protein sources, through increased consumption of fish (in line with the recommendation to consume at least two portions of fish per week), lean meats and vegetable protein sources such as legumes.

Contribution to knowledge

There is some evidence to suggest that dietary magnesium and protein intakes may beneficially affect stroke risk and risk factors including blood pressure and cholesterol levels. However, to the best of my knowledge no other study has previously assessed these associations in parallel in one cohort of men and women from the general population. In addition previous research has focused on recording dietary intakes through 24hr recall and FFQ whereas in this thesis magnesium and protein intakes were derived from 7 day food diaries. Finally, there has been a lack of research in the UK population specifically, and there is evidence to suggest that even within Europe there are significant differences in consumption patterns, both of specific items and frequency/portions consumed (309, 422).

There is a current lack of knowledge surrounding the contributing factors to protein intake in the UK diet, specifically subtypes of protein (plant and animal). Little is known about how protein intake differs between men and women, and this may have implications for health outcomes due to the quality of protein consumed. Previous research on the correlation of urinary urea and dietary protein intake in an exclusively older population is lacking. Few studies have previously investigated the associations and correlations of magnesium and protein intakes and biomarkers in older population.

Therefore this thesis aimed to fill these identified gaps in the existing literature.

7.1 Implications of findings

The findings in relation to dietary magnesium intake and blood pressure may have significant health implications. In men in the EPIC-Norfolk cohort, a significant difference of -7 mmHg SBP and -4.3 mmHg DBP was reported between extreme quintiles of magnesium intake, the difference in magnesium intake between quintiles was 250 mg an amount that can be achieved through dietary modification. Independently reductions of 10 mmHg SBP and 5 mmHg DBP have been associated with a ~33% and 34% reduction in stroke risk (423, 424), and also reduced risk of stroke death by 50-60% (425). Correlations between dietary magnesium intake and blood pressure were also seen in the older NU-AGE cohort of men and women aged 65-79 years. Dietary total protein and urinary urea were also related to lower DBP in men, only, in the NU-AGE cohort.

There is some evidence to suggest that in a very old population, aged >80, treatment with antihypertensive medication may infer higher risk than the benefit gained from blood pressure reduction (426). The findings of the present study do not cover this age range, but may indicate a potential for, the increasingly ageing population, to increase their magnesium intake as a blood pressure lowering strategy (426). By 2037 it is predicted that 1 in 12 of the UK population will be over 80, approximately 6 million people, double the current number (427). Therefore if the currently identified relationship remains the same in older age, there may be a potential benefit of dietary modification on blood pressure by increasing magnesium intake.

In terms of effects on women, differences in blood pressure were smaller with dietary magnesium and protein intakes. However, even small reductions in blood pressure have been indicated to reduce the risk of hypertension and stroke itself. For example a -2 mmHg decrease in DBP was equated to a 17% reduction in prevalence of hypertension and a 15% reduction in stroke risk (428). The difference in effects of dietary intake on blood pressure between men and women are of interest, these differences are summarised in **Table 7.0**. Men tend to have higher blood pressure than their age-matched pre-menopausal women, so this may be one contributing factor to the larger effect seen in men. Previous studies have also highlighted that reduction in blood pressure associated with dietary intakes is

often greater in individuals with elevated blood pressure initially (108). The potential mechanisms of the difference in blood pressure regulation between men and women are unclear (197) but may be related to changes and differences in hormone levels of men and women.

A study investigating the effects of different statin medications on LDL cholesterol indicated that on average LDL was reduced by 1.8 mmol/L and this equated to approximately 17% reduction in stroke risk (280). Differences of this magnitude were not identified during the current analyses, however modest differences of -0.21 mmol/L LDL were seen between extreme quintiles of magnesium intake in both men and women. For plant protein intake differences of -0.20 mmol/L and -0.28 mmol/L of LDL for men and women respectively were reported. In men animal protein, and total protein, was related to a slight increase in LDL levels between extreme quintiles of intake, whereas in women the difference was a consistently lower LDL in those with higher total and animal protein as percentage of energy. This finding may be influenced by the differences in the food items consumed that contribute to protein intake. Within this thesis it was identified that in terms of animal protein sources, men were higher consumers of red and processed meat, which are sources of saturated fat whereas women were on average consuming more fish and poultry which are leaner sources of more healthful fatty acids. Thus it may be of relevance to highlight the importance of the source of protein consumed in terms of health benefits.

As with blood pressure, some differing effects of dietary magnesium and protein intakes were shown in relation to lipid profiles of men and women. This may have occurred for a number of reasons, including a difference in dietary intakes which influenced the associations. Mechanistic differences between men and women that may have led to these discrepancies could be related to the ways in which men and women deposit adipose tissue (429). For example central abdominal adipose tissue, which is more prevalent in men, provides a plentiful supply of free fatty acids and cytokines to the liver which may increase the risk of developing dyslipidaemia (429). This may also contribute to an increase in blood pressure in men. Although as women age distribution of fat changes and their central adiposity increases.

The combined implications of reduction in blood pressure and potential modest reductions in lipid levels that may be achieved through consuming a diet rich in both high quality protein and magnesium are not known but could potentially have a greater influence on stroke risk than the components individually.

Table 7.0 Summary of results highlighting similarities and differences in direction of effect between men and women.

Dietary Intakes	Men significant[1]	Men non-significant	Both significant	Both non-significant	Women significant	Women non-significant
Magnesium	SBP, DBP TC Stroke risk (intake groups)	TG, HDL	HDL:LDL	LDL Stroke risk (quintiles)	TG, HDL	SBP, DBP TC Stroke risk (intake groups)
Total protein		SBP, DBP TG, LDL, HDL:LDL	HDL	TC Stroke risk (quintiles)	SBP, DBP TG, LDL, HDL:LDL	
Plant protein	HDL:LDL		DBP TC, LDL	SBP TG, HDL Stroke risk (quintiles)		
Animal protein		SBP, DBP TG, HDL, HDL:LDL		TC, LDL Stroke risk (quintiles)	SBP, DBP TG, HDL, HDL:LDL	TC, LDL
Ratio plant:animal protein	TC, LDL			SBP, DBP TG, HDL, HDL:LDL Stroke risk (quintiles)		

Abbreviations: DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; TC, total cholesterol; TG, triglyceride; SBP, systolic blood pressure

[1]significant inverse effect for SBP, DBP, TC, TG and LDL. Significant positive effect for HDL and HDL:LDL.

The findings of the dietary intake and biomarker study, Chapter Six, indicated that dietary magnesium intake may be correlated with blood pressure in older adults, however, as there was little significant correlation between serum magnesium and blood pressure in men, women or the whole population serum magnesium would not be recommended for predicting blood pressure in an older UK population. Dietary protein and urinary urea may be associated with DBP in older men and urinary urea in whole populations also. This finding would suggest a potential benefit of urinary urea, due to its objectivity, in predicting DBP. Urinary urea is an objective measure, which relates to dietary protein intake, and may thus be less prone to bias than self-reported dietary intake.

The findings in context of other dietary components

The results of the current thesis are comparable to the established findings of other studies investigating the effects of different dietary nutrients and patterns on stroke risk and risk factors. For example the DASH combination diet comprising of low-fat dairy, modest saturated, total fat and cholesterol intakes and high in fruit and vegetables consumption has been associated with decreased blood pressure (108). This diet, consumed for 8 weeks, lead to reductions in SBP of 5.5 mmHg and 3.0 mmHg for DBP ($P<0.001$) compared to the control diet which was representative of typical dietary intake in US. In addition a diet high in fruit and vegetables, and lower in sweet and snack foods (108), resulted in reduced SBP of 2.8 mmHg ($P<0.001$) and DBP of 1.1 ($P=0.07$) compared with the control group. For those with existing hypertension the reductions in SBP and DBP were greater (108).

Plasma vitamin C, a marker of fruit and vegetable intake, has been significantly inversely associated with both blood pressure and stroke risk (110, 111). With every 20 µmol/L increase in plasma vitamin C, approximately equivalent to 1 serving of fruit or vegetable/d, a decrease of -0.9 mmHg SBP and -0.5 mmHg DBP was seen ($P<0.001$ for both) (111). In addition a further study showed that plasma vitamin C was also inversely associated with stroke risk, after full adjustment for confounding factors and exclusion of those reporting stroke within 2 years of follow up the RR for stroke was 0.60 (95% CI 0.44-0.81) in the highest quartile of plasma vitamin C, levels ≥66 µmol/L (110).

A RCT on soy protein intake reported beneficial effects on serum lipid levels (127). A diet consisting of 24 g soy protein and 70-80 mg soy isoflavones lead to a reduction in total cholesterol and triglyceride levels after 6 weeks of consumption. Total cholesterol was -0.17 mmol/L ($P<0.05$) and triglycerides were -0.14 mmol/L lower than the control diet which provided 24 g dairy protein ($P<0.05$). But no significant effect on LDL or HDL levels were shown (127).

7.2 Strengths and limitations of the study

To the best of my knowledge this is the first study to investigate the associations of dietary magnesium and protein intakes on stroke risk and risk factors for stroke in one general population cohort of men and women. Additionally this study was also able to assess the associations of dietary magnesium and protein with blood pressure in an exclusively older population, whilst also exploring the potential correlations with biomarkers of intake, serum magnesium and urinary urea.

The strengths of this study include the large cohort size and prospective design of EPIC-Norfolk cohort. This design reduces the susceptibility of the study to selection bias and the prospective nature allows for risk of stroke to be investigated. The EPIC-Norfolk cohort used in this thesis was a sub-cohort of the larger EPIC-Norfolk cohort. The cohort was derived from a random sub-sample of 4,000 participants whom were representative of the larger cohort. To this sub-sample, those with an incident stroke were also included. Therefore there is potential for this sampling procedure to have skewed the cohort to be less representative of the original sample. The strengths of the NU-AGE cohort include its inclusion of potentially objective measures of dietary intake; urinary urea, and serum magnesium. It also allowed analysis in an exclusively older population, and as EPIC-Norfolk cohort is based on baseline food intakes, recorded between 1993 and 1997, there may be differences in dietary intake patterns and foods consumed in today's modern diet that may influence the associations of magnesium and protein on blood pressure.

Statistical analyses were adjusted for a number of relevant confounding factors, which enables identification of potential associations independent of other known associated components. However, despite the care taken to develop statistical models it is possible for residual confounding to occur.

The 7-day food diary reduces the risk of recall bias, and is validated in the EPIC-Norfolk cohort to more accurately represent dietary intakes, including protein and some micronutrients, than 24hr recall and FFQs. The 7-day food diary was utilised in both the EPIC-Norfolk and NU-AGE cohorts. However, due to the nature of self-reported food intakes it is possible that there was response bias, in the form of either consuming a diet

perceived to be more healthful during the recording period, rather than habitual diet. Or the conscious and/or unconscious bias of foods and portion sizes. When compared with the UK population and dietary guidelines this EPIC-Norfolk cohort was generally representative of the UK population. Dietary intakes, and lifestyle factors, were only measured at one time-point, baseline, in these analyses. This means that changes in dietary habits, lifestyle or medical history which may have occurred during the following up period of the study (mean 9.58 years) cannot be accounted for.

Some participants had missing data for variables of interest such as smoking status or medication use. In these cases participants were reclassified into an alternative category. For example those with missing smoking status were reported as current smokers, and those with missing aspirin or antihypertensive medication use were reclassified to 'no' category. Therefore it is possible that some individual observations were misclassified. In addition participants with missing values for certain variables including history of stroke at baseline, blood pressure and lipid levels were excluded from analysis, which may have introduced an element of bias. Selection bias is also possible, although the whole EPIC-Norfolk cohort was representative of the UK population. Additionally, the sub-cohort in this thesis is primarily comprised of a further random sample of the EPIC-Norfolk cohort. However, truncation of the sample distribution would likely only attenuate the observed associations and therefore associations may actually be stronger than are presented.

Due to the nature of the study design it is not possible to infer causality between exposure (magnesium and protein from diet and biomarkers) and outcome (blood pressure, lipid levels and stroke risk).

It would also be beneficial to additionally investigate the association of urinary magnesium alongside serum and dietary intakes with risk factors for stroke. As urinary magnesium may correlate more strongly with dietary intakes, and may therefore be related to blood pressure and stroke risk.

In terms of dietary protein intake, it would be of interest to further explore the differences in effects of subtypes of protein, to identify if the quality of protein is more important than the quantity. Further sub classification of protein into specific amino acids, may be relevant

to aid in hypothesis generating of potential mechanisms of action of dietary protein on stroke risk and risk factors. However, specific amino acid deficiencies and low intakes are rare, due to the widespread presence of amino acids in all food items. Therefore it would be difficult through diet alone to increase the intakes of only specific amino acids, and it would be preferred to obtain dietary needs through food intake as opposed to supplementation where possible.

At present the current analyses allow for the understanding of the independent associations of dietary magnesium and protein intakes with stroke risk factors and stroke risk in one population. As the results of the analyses conducted in this thesis indicated that both nutrients were independently associated with stroke risk factors, blood pressure and lipid levels, it may be necessary to adjust for dietary intake of each variable in future analyses. Prior to this it would be of benefit to investigate the collinearity of the protein and magnesium in the respective regression models using variance inflation factor. If collinearity is identified a model incorporating an interaction term between dietary magnesium and protein intake may be necessary.

The current analyses could be enhanced by assessing the associations by stroke subtypes. This would enable identification in differences in risk and prevention of subtypes, as the mechanisms of action may potentially be different.

As these analyses were conducted at one time point, it would, be interesting to map changes in blood pressure, lipid profile and incidence of stroke over time. In this way it may be possible to determine if those with habitually low magnesium intakes across follow-up were at increased risk of stroke, or presented with hypertension or abnormal lipid profile at later follow-ups than those with adequate intakes. A similar approach could be taken in regard to dietary protein intake.

7.3 Future directions

As has been mentioned in the previous section, one of the limitations of this study was that due to the design it is not possible to infer causality. Therefore, further research would be required to assess, using randomised controlled trial, whether increased dietary magnesium intake could lead to clinical reductions in blood pressure. Foods which commonly provide high proportions of UK magnesium intake include cereals and wholegrains, thus these could be encouraged and less commonly consumed but good sources of magnesium such as nuts which can be grown in the UK including cobnuts, and leafy green vegetables which can also be sourced in abundance locally could also be investigated and proposed for consumption. This would then also improve the sustainability of increasing dietary intake recommendations.

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Appendices

Appendix 1: Published paper

Bain, L. K., Myint, P. K., Jennings, A., Lentjes, M. A., Luben, R. N., Khaw, K. T., Wareham, N. J. and Welch, A. A. The relationship between dietary magnesium intake, stroke and its major risk factors, blood pressure and cholesterol, in the epic-norfolk cohort. *International Journal of Cardiology* 2015;196:108-14. doi:10.1016/j.ijcard.2015.05.166

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The relationship between dietary magnesium intake, stroke and its major risk factors, blood pressure and cholesterol, in the EPIC-Norfolk cohort☆

Lucy K.M. Bain [a,1], Phyo K. Myint [b,1], Amy Jennings [c,1], Marleen A.H. Lentjes [d,1], Robert N. Luben [d,1], Kay-Tee Khaw [d,1], Nick J. Wareham [d,1], Ailsa A. Welch [a,*,1]

[a] Department of Population Health and Primary Care, Norwich Medical School, University of East Anglia, Norwich, UK
[b] Aberdeen Gerontological and Epidemiological INterdisciplinary Research Group (AGEING), Epidemiology Group, Institute of Applied Health Sciences, School of Medicine & Dentistry, Aberdeen, UK
[c] Department of Nutrition, Norwich Medical School, University of East Anglia, Norwich, UK
[d] Department of Public Health and Primary Care, Institute of Public Health, Cambridge University, Cambridge, UK

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ABSTRACT

Background: Dietary magnesium could modify the major stroke risk factors, high blood pressure (BP) and cholesterol, but has been understudied in both sexes in a single population. This study aimed to investigate if dietary magnesium intake was associated with BP, total cholesterol (TC) and incident stroke risk in an adult population.
Methods: We conducted cross-sectional analyses in a case–cohort study of 4443, men and women aged 40–75, representative of 25,639 participants years of the EPIC (European Prospective Investigation into Cancer)-Norfolk cohort. The cohort included 928 stroke cases (42,556.5 person years). Dietary data from 7 day food diaries were analysed using multivariate regression to assess associations between quintiles or data-derived categories of dietary magnesium intake and BP, TC and stroke risk, adjusted for relevant confounders.
Results: We observed differences of −7 mm Hg systolic BP (P trend ≤ 0.01) and −3.8 mm Hg diastolic BP (P trend = 0.01) between extreme intakes of magnesium in men, a significant inverse association with TC was observed (P trend = 0.02 men and 0.04 women). Compared to the bottom 10%, the top 30% of magnesium intake was associated with a 41% relative reduction in stroke risk (HR 0.59; 95% CI 0.38–0.93) in men.
Conclusions: Lower dietary magnesium intake was associated with higher BP and stroke risk, which may have implications for primary prevention.

1. Introduction

Stroke accounts for more than 5.5 million deaths annually and by 2020 predictions estimate that the global burden of stroke will account for 61 million disability-adjusted life years [1].

Elevated BP[2] is a significant modifiable risk factor for stroke with an approximate fourfold increase in stroke risk in hypertensive individuals compared with the normotensive population [2]. Although established evidence indicates that elevated BP, hypertension and circulating cholesterol can be modified by dietary intake including: salt, alcohol, saturated fat and cholesterol [2] other dietary components, including magnesium, which is abundantly available in nuts, green leafy vegetables and whole grains, have been less extensively studied.

Magnesium has a number of metabolic roles in the body and may influence BP and blood lipids through different mechanisms [3,4]. Magnesium may serve as a natural calcium channel blocker, exhibit beneficial effects on platelet coagulation, have a potential role in vasodilation and has been associated with reduced coronary artery calcification [4,5]. Other proposed mechanisms include increased peroxidation of lipoproteins with subsequent acceleration of atherosclerotic plaque formation and low magnesium may facilitate an increase in inflammation which is associated with negative changes in lipid profile [3,4]. Higher magnesium intake has been associated with lower risk of Type II diabetes [6], metabolic syndrome [7] and cardiovascular disease (CVD) [8].

Two recent meta-analyses have investigated the effects of dietary magnesium on stroke risk and CVD risk respectively [8,9] showing inconsistent findings. The reason for these inconsistencies may be due to estimation of magnesium intakes from less precise methods of recording diet such as Food Frequency Questionnaires (FFQs) and 24 hour recalls. However, it has been increasingly suggested that the detailed 7-day diary represents dietary intakes more precisely [10].

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* Corresponding author at: Department of Population Health and Primary Care, Norwich Medical School, University of East Anglia, Norwich NR4 7TJ, UK.
E-mail address: a.welch@uea.ac.uk (A.A. Welch).

[1] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

[2] Abbreviations: BMI — body mass index, BP — blood pressure, CVD — cardiovascular disease, DBP — diastolic blood pressure, DINER — Data Into Nutrients for Epidemiological Research, EPIC-Norfolk — European Prospective Investigation into Cancer-Norfolk, FFQ — Food Frequency Questionnaire, HDL — high density lipoprotein, HLQ — Health and Lifestyle Questionnaire, LDL — low density lipoprotein, MI — myocardial infarction, SBP — systolic blood press, TC — total cholesterol, and WHR — waist–hip ratio.

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Therefore, the purpose of this study was to determine whether dietary magnesium intake, estimated using a 7-day diary, was associated with BP, lipid profile and stroke risk in an adult general population of 4443 (representative of larger cohort 25,639) men and women.

2. Subjects and methods

2.1. Study population

The present study population is comprised of a randomly selected representative sample (n = 4000) of the EPIC-Norfolk cohort (n ~ 25,639), which will herein be referred to as EPIC-Norfolk sub-cohort. EPIC-Norfolk has previously been described in detail and the characteristics of the sample were comparable with other representative UK populations with the exception of a lower proportion of current smokers [10]. Ethical approval for the study was obtained from the Norwich Ethics Committee, and participants provided informed consent.

Briefly this sub-cohort (n = 4920) is comprised of a representative random sample of 4000 men and women with complete data for food diaries from the EPIC-Norfolk cohort (n = 25,369) and 1102 stroke cases (n = 182 part of 4000 random sample previously mentioned) giving a total of 4920. Participants were resident in the Norfolk area at recruitment between 1993 and 1997 and recruited through participating General Practices (n = 35) [10]. Participants were excluded from analyses if they had reported prevalent stroke at baseline or had missing values for any variables included in the multivariate model (n = 477). Participants with missing values for smoking status, aspirin medication use for >3 months, and magnesium from supplements (including medication) were recoded and classified as 'current smoking' (n = 37) and 'no' aspirin (n = 813) and 'no' supplements (n = 2) to reduce the risk of bias due to under-reporting. Therefore 4443 participants remained for analysis in this study.

2.2. Anthropometric measures

At baseline participants attended a health check, which took place either at a clinic or the participant's GP surgery, where a number of anthropometric measurements were taken by trained staff according to standardised protocols [10]. This included height to the nearest mm, using a free-standing stadiometer. Weight was recorded to the nearest 0.2 kg with participants wearing light clothing and no shoes. From this measurement BMI was calculated. Waist and hip measurements were also recorded to the nearest mm [10].

2.3. Clinical and biological measures

BP was taken after participants had been seated for 3 min. Two readings were taken using Accutorr Sphygmomanometer (Datascope, UK) with the participants arm in the horizontal position in line with the mid-sternum [10]. At the clinic visit, a non-fasting venous blood (42 ml) sample was taken from which biochemical analysis for serum cholesterol was conducted [10].

Stroke cases were defined as ICD-9 430–448 or ICD-10 60–69. Fatal and non-fatal stroke incidence was established using death certificate data and linkage with hospital records, using ICD-10 60–69 this method of stroke ascertainment has been shown to have high sensitivity and specificity [11]. The current study is based on follow-up to 31st March 2008. Numbers of stroke are given in the tables.

2.4. Lifestyle factors

Information was obtained from participants on a number of lifestyle variables via a Health and Lifestyle Questionnaire (HLQ). This included smoking status which was categorised as current, if participants answered "yes" to the question "Do you smoke cigarettes now?", never if they answered "no" to the question "Have you ever smoked as much as one cigarette a day for as long as a year". All other participants with valid data were classified as former smokers (missing data were treated as 'current'). Physical activity was assessed by the use of a short physical activity questionnaire which assessed typical activity over the previous 12 months. Physical activity status took account of both work and leisure related activities and participants were ranked into one of four categories (inactive, moderately inactive, moderately active and active) [10]. The repeatability and validity of these was confirmed against heart-rate monitoring [12].

Education level was determined from the HLQ and was defined at the highest qualification obtained at that time. Participants were ranked into one of four categories: 'degree or equivalent', 'A-level or equivalent', 'O-level or equivalent', and 'less than O-level or equivalent'.

2.5. Previous medical history

The presence of a number of existing underlying medical conditions was ascertained using the HLQ. Conditions of interest included; stroke, cancer, myocardial infarction and diabetes amongst others. In conjunction with this, participants were requested to detail any medication that they were currently taking.

2.6. Dietary assessment method

Participants were requested to record all food and drink items consumed within the 7 day period in a food record diary. Included in each food diary were colour photographs of 17 foods, each with three incremental portion sizes. Participants were requested to indicate which photograph best represented their portion size for each of the items. They were also asked to record the weight of food items or use household measures to describe the portion size. The use of dietary supplements was also recorded within the diary, and data was input into a specifically designed program ViMiS (vitamin and mineral supplements) [13]. The 7-day diary was chosen after validation studies showed its reproducibility and relative validity and indicated that the diet diaries provide a more accurate representation of dietary intakes, over FFQs [10,14]. These studies indicate that FFQs overestimate dietary intakes of a number of food groups including fruit and vegetables, milk and cheese which influence the magnesium intake estimates.

A specific program, DINER (Data Into Nutrients for Epidemiological Research), was developed for entry of dietary information from the 7-day food diaries [15]. DINER allows the detailed information provided in diet diaries to be translated into structured data files for nutritional analysis. The program is more flexible than other software which enables the detail of the diary, including cooking method, type of fat used and commercial brand names of products, to be retained [16]. Due to the classification structure used to code food items DINER is also able to adapt to changes in food items available on the consumer market [15]. The input of items from the food diaries requires a high level of detail, which reduces the risk of bias between coders, and analysis of consistency has echoed this [15]. Nutritionists, trained to use the DINERMO program, checked the entered data after which nutrient quantities were calculated and checked for a final time [17].

The ratio of calcium to magnesium intake was calculated by dividing dietary calcium intake by dietary magnesium intake.

2.7. Statistical methods

All statistical analyses were conducted using the statistical software Stata; version 11 (StataCorp, College Station, TX, 2009). Continuous data are presented as mean with standard error and categorical data as number and percentage. A two-sided P-value of ≤0.05 was considered statistically significant. Independent sample *t*

test was used to assess differences in baseline characteristics between men and women.

To account for sex differences associated with a number of variables of interest including BMI and WHR sex specific analyses were conducted.

Multiple regression analysis with multivariate adjustment was employed to assess differences in SBP, DBP and total cholesterol TC with sex specific quintiles of dietary magnesium intake.

2.7.1. Statistical models

Model 1 comprised of age, BMI, smoking, physical activity (PA) levels, education (all outcomes); use of antihypertensive medications (BP only); baseline reported myocardial infarction (MI) or diabetes, family history of stroke or MI, and use of statin medication (TC only) [2,18,19]. Model 2, additionally adjusted for dietary factors, including total energy intake in order to demonstrate the effect of magnesium intake independent of total caloric intake, as well as previous incident myocardial infarction (MI) or diabetes at baseline, family history of stroke and MI (BP model only). Alcohol intake, dietary potassium and sodium were included due to their associations with BP [2] and total fat (TC model only). The use of calcium supplements and the ratio of calcium to magnesium intake were included; these two ions antagonise each other, may compete during intestinal absorption and the Ca:Mg ratio may be important for total mortality and coronary heart disease [20].

For stroke risk, Model 1 comprised age, BMI, smoking, PA, education and alcohol intake. Model 2 additionally adjusted for serum TC, baseline reported MI or diabetes and family history of stroke or MI. Model 3 included the addition of SBP and DBP, use of aspirin medication >3 months, use of antihypertensive medication, the ratio of dietary Ca:Mg and the use of calcium and magnesium supplements.

A modified Prentice-weighted Cox regression analysis, for case–cohort studies, was used to calculate hazard ratios with 95% CIs for the risk of incident of stroke in association with dietary magnesium intake [21]. This modified method accounts for the potential overlap of participants with incident stroke and also randomly present in the representative sub-cohort. Analyses were conducted by sex-stratified data derived categories of magnesium intake with the lowest 10% of intakes (<214 mg/day and <180 mg/day for men and women), forming the reference category, and subsequent 30% groups of magnesium intake. This approach was taken as we hypothesised that the lowest risk of incident stroke would be in those with the highest dietary magnesium intakes.

Sensitivity analysis was conducted excluding those taking antihypertensive and statin medication respectively.

Total energy was not included as a covariate in cox regression analysis. This was for a number of reasons, including that in the cox regression we adjusted for classical risk factors for stroke and have previously adjusted for total energy in early BP analyses, which indicated that dietary magnesium intake has an effect on BP independently of total energy, specifically for men, and BP was included in cox regression analyses. Additionally with the inclusion of total energy there is potential for collinearity, as a number of covariates included in the model such as BMI, alcohol intake and physical activity are highly correlated with total energy intake. There is also the potential for over adjustment, and for these reasons we chose not to include total energy in the cox regression models.

3. Results

In the 4443 participants included in these analyses 45.0% were male, with an age range of 39–78 years. Mean BP was 140/85 (SD 18.5/11.5) and 136/82 (SD 19.5/11.4) mm Hg for males and females respectively (Table 1). There was a total of 928 incident strokes during follow-up (mean 9.58 years; total person years 42,556.5) between 1993 and 2008.

Men had significantly higher SBP, DBP and BMI and women had significantly higher TC levels (P for all < 0.001), and BMI (P = 0.01), but not family history of stroke or MI (P = 0.35 and 0.17 respectively), and antihypertensive or lipid lowering medication use (P = 0.89 and 0.34 respectively). This illustrates the need to conduct sex-stratified analyses.

Both men and women with the lowest 10% of dietary magnesium intake, compared with the remaining 90% of intakes, tended to be older (64 vs. 61 years, and 63 vs. 60 years for men and women respectively), had a higher percentage of current smokers (18.6% vs. 10.9% and 21.1% vs. 12.0% for men and women respectively), inactive people (42.7% vs. 31.0% and 47.4% vs. 31.2% for men and women respectively) and people taking antihypertensive medication (25.6% vs. 20.3% and 30.2% vs. 20.2% for men and women respectively). There was no substantial difference in BMI, use of statin or aspirin medication and MI or diabetes at baseline between lowest 10% of magnesium and remaining intakes. Across quintiles of dietary magnesium intake a significantly higher intake of fruit, vegetables and bread and cereals was seen in men and women (P < 0.001 for all).

In men but not women, there were inverse associations between dietary magnesium intake and SBP and DBP that remained significant after analysis that accounted for age, dietary sodium intake and use of aspirin or antihypertensive medication (Table 2). There were differences of −7 mm Hg and −3.8 mm Hg between Quintile-1 and

Table 1
Baseline characteristics by sex in 4443 men and women, aged 40–75 years in EPIC-Norfolk cohort (1993–1997).

	Men n = 2000	Women n = 2443	P-value[a]
Age (years)	61.1 (±9.53)	60.4 (±9.71)	0.02
BMI (kg/m$^{2)}$	26.5 (±3.18)	26.2 (±4.24)	<0.01
Family history stroke (%)	465 (23.3%)	601 (24.6%)	0.29
Family history MI (%)	720 (36.0%)	934 (38.2%)	0.13
Family history DM (%)	222 (11.1%)	305 (12.5%)	0.16
Blood pressure (mm Hg)			
SBP	140 (±18.5)	136 (±19.5)	<0.001
DBP	85.3 (±11.5)	81.8 (±11.4)	<0.001
PP	54.2 (±11.2)	54.0 (±11.4)	0.66
Antihypertensive use (%)	417 (20.9%)	516 (21.1%)	0.83
Aspirin use (%)	271 (13.6%)	197 (8.06%)	<0.001
Blood lipids (mmol/L)			
Total cholesterol	6.07 (±1.10)	6.36 (±1.22)	<0.001
Smoking (%)			
Current	234 (11.7%)	314 (12.9%)	<0.001
Former	1114 (55.7%)	774 (31.7%)	
Never	652 (32.6%)	1355 (55.5%)	
Physical activity (%)			
Inactive	644 (32.2%)	800 (32.8%)	<0.001
Moderately inactive	476 (23.8%)	790 (32.3%)	
Moderately active	440 (22.0%)	514 (21.0%)	
Active	440 (22.0%)	339 (13.9%)	
Education level (%)			
0 — No qualifications	667 (33.4%)	1086 (44.5%)	<0.001
1 — O-level or equivalent	165 (8.3%)	249 (10.2%)	
2 — A-level or equivalent	887 (44.4%)	822 (33.7%)	
3 — Degree or equivalent	281 (14.1%)	286 (11.7%)	
Dietary factors			
Total energy (kcal/day)	2218 (±505)	1685 (±384)	<0.001
Magnesium (mg/day)	318 (±92.0)	265 (±73.2)	<0.001
Ca:Mg ratio	2.93	2.93	0.96
Potassium (mg/day)	3423 (±819)	2962 (±683)	<0.001
Alcohol (g/day)	15.9 (±20.8)	7.70 (±11.7)	<0.001
Sodium (mg/day)	3150 (±864)	2405 (±660)	<0.001
Calcium supplement use (%)	34 (1.70%)	160 (6.55%)	<0.001
Magnesium supplement use (%)	22 (1.10%)	53 (2.17%)	<0.01

Values are mean and standard deviations were continuous and number and percentage were categorical.

[a] P-value difference between males and females.

Quintile-5 in SBP and DBP (P ≤ 0.01 and P = 0.01 respectively). In women there were no significant associations between dietary magnesium intake and SBP or DBP (Table 2).

Significant inverse associations between dietary magnesium intakes and TC were identified for both genders (P = 0.02 in men and P = 0.04 in women) after adjustment for anthropometric and lifestyle factors (Table 2). However, these associations were attenuated with the addition of dietary factors; alcohol intake, total fat intake, ratio of Ca:Mg, total energy and calcium supplement intake to the multivariate model but remained significant (P = 0.02 in men and P = 0.04 in women) (Table 2).

Sensitivity analysis excluding those on antihypertensive medication (n = 1583 men and 1927 women) or statin medication (n = 1973 men and 2400 women) provided similar results.

Stroke risk showed a non-significant inverse trend across quintiles of dietary magnesium intakes in men and women after adjustment (Table 3). In further analyses examining magnesium intake by categories there was a significant trend across categories in men. In those in the highest 30th percentile of dietary magnesium intake (Table 4), in men, but not in women (Table 4), there was a 41% relative reduction of stroke risk (HR 0.59; 95% CI 0.38–0.93 (P = 0.04)) compared to the lowest 10% of magnesium intakes. Although stroke risk was also lower in women this was not significant.

Sensitivity analyses excluding those taking antihypertensive medication attenuated the association of stroke risk in men to be non-significant and strengthened the association in women to be significant and separately excluding those taking statin medication attenuated the association in men to be non-significant.

4. Discussion

The main findings of this case–cohort study of British adults suggest that, after adjustment for several important confounding factors including age, smoking status, history of CVD, medication use, total energy intake and other dietary variables, there was a strongly significant association (P ≤ 0.01) between dietary magnesium intake and SBP and DBP in men, but not in women. There was also an association with TC in both men and women (P = 0.001 and P ≤ 0.01 respectively) which was attenuated but remained significant after adjustment for other dietary factors (P = 0.02 and P = 0.04 for men and women respectively). Furthermore in relation to stroke risk specifically, we identified a significant decrease in risk (HR 0.59; 95% CI 0.38–0.93 P = 0.04) in men with

Table 2
Association of quintiles of dietary magnesium intake (range and mean quintile intake) and blood pressure and total cholesterol (means and SE) in 4443 men and women, aged 40–75 years in EPIC-Norfolk cohort (1993–1997).

Men		Q1	Q2	Q3	Q4	Q5	P for trend
		85–242 mg 206 mg n = 400	243–284 mg 266 mg n = 400	285–328 mg 307 mg n = 400	329–385 mg 355 mg n = 400	386–829 456 mg n = 400	
SBP	Unadjusted	143 (±0.98)	140 (±0.97)	140 (±0.88)	139 (±0.90)	136 (±0.87)	<0.001
	Model 1[a]	140 (±0.87)	139 (±0.86)	140 (±0.86)	140 (±0.86)	139 (±0.87)	0.64
	Model 2[b]	143 (±1.16)	140 (±0.90)*[c]	140 (±0.85)*	138 (±0.89)**	136 (±1.18)***	0.002
DBP	Unadjusted	86.1 (±0.58)	85.9 (±0.60)	85.4 (±0.57)	85.2 (±0.56)	84.1 (±0.56)	0.008
	Model 1	85.4 (±0.57)	85.4 (±0.56)	85.3 (±0.56)	85.5 (±0.56)	85.0 (±0.57)	0.68
	Model 2	87.15 (±0.76)	86.1 (±0.59)	85.1 (±0.55)*	84.9 (±0.58)*	83.4 (±0.77)**	0.01
Women		Q1	Q2	Q3	Q4	Q5	
		48–204 mg 176 mg n = 489	205–240 mg 223 mg n = 489	241–274 mg 258 mg n = 489	275–319 mg 295 mg n = 489	320–692 mg 374 mg n = 488	
SBP	Unadjusted	140 (±0.90)	135 (±0.85)	137 (±0.93)	135 (±0.89)	133 (±0.81)	<0.001
	Model 1	136 (±0.79)	135 (±0.77)	137 (±0.77)	136 (±0.77)	135 (±0.78)	0.85
	Model 2	137 (±1.07)	135 (±0.82)	137 (±0.77)	135 (±0.81)	135 (±1.09)	0.45
DBP	Unadjusted	83.5 (±0.51)	81.5 (±0.50)	82.4 (±0.55)	80.9 (±0.53)	80.7 (±0.48)	<0.001
	Model 1	82.0 (±0.49)	81.3 (±0.48)	82.6 (±0.48)	81.4 (±0.48)	81.7 (±0.49)	0.71
	Model 2	82.5 (±0.67)	81.6 (±0.51)	82.5 (±0.48)	81.1 (±0.51)	81.2 (±0.68)	0.26
Men		Q1	Q2	Q3	Q4	Q5	
		85–242 mg 206 mg n = 400	243–284 mg 266 mg n = 400	285–328 mg 307 mg n = 400	329–385 mg 355 mg n = 400	386–829 456 mg n = 400	
Total cholesterol	Unadjusted	6.21 (±0.06)	6.16 (±0.06)	6.02 (±0.05)	6.05 (±0.06)	5.91 (±0.05)	<0.001
	Model 1[d]	6.17 (±0.06)	6.16 (±0.06)	6.03 (±0.05)	6.06 (±0.05)	5.93 (±0.06)**	0.001
	Model 2[e]	6.18 (±0.07)	6.16 (±0.06)	6.03 (±0.05)	6.06 (±0.06)	5.94 (±0.07)*	0.02
Women		Q1	Q2	Q3	Q4	Q5	
		48–204 mg 176 mg n = 489	205–240 mg 223 mg n = 489	241–274 mg 258 mg n = 489	275–319 mg 295 mg n = 489	320–692 mg 374 mg n = 488	
Total cholesterol	Unadjusted	6.67 (±0.06)	6.35 (±0.05)	6.28 (±0.05)	6.32 (±0.05)	6.16 (±0.05)	<0.001
	Model 1[c]	6.51 (±0.05)	6.33 (±0.05)*	6.31 (±0.05)*	6.36 (±0.05)*	6.26 (±0.05)**	0.005
	Model 2[d]	6.52 (±0.06)	6.34 (±0.05)*	6.31 (±0.05)*	6.35 (±0.05)	6.25 (±0.06)*	0.04

[a] Model 1: age, BMI, smoking status, physical activity, education level, antihypertensive medication use.
[b] Model 2: model 1 + baseline MI or diabetes, family history stroke, family history MI, alcohol intake, dietary sodium, potassium, ratio Ca:Mg, total energy and calcium supplement use (including contribution from medication).
[c] Model 1: age, BMI, smoking status, physical activity, education level, baseline MI or diabetes, family history stroke, family history MI, statin medication use.
[d] Model 2: model 1 + alcohol, dietary total fat intake, ratio Ca:Mg, total energy and calcium supplement use (including contribution from medication).
[e] P value for significance compared with Q1: * = P value ≤ 0.05, ** = P value ≤ 0.005, *** = P value ≤ 0.001.

Table 3
Quintiles of dietary magnesium intake (range and mean quintile intake) at baseline (1993–1997) and stroke risk (HR and 95% CI), follow-up March 2008, in 4443 men and women, aged 40–75 in EPIC-Norfolk cohort.

Men	Q1	Q2	Q3	Q4	Q5	P trend
	85–242 mg 206 mg n = 400	243–284 mg 266 mg n = 400	285–328 mg 307 mg n = 400	329–385 mg 355 mg n = 400	386–829 mg 456 mg n = 400	
Stroke events	126 (30.6)	111 (26.9)	93 (22.6)	85 (20.6)	75 (18.3)	
Model 1[a]	1.0 (reference)	0.85 (0.60–1.20)	0.70 (0.49–1.00)	0.86 (0.60–1.24)	0.80 (0.55–1.16)	0.22
Model 2[b]	1.0 (reference)	0.86 (0.60–1.20)	0.68 (0.47–0.99)	0.81 (0.56–1.17)	0.74 (0.50–1.09)	0.11
Model 3[c]	1.0 (reference)	0.87 (0.61–1.25)	0.73 (0.50–1.06)	0.80 (0.55–1.17)	0.81 (0.53–1.22)	0.21
Women	Q1	Q2	Q3	Q4	Q5	
	48–204 mg 176 mg n = 489	205–240 mg 223 mg n = 489	241–274 mg 258 mg n = 489	275–319 mg 295 mg n = 489	320–692 mg 374 mg n = 488	
Stroke events	152 (30.5)	102 (20.5)	87 (17.5)	82 (16.5)	88 (17.7)	
Model 1[a]	1.0 (reference)	0.74 (0.53–1.05)	0.74 (0.51–1.06)	0.84 (0.59–1.20)	0.83 (0.57–1.20)	0.39
Model 2[b]	1.0 (reference)	0.71 (0.50–1.01)	0.71 (0.49–1.03)	0.82 (0.57–1.17)	0.76 (0.52–1.11)	0.23
Model 3[c]	1.0 (reference)	0.72 (0.50–1.04)	0.73 (0.50–1.08)	0.86 (0.59–1.26)	0.82 (0.54–1.24)	0.45

[a] Model 1: age, BMI, education status, physical activity, smoking status, and alcohol intake.
[b] Model 2: model 1 + serum total cholesterol, baseline MI or diabetes, family history stroke, or MI.
[c] Model 3: model 2 + SBP, DBP, aspirin use >3 months, antihypertensive medication, ratio Ca:Mg and magnesium and calcium supplement use (including contribution from medication).

dietary magnesium intakes ≥354 mg/day (the highest 30%) compared to those with intakes ≤214 mg (the lowest 10%).

Compared with our findings, previous studies have shown a significant inverse association between dietary magnesium intake and SBP and DBP in men has also been reported [22] with differences of −6.4 mm Hg and −3.1 mm Hg for SBP and DBP respectively between those with the highest and lowest intakes in 615 older Japanese men (aged 63–82 years) using 24 hour recall. Using 7 day food diary data, a more robust measure of dietary intakes, in a representative general population of middle and older age we identified slightly greater differences in BP between extreme quintiles of magnesium intakes with a difference of −7 mm Hg ($P < 0.001$) and −3.8 mm Hg ($P < 0.001$) for SBP and DBP respectively. We identified between quintiles differences were 250 mg/day for men, and 198 mg/day for women, the equivalent to approximately 2 slices of wholemeal bread with peanut butter and 9 Brazil nuts, therefore achievable through dietary intakes [23]. We also noted a tendency towards lower fruit, vegetable and bread and cereal intake in those with the lowest dietary magnesium intakes which may be relevant for identifying individuals whom may benefit from increased intake.

In women a potential benefit from increased consumption of magnesium has previously been reported [24–26]. Witteman et al. [24] and Song et al. [25] showed a reduction in relative risk, RR 0.77, 95% CI 12%–33% and RR 0.93, 95% CI 0.86–1.02 respectively for developing hypertension in prospective studies using FFQs. A meta-analysis of RCTs using oral magnesium supplements also reported a dose dependent effect of supplementation on blood pressure [27]. This is in contrast to the current study where we did not find any significant trends between magnesium intake and BP in women. This discrepancy may be due to differences in the models used. For example Witteman et al. [24] did not adjust for lifestyle factors including physical activity levels and smoking status which are known to influence BP. We further

Table 4
Stroke risk (HR and 95% CI) by magnesium groups (range and mean intake), bottom 10% (Group 1 reference category) and 3 groups of 30% intakes each, in 4443 men and women, aged 40–75 in EPIC-Norfolk cohort.

Men	Group 1	Group 2	Group 3	Group 4	P trend
	85–214 mg 181 mg n = 199	215–285 mg 254 mg n = 605	286–353 mg 318 mg n = 591	354–828 mg 427 mg n = 605	
Stroke events	65 (32.7%)	157 (26.0%)	123 (20.8%)	104 (17.2%)	
Model 1[a]	1.00	0.73 (0.50–1.07)	0.63 (0.43–0.94)*	0.67 (0.44–1.01)*	0.07
Model 2[b]	1.00	0.72 (0.48–1.07)	0.61 (0.41–0.92)*	0.61 (0.40–0.94)*	0.03
Model 3[c]	1.00	0.67 (0.45–1.01)*	0.60 (0.40–0.90)*	0.59 (0.38–0.93)*	0.04
Women	Group 1	Group 2	Group 3	Group 4	
	48–180 mg 156 mg n = 232	181–240 mg 213 mg n = 745	241–294 mg 267 mg n = 740	295–691 mg 352 mg n = 726	
Stroke events	73 (31.5%)	165 (22.2%)	126 (17.0%)	115 (15.8%)	
Model 1[a]	1.00	0.73 (0.49–1.08)	0.70 (0.46–1.06)	0.74 (0.48–1.12)	0.27
Model 2[b]	1.00	0.67 (0.45–1.00)*	0.65 (0.43–0.98)*	0.66 (0.43–1.02)	0.14
Model 3[c]	1.00	0.65 (0.43–0.99)*	0.65 (0.42–1.01)	0.69 (0.44–1.09)	0.27

[a] Model 1: age, BMI, education status, physical activity, smoking status, alcohol intake.
[b] Model 2: model 1 + serum total cholesterol, baseline MI or diabetes, family history stroke, or MI.
[c] Model 3: model 2 + SBP, DBP, aspirin use >3 months, antihypertensive medication, ratio Ca:Mg and magnesium and calcium supplement use (including contribution from medication).
* P value for significance compared with reference (Group 1): P value ≤ 0.05.

explored why we might have identified differences between genders. It may be due to the fact that older age, higher BMI, and higher levels of physical inactivity were more prevalent in women with low magnesium intakes, which may in part explain why a significant effect was shown in men but not women. As highlighted in the results section differences were identified between those with the lowest 10% of intakes and those with higher intakes, however, we took these factors into account during our analyses. It is also of note that differences identified were largely the same for both men and women, although there was a higher percentage of women, with the lowest 10% magnesium intakes, using antihypertensive medication which may influence the findings due to modifying effect of medication on future risk. In addition there was a narrower range of magnesium intakes for women (644 mg/day) compared with men (744 mg/day) which may attenuate the results. It may also be that the cohort was insufficiently powered to detect an affect in women.

A number of intervention trials, using oral magnesium supplements, have reported significant reductions in BP ranging from 2.0–12.0 to 2.7–8.0 mm Hg for SBP and DBP respectively [28–32]. Although, the supplement doses were comparable with dietary intake, ranging between 200 mg/day to 600 mg/day, the formulations used were inconsistent.

Limited studies have previously investigated associations between dietary magnesium intake and TC or subfractions and two previous studies found no association with TC unlike our study which found that TC was ≈4% lower in Quintile-5 compared with Quintile-1 [33–36]. However, a higher magnesium intake has been related to beneficial increases in high density lipoprotein (HDL) concentrations [33,35].

Although several studies have previously investigated stroke risk and dietary magnesium intakes, to our knowledge none have included populations of both men and women simultaneously or included risk factors as well as stroke risk [26,33–35,37–42]. Previous studies in large populations of American, Taiwanese and Northern European cohorts, which have mainly used FFQs, have reported no associations [26,33,34,38,40–42]. However, several studies found significant associations in men [35], women [43], and men and women [40]. Additionally, a meta-analysis by Larsson et al. (2012) [9], in 241,378 people, reported an inverse association between dietary magnesium intake, recorded by FFQ, and risk of stroke. A more recent meta-analysis, of CVD, but not stroke specifically, by Del Gobbo et al. [8] in 313,041 participants, concluded that there was no significant association between dietary magnesium intake and CVD. However, a significant inverse association was identified in relation to circulating magnesium and CVD incidence potentially indicating mechanisms that could also affect stroke risk. In addition Guasch-Ferré et al. [44] recently reported that an increase in magnesium intake was associated with a decrease in both CVD mortality and total mortality in individuals at high CVD risk. Our results indicated a non-significant trend across quintiles of dietary magnesium intake and stroke risk in men and women (Table 3). However, when we compared men with the lowest 10% of magnesium intake with the remainder of the cohort, we identified a significant inverse trend ($P = 0.04$) across groups in men only (Table 4). This finding would suggest that it is the very lowest magnesium intakes that may infer the greatest risk of stroke incidence, and the current findings suggest an association between lower dietary magnesium intake and higher stroke risk.

4.1. Strengths and limitations

The strengths of the present analyses include; the size of the cohort, the representativeness of the UK general population and prospective design for the stroke analyses, which reduces the susceptibility of the study to selection bias. The study design also reduces the likelihood of measurement error, due to the recording of dietary intake at baseline prior to the onset of stroke. Additionally robust and systematic adjustment for a number of potential confounding factors allowed for the identification of dietary associations independent of known risk factors. It is possible that factors not included in the model may also influence associations such as medication use; proton pump inhibitors, and diuretics. The use of quantitative 7-day food diaries is likely to have provided a more accurate representation of micronutrient intakes, including magnesium intake, compared with FFQ and 24-hour recall methods [45]. To our knowledge our study is the only one to use dietary intake values from 7-day diaries as opposed to estimates from FFQs. Seven day food diaries have been shown to more accurately represent dietary intakes of a number of food groups that contribute to magnesium intake, including fruit and vegetables, and micronutrient intakes including potassium, carotene and vitamin C in validation studies [14,45,46]. Despite this, dietary intakes do not account for variation in bioavailability and absorption of magnesium, potentially the use of a biomarker would strengthen the findings [47] and we were also unable to take into account possible contributions of magnesium from drinking water [48]. Furthermore in the same population we were able to examine the complex relationship between magnesium intake, BP and cholesterol and stroke risk taking into account potential relevant risk specific confounders.

Selection bias may be possible although the whole EPIC-Norfolk cohort was representative of the UK population, with comparable cohort characteristics, and the sub-cohort for these analyses was representative of the EPIC-Norfolk cohort. Furthermore, truncation of the sample distribution due to potential healthy responder bias would likely only attenuate the observed associations and therefore associations may actually be stronger than are presented. It should also be noted that, as with other cross-sectional and observational longitudinal studies, it is not possible to infer causation from these findings. However, the prospective relationship observed with dietary magnesium intake and stroke risk reduces the likelihood of reverse causality. Furthermore the associations were in agreement with the existing literature. Residual confounding is possible but, the likelihood is reduced due to previous validation of dietary methods and results of the EPIC-Norfolk cohort [46,49].

4.2. Summary

To our knowledge this is the first study to investigate the association of dietary magnesium with BP, TC and stroke risk in a UK general population of both genders. The results suggest that increased dietary magnesium could positively impact on BP and stroke risk in men and total cholesterol levels in both genders. Our findings suggest that men with the lowest magnesium intakes are at the greatest risk of stroke, lower magnesium intake was also associated with higher blood pressure. Therefore a higher dietary magnesium intake may be beneficial for prevention of stroke in men and warrants further investigation.

Author contributions

Contribution of each author: The research question was formulated by AAW, PKM and LKMB who also analysed the data and wrote the manuscript. KTK and NJW are principal investigators of the EPIC-Norfolk. The data collection was organised by AAW, RNL. RNL performed the record linkage. MAHL obtained data from both food and supplement sources using the 7-day diet diaries. All authors contributed to the manuscript and commented on the final version.

Conflict of interest

No authors declare a conflict of interest.

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