Investigating the impact of nutrition and body composition on mortality risk, diseases of ageing and inflammation in the EPIC-Norfolk cohort

Angela Mulligan

Registration number: 100425752

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Norwich Medical School,

University of East Anglia,

Norwich, England

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Primary supervisor: Professor Ailsa Welch

Secondary supervisor: Dr Marleen Lentjes

Abstract

In England, heart disease, cancer and respiratory diseases are currently among the top five causes of mortality. Additionally, the number of older people is growing, many of whom suffer from a poor quality of life, often associated with the presence of multiple long-term conditions (MLTCs), and potentially linked to age-related inflammation.

I investigated the impact of diet and changes in body composition on mortality risk, chronic conditions and inflammation, to ascertain how these lifestyle factors could benefit the ageing process. I used anthropometric, biochemical and dietary data from the Norfolk centre of the European Prospective Investigation into Cancer and Nutrition (EPIC-Norfolk), a general population-based cohort of more than 25,000 middle- and older-aged men and women, with a long follow-up on measures of exposure and disease outcomes. I carried out research where there were conflicting findings in the existing literature and for previously unexplored hypotheses.

I researched the effects of (1) changes in weight and (2) waist circumference on mortality risk, and (3) associations of dietary intakes and plasma concentrations of vitamin E with measures of skeletal muscle mass, bone density status and fracture risk. I also (4) validated the Dietary Inflammatory Index against C-reactive protein (CRP) and several nutritional biomarkers, and additionally assessed if a more pro-inflammatory diet was associated with having MLTCs.

Results showed that weight loss, but conversely waist circumference gain were significantly associated with higher mortality risk; higher intakes and blood concentrations of vitamin E were protective for musculoskeletal health; and a more anti-inflammatory diet was significantly associated with higher odds of having MLTCs, though a lower concentration of CRP and higher circulating vitamin concentrations.

Taken together, this research provides a valuable contribution to understanding the impact of nutrition and body composition on mortality risk, diseases of ageing and inflammation.

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List of abbreviations

1HE	First health examination
2HE	Second health examination
24hDR	24-hour diet recall
7dDD	7-day diet diary
BMI	Body mass index
BUA	Broadband ultrasound attenuation
CI	Confidence interval
CRP	C-reactive protein
CVD	Cardiovascular disease
DII®	Dietary Inflammatory Index
DINER	Data Into Nutrients for Epidemiological Research
EFSA	European food safety authority
EPIC	European Prospective Investigation into Cancer and Nutrition
FETA	FFQ EPIC Tool for Analysis
FFM	Fat-free mass
FFQ	Food frequency questionnaire
HLQ	Health and lifestyle questionnaire
HR	Hazard ratio
hs-CRP	High-sensitivity C-reactive protein
IHD	Ischaemic heart disease
ISCED	International Standard Classification of Education
MLTCs	Multiple long-term conditions
MUFAs	Monounsaturated fatty acids
NDNS	National Diet and Nutrition Survey
OADR	Old-age dependency ratio
OR	Odds ratio
PUFAs	Polyunsaturated fatty acids
QOF	Quality and Outcomes Framework

RNI	Reference nutrient intake
SMM	Skeletal muscle mass
SO	Sarcopenic obesity
ViMiS	Vitamin and mineral supplement
WC	Waist circumference
WHO	World Health Organization
Wt	Weight

Chapter 1. Introduction

1.1 The potential for diet to impact on ageing

The World Health Organization (WHO) states that the consumption of a healthy diet throughout the life-course helps to prevent a number of non-communicable diseases including diabetes, heart disease, stroke and cancer (1). However, research shows that most people in the UK do not meet government dietary recommendations. Data from the most recent UK National Diet and Nutrition Survey (NDNS) shows that intakes of saturated fat, sugar, and salt are above the government recommendations whereas intakes of fibre, oily fish, and fruit and vegetables, are below government recommendations (2).

My research has focused on each of the four principal methods used in the assessment of nutritional status, from the classically used acronym, ABCD: Anthropometry, Biological markers, Clinical symptoms and Dietary intake. A review by Hu (3) summarised the recent findings on dietary-related components that have an effect on chronic conditions, healthy ageing and longevity, which link with ABCD. He highlights five important factors, which are mainly based on observations from large cohort studies. These include (1) maintaining a healthy weight throughout the lifespan; (2) consumption of macronutrients from high-quality sources; (3) adherence to some traditional diets and dietary patterns, focussing on the consumption of minimally processed plant foods and healthy fats in combination with a low intake of red and processed meats and added sugars; (4) consumption of plant foods rich in phytonutrients such as polyphenols; and (5) adherence to a healthy lifestyle, to include not smoking and taking regular exercise, and maintaining a healthy weight, as noted previously, as well as moderate alcohol consumption. Research using prospective data from the Norfolk cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Norfolk) has estimated the combined impact of four behaviours, two dietary-related (moderate alcohol intake and at least five servings of fruit and vegetables a day) and two lifestyle-related (not smoking and not being physically inactive), amounts to 14 additional years of life (4). This study illustrates that modest and achievable changes in lifestyle could have a considerable effect on the health of populations.

The research presented in this thesis addresses the themes within Hu's paper (3) regarding the effects of dietary-related components on ageing, namely the importance of maintaining a healthy weight and the consumption of a healthy diet, in the form of predominantly plant-based, anti-inflammatory foods and nutrients, which are components of the Dietary Inflammatory Index (DII®). I have used anthropometric, biochemical, clinical and dietary data in my research to ascertain how the aforementioned lifestyle factors could benefit the ageing process.

1.2 Age distribution in the UK and relevance to the development of chronic diseases

Worldwide and within the UK, the number of older people is growing. In the UK, those aged 85 years and over was estimated to be 1.6 million in 2021 (2.5% of the UK population); by 2036, this is projected to increase to 2.6 million (3.5% of the UK population) (5). The population pyramid in Figure 1 below shows the age structure of the population in mid-2020 and the projected age structure in mid-2030 (6).

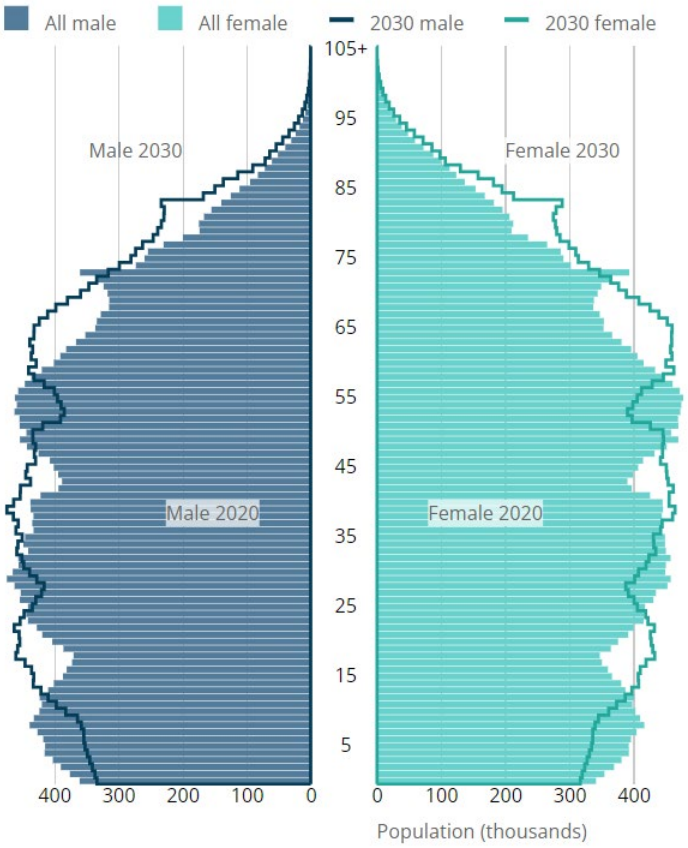


Figure 1 Age distribution of the UK population at mid-2020 and mid-2030 (6)

The UK's age structure is shifting towards older ages due to declining fertility rates and people living longer. The old-age dependency ratio (OADR) is a traditional way of assessing the impact of an ageing population. This is a simple ratio of the number of people of pensionable age and over (aged ≥ 65 years) per 1,000 people who are aged 16 years to State Pension age. The UK OADR during 2020 was 280 and is projected to be 352 by 2041 (7).

Figure 2 below, taken from the Chief Medical Officer's Annual Report of 2023 (8), using data from the Global Burden of Disease Study 2019 (9), illustrates that people have more morbidity and disability in old age (8) and the prevalence of individual chronic conditions is increasing (10). The main aim of prevention and treatment for an older-aged population, as stated in this report, should be to reduce the period spent living with ill health by delaying the onset of disease, thereby increasing people's independence in older age and decreasing their period of dependence on others.

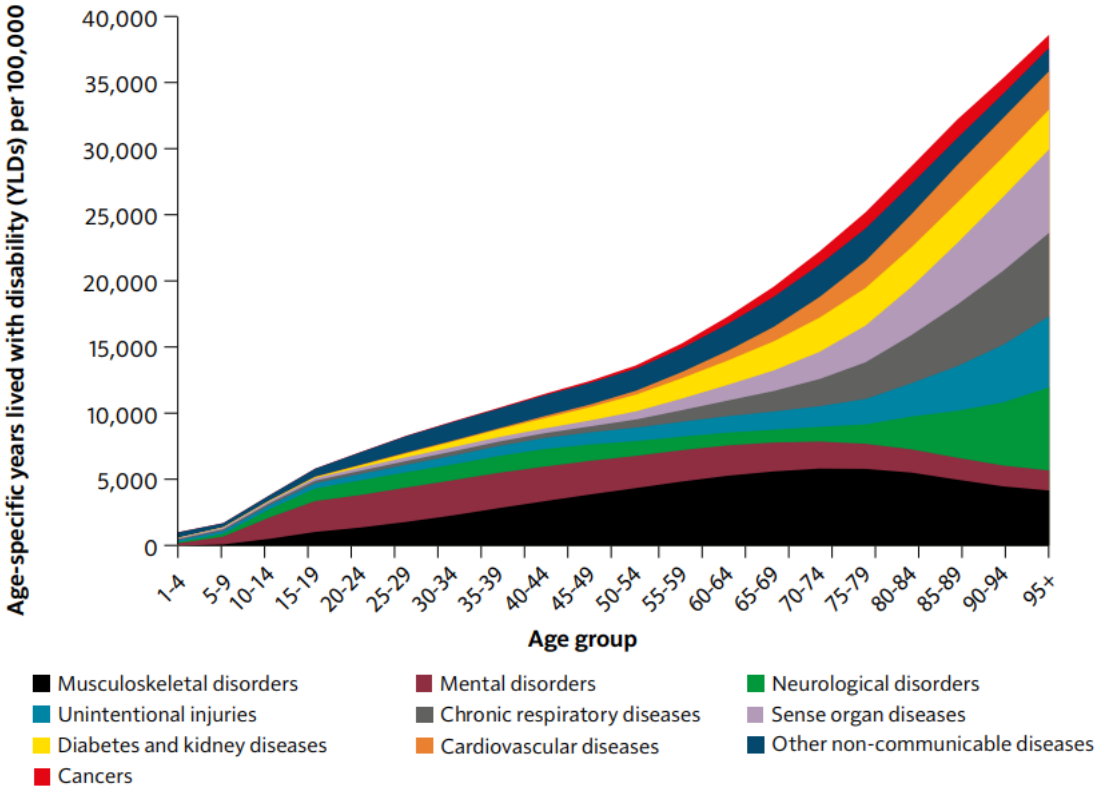


Figure 2 Age-specific years lived with disability (YLDs) for the ten main causes of morbidity by age group in England in 2019 (8).

1.3 Chronic diseases and multiple long-term conditions

In the UK, life expectancy at birth in 2020 to 2022 was 78.8 years for males and 82.8 years for females (11). Although people are living longer, their quality of life in later years is not always good, which may be a result of poor nutrition and/or a lack of physical activity. Due to differences in data sources of the prevalence of chronic diseases, I am citing data from two recent papers. Valabhji *et al.* used data on individuals registered with a GP surgery in England, who were alive on 31st March 2020 (12); in Figure 3 below, I have shown the prevalence of the 35 conditions in adults that the researchers included.

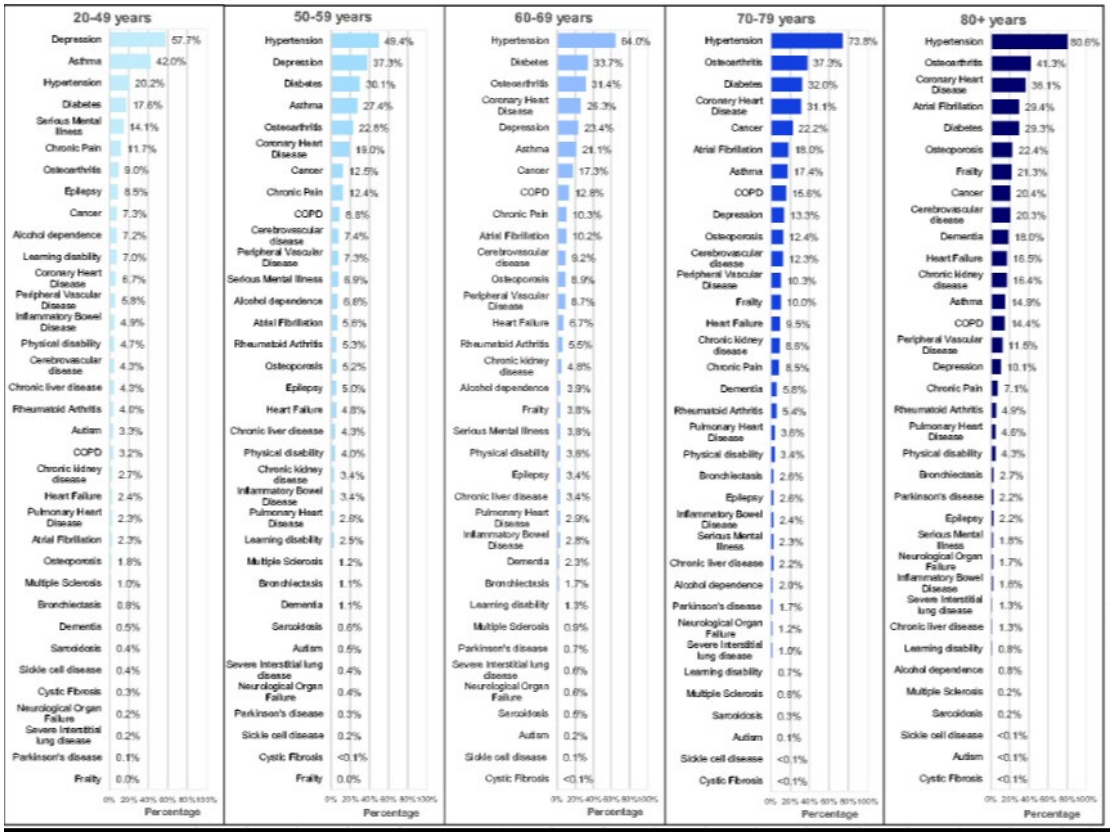
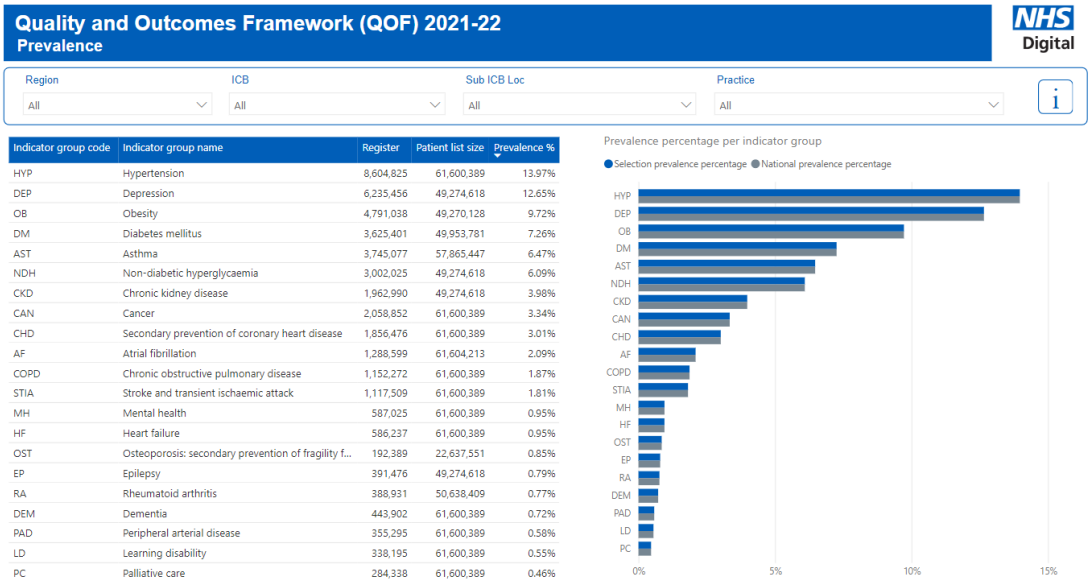


Figure 3 Prevalence of the 35 long-term conditions in adults with MLTCs, by age group (12)

The Quality and Outcomes Framework (QOF) is a voluntary annual reward and incentive program for all GP surgeries in England, detailing practice achievement results and is a useful source of prevalence data for several chronic conditions. Figure 4 shows the prevalence of several conditions in 2021-2022 (13).



Indicator group code	Indicator group name	Register	Patient list size	Prevalence %
HYP	Hypertension	8,604,825	61,600,389	13.97%
DEP	Depression	6,235,456	49,274,618	12.65%
OB	Obesity	4,791,038	49,270,128	9.72%
DM	Diabetes mellitus	3,625,401	49,953,781	7.26%
AST	Asthma	3,745,077	57,865,447	6.47%
NDH	Non-diabetic hyperglycaemia	3,002,025	49,274,618	6.09%
CKD	Chronic kidney disease	1,962,990	49,274,618	3.98%
CAN	Cancer	2,058,852	61,600,389	3.34%
CHD	Secondary prevention of coronary heart disease	1,856,476	61,600,389	3.01%
AF	Atrial fibrillation	1,288,599	61,604,213	2.09%
COPD	Chronic obstructive pulmonary disease	1,152,272	61,600,389	1.87%
STIA	Stroke and transient ischaemic attack	1,117,509	61,600,389	1.81%
MH	Mental health	587,025	61,600,389	0.95%
HF	Heart failure	586,237	61,600,389	0.95%
OST	Osteoporosis: secondary prevention of fragility f...	192,389	22,637,551	0.85%
EP	Epilepsy	391,476	49,274,618	0.79%
RA	Rheumatoid arthritis	388,931	50,638,409	0.77%
DEM	Dementia	443,902	61,600,389	0.72%
PAD	Peripheral arterial disease	355,295	61,600,389	0.58%
LD	Learning disability	338,195	61,600,389	0.55%
PC	Palliative care	284,338	61,600,389	0.46%

Figure 4 Prevalence of single conditions reported by GP surgeries in England in 2021 - 2022(13)

However, as people grow older, they often suffer from more than one chronic condition. Work to understand combinations of individual chronic diseases that form clusters of multiple long-term conditions is relatively recent. The National Institute for Health and Care Excellence (NICE) defines multiple long-term conditions (MLTCs), or multi-morbidity, as the presence of two or more long-term health conditions in a person (14). I will be using the term MLTCs throughout my thesis. These conditions can include defined physical or mental health conditions, such as diabetes or schizophrenia; ongoing conditions, such as a learning disability; symptom complexes, such as frailty or chronic pain; sensory impairment, such as sight or hearing loss; alcohol or substance misuse (14).

Using data for England, in 2015, 54.0% of people aged 65 and over suffered from MLTCs; by 2035 this is predicted to rise to 67.8% (15); the prevalence of MLTCs in 2035 was predicted at 52.8% for people aged 65-74, 75.9% for those aged 75-84, and 90.5% for those aged 85 and over. Kingston *et al.* estimate that there will be 17.0% of people aged 65 and over living with four or more conditions in 2035, compared with 9.8% in 2015; people aged 75 and over will make the greatest contribution to this number (15); most people aged 65 and over will be affected by arthritis (62.6%), hypertension (55.9%), respiratory disease (24.4%), cancer (23.7%) and diabetes (21.6%). Valabhji *et al.* (12) used data on individuals registered with a GP surgery in England to provide information on the most common combinations of MLTCs, where

the combinations may occur either uniquely or together with one or more additional condition; asthma and depression were the most common combination for those aged 20–49 years, with a prevalence of 24.8%. For those aged 50–59 years and 60–69 years, diabetes with hypertension was the most common combination, with prevalences of 18.8% and 24.8%, respectively. Hypertension and osteoarthritis was the most common combination in those aged 70–79 years and 80+ years, with prevalences of 27.3% and 33.6%, respectively.

As noted earlier, classifying MLTC is a relatively new field and understanding how to develop classifications is complex, as it is difficult to decide upon what conditions to include, which is in turn dependent upon data availability. In my recent work that forms part of this thesis, in the creation of the MLTC score, I included the most prevalent chronic conditions from the QOF 2021 – 2022 report (13) and those from the study carried out by Valabhji *et al.* (12), where data were available from the EPIC-Norfolk study. I included obesity as one of the conditions, which is associated with several chronic conditions (16,17), as is being overweight, and both are major risk factors for global mortality (18,19). It has been estimated that worldwide, at least 2.8 million people die annually because of being overweight or obese (20). Adult obesity has more than doubled throughout the world since 1990 (21). Bell and colleagues have estimated the annual cost to the UK of people living with obesity at more than £74 billion, with individuals responsible for more than 53%, as a result of a reduction in their years of quality of life, and their families and friends, through informal social care. Cost to the NHS is 11%, and the wider society deals with more than 9% of the cost, as a result of lower productivity (22). Work presented within this thesis relates to weight change and mortality risk.

Figure 5 shows the percentage of adults by weight classification. In England in 2021, 26% of all adults were categorised as obese (23). A high waist circumference (WC), indicating abdominal/central obesity or visceral adiposity, is also a risk factor for developing a number of chronic conditions, including cardiovascular disease (CVD), hypertension and type 2 diabetes mellitus (24). The work presented in my thesis also aims to understand the relevance of changes in WC on the risk of all-cause and CVD mortality.

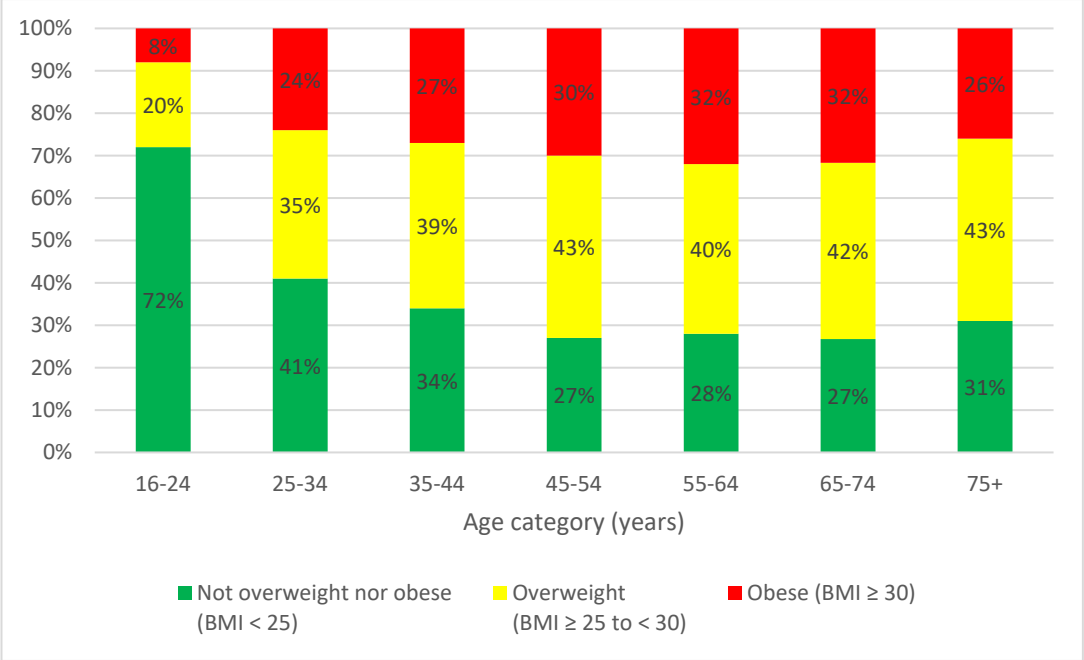


Figure 5 Percentage of adults who were not overweight nor obese, overweight or obese in England in 2021, by age category (23)

A recent systematic review of 17 studies of adults in the UK, aged 18 – 90+ years [23] suggests that MLTCs are associated with increased healthcare costs and utilisation and that patients with four or more conditions have more than 14 times the odds of having an unplanned and possibly preventable hospitalisation, independent of age, than those without comorbidities (25). The economic costs associated with MLTCs provides further motivation for focussing efforts on delaying the onset of chronic diseases.

1.4 Main causes of deaths in England

Mortality data from the Office for National Statistics for 2022 show that the top five leading causes of death in England were (1) dementia and Alzheimer's disease, (2) ischaemic heart diseases, (3) chronic lower respiratory diseases, (4) cerebrovascular diseases and (5) malignant neoplasm of trachea, bronchus and lung (26). Ischaemic heart disease was the leading cause of death in men, accounting for 13.2% of all male deaths whereas dementia and Alzheimer's disease was the leading cause of death in women (15.1% of all female deaths) (5). The main causes of death in men and women for selected age groups are shown in Table 1.

Table 1 Main causes of death by sex and older age groups, using 2022 death registration data for England (26)

Age group	Men	Women
35 – 49 years	Accidental poisoning	Malignant neoplasm of breast
50 – 64 years	Ischaemic heart diseases	Malignant neoplasm of breast
65 – 79 years	Ischaemic heart diseases	Malignant neoplasm of trachea, bronchus and lung
≥ 80 years	Dementia and Alzheimer's	Dementia and Alzheimer's

1.5 Weight changes, anthropometry and ageing

Studies have reported that weight loss, compared to maintaining a stable weight, is associated with a higher mortality risk, especially in middle-aged and older adults (27–33), although some of these studies did observe that weight gain was also associated with a higher mortality risk (27,29). A systematic review and meta-analysis of 26 papers by Karahalios *et al.* observed that weight loss was significantly associated with a higher risk of all-cause (pooled hazard ratio (HR): 1.45; 95% confidence interval (CI): 1.34 - 1.58) and CVD mortality (1.50; 1.32 - 1.70); weight gain was also significantly associated with a higher risk of CVD (1.21; 1.07 - 1.36) and all-cause mortality (1.07; 1.01 - 1.13) (34). However, the authors noted that substantial heterogeneity existed, mainly due to the method used to determine body size and the percentage of baseline participants available in the final analysis.

Although body mass index (BMI) is commonly used to define overweight and obesity, it does not take fat mass distribution into account. As body composition changes with age, it is important to be able to assess the impacts of these changes, using alternative measures. WC strongly correlates with central or abdominal obesity and is a commonly used clinical measure of body fat distribution (35,36).

Studies have shown that a higher WC is associated with higher CVD risk (37–39) and CVD mortality (40). WC has also been positively associated with all-cause mortality in several studies (41–47) with a few exceptions (48,49). However, data are limited and conflicting with regard to the effects on mortality of changes in body fat distribution. De Hollander *et al.* found that a decrease in WC was associated with a HR of 1.52 (95% CI: 1.01 – 2.31) for all-cause mortality in older adults, aged 70 – 77 years (50). Findings from the Melbourne Collaborative Cohort Study of men and women aged 40–69 years at baseline found that a loss in WC over approximately 12

years was associated with an increased risk of all-cause mortality (HR: 1.26; 1.09 – 1.47), particularly in older adults, compared to those who had minimal changes (51). Changes in WC were positively associated with all-cause mortality (HR per 5 cm for both sexes was 1.09 (1.02 - 1.16)) in healthy middle-aged men and women throughout the range of concurrent changes in BMI (52). An increase in WC over six years in middle-aged Danish and Swedish women was associated with both all-cause and CVD mortality (53). As changes in weight are accompanied by changes in fat distribution, studying the association of these changes with mortality in an ageing population seems justifiable and I have therefore included this area of research as part of my thesis.

Weight and weight change are also factors that are included in nutritional status assessment, such as sarcopenia and frailty. Sarcopenia is a relatively newly diagnosed chronic condition, characterised by the loss of muscle mass, strength and function with increasing age. There is currently no international agreement on the definition of sarcopenia. However, the Global Leadership Initiative in Sarcopenia recently established that the global conceptual definition of sarcopenia should include muscle mass, strength and muscle-specific strength as components (54). With such a definition, the committee also agreed that the prevalence of sarcopenia becomes higher with age, increasing the risk of impaired physical performance (54). Losses in bone density, skeletal muscle mass (SMM) and strength occur gradually from the age 30 years, with increasing rates of loss in those over the age of 60 years (55,56). Lower SMM is a risk factor for sarcopenia, and it is also related to osteoporosis and fracture risk. Therefore, as the percentage of older people in our society grows, so does the prevalence of sarcopenia, frailty and fractures. A relative or absolute increase in body fat can accompany the age-related loss of SMM, which can result in sarcopenic obesity (SO). The European Society for Clinical Nutrition and Metabolism and the European Association for the Study of Obesity recently recommended that SO is defined as the concurrence of excess adiposity and low muscle mass/function (57).

1.6 Vitamin E, weight loss and sarcopenia

Weight loss is associated with sarcopenia, and it is therefore possible that intakes and concentrations of vitamin E would also potentially decrease with weight

loss, through a reduction in dietary intake. Vitamin E is a fat-soluble vitamin, with both antioxidant and anti-inflammatory potential, and as skeletal muscle is the organ with the highest consumption of oxygen in the body, it is feasible that this vitamin might have the potential to offer some protection regarding musculoskeletal health in middle-aged and older adults.

A study by Hamulka *et al.* (58) found that a 6-week weight loss program in overweight and obese adults resulted in significant decreases in fat intake, weight, fat mass (FM), WC and α-tocopherol concentrations, although no significant changes in α-tocopherol intakes were observed. Average intake of α-tocopherol for approximately 90% of the study participants was inadequate (< 12 mg/day) at baseline and for 83% at the end of weight loss program while low α-tocopherol status (< 20 µmol/L) was found in 60% of the women and 63% of the men at baseline, increasing to 78% and 68% after 6 weeks, respectively. The researchers concluded that there was an increased risk of developing diseases caused by oxidative stress in these overweight and obese adults, who had a low baseline α-tocopherol status, as a result of their reduced calorie diet. However, this was a small study of 60 participants and further larger studies are required to confirm these findings, in addition to investigating changes in other fat-soluble vitamins, before recommending that long-term reduced calorie diets for obese adults need to be monitored to avoid α-tocopherol deficiency and considering higher dietary α-tocopherol requirements for obese people.

1.7 Diet, inflammation, ageing and MLTCs

The prevalence of MLTCs in ageing populations is increasing. 'Inflamm-aging' is used to characterise chronic low-grade inflammation that typically occurs with increasing age (59). The development of lifestyle-related chronic diseases such as cancer (60), CVD (61), diabetes (62), musculoskeletal conditions (63) and depression (64) has been shown to be promoted by low-grade chronic inflammation. Dietary components are one of the key factors affecting an individual's inflammatory status (65). The DII® is a literature-based dietary score that was developed to measure the potential impact of a diet on the inflammatory status of an individual (66). Research into the potential beneficial associations of consuming an anti-inflammatory diet and

the prevalence of chronic conditions continues, with most studies focusing on individual diseases. However, as low-grade chronic inflammation is related to several chronic diseases that contribute to MLTCs, further research into the potential associations between the consumption of an inflammatory diet and MLTCs seems worthy of investigation. Despite this knowledge, I was unable to find any articles that had investigated the relationship of an inflammatory diet and MLTCs and so I embarked on research into this area, which is included in my thesis.

1.8 Aim and objectives

The overall aim of this thesis was to assess the impact of diet and changes in body composition on mortality risk, chronic conditions and inflammation in community-dwelling, middle-aged and older men and women, participating in the EPIC-Norfolk cohort study, to ascertain how these lifestyle factors could benefit the ageing process. Taking Hu's components known to have an effect on chronic conditions, healthy ageing and longevity (3), I have investigated the importance of maintaining a healthy weight and the consumption of a healthy diet, using the DII®, with the aid of anthropometric, biochemical, clinical and dietary data, to determine how the aforementioned lifestyle factors could contribute to healthy ageing.

1. To investigate if measured weight change over approximately four years is associated with long term mortality from all causes, as well as from cardiovascular disease, cancer and respiratory causes.
2. To examine the association between changes in waist circumference and all-cause and cardiovascular mortality over a period of approximately four years, and to examine these changes in relation to concurrent changes in weight.
3. To investigate the potential associations of reported dietary vitamin E intake (α-tocopherol equivalents), as well as plasma concentrations of both α- and γ-tocopherol and the ratio of α:γ-tocopherol, with measures of SMM and bone density status concurrently. Additionally, to examine both dietary and plasma concentrations of vitamin E in relation to fracture risk during 18.5 years of follow-up.
4. To investigate the Dietary Inflammatory Index (DII®) and its associations with biomarkers of nutrients with antioxidant potential and an inflammatory

biomarker and to assess if a more pro-inflammatory diet is associated with having multiple long-term conditions (MLTCs).

Chapter 2. Methods

2.1 The EPIC-Norfolk study

The data for this thesis have come from the EPIC-Norfolk study, which was co-ordinated in Cambridge. This cohort study is part of a ten country, 23 centre (Figure 6), 500,000 participant collaboration, which was initially set up to examine the relationship between diet and the risk of developing cancer (67).

Figure 6 Countries and centres participating in EPIC

https://www.epic-norfolk.org.uk/about-epic-norfolk/international-epic/

However, the EPIC-Norfolk study broadened its scope from the beginning, to additionally investigate both dietary and non-dietary determinants of other chronic diseases such as diabetes, CVD, arthritis and osteoporosis, as well as death (68).

The EPIC-Norfolk study was designed as a prospective cohort study. Participants were recruited from the Norfolk region of East Anglia because of the low migration rate and as one main hospital serviced the area, enabling easier follow-up (68). Thirty-five general practitioners' surgeries were used to recruit participants, aged between 39 and 79 years, based in Norwich city, as well as market towns and rural areas of Norfolk, from 1993 to 1997. As virtually all the UK population are registered with a general practice through the National Health Service, general practice age-sex

registers act as a population sampling frame. The National Health Service is used by almost all UK residents throughout their lives and therefore general practice registers approximate population registers.

The study has ethics committee approval from the Norfolk District Health Authority Ethics Committee (Ref: 98CN01) and all participants gave written, informed consent for study participation, including access to medical records.

Proposals for projects are welcomed, and the management committee aim to make data and samples as widely available as possible whilst safeguarding the privacy of the EPIC-Norfolk participants, protecting confidential data and maintaining the reputations of studies and participants. Data from the EPIC-Norfolk cohort study are available upon request to the management committee at https://www.epic-norfolk.org.uk/for-researchers/data-sharing/data-requests/. Requests for data should fulfil a number of criteria, including that the work is within the bounds of consent given by participants.

The design and recruitment of the study have been previously described in detail (68). In brief, 77,630 invitations were sent, with 30,445 (40%) consenting to participate in the study, of whom 25,639 completed a health and lifestyle questionnaire (HLQ) and attended a health examination (1HE). Approximately 4 years later, 15,786 attended a second health examination (2HE).

2.2 Self-reported health and lifestyle data

Participants completed five HLQs during the study, from which the following variables have been obtained. Only data from HLQ1 (1993-1997) and HLQ2 (1998-2000) have been used for the papers included in this thesis. Chapters 3, 4 and 5 used data from both of these time-points whereas Chapter 6 only used data from the 1HE.

2.2.1 Social class

Information on a participant's current and past occupation was only collected at baseline. Occupational social class was defined according to the Registrar General's classification. Non-manual occupations were represented by codes I (professional), II (managerial and technical), and IIIa (non-manual skilled) occupations while manual occupations were represented by codes IIIb (manual skilled), IV (partly skilled) and V

(unskilled) occupations (69). The following priority order for assigning social class was used for men: own current employment, own past employment, partner's current employment or partner's past employment. The priority order for assigning social class for women was partner's current employment, partner's past employment, own current employment, own past employment. Where indicated, social class is dichotomised as follows: professional, managerial and technical and non-manual skilled occupations (codes I, II and IIIa, respectively) are classed as non-manual, while manual skilled, partly skilled and unskilled (codes IIIb, IV and V, respectively) are classed as manual.

2.2.2 Educational status

Data on the education level attained was only collected at baseline, using the question "Do you have any of the following qualifications?" followed by a list of common UK qualifications. Participants were categorised according to the highest qualification attained, into the following four groups: those with no formal qualifications; those with 'O' level or equivalent; those with 'A' level or equivalent; and those with degree level qualifications or equivalent. These categories correspond to the International Standard Classification of Education (ISCED) 1997 of bachelor/master/doctoral or equivalent (ISCED 5A-6), post-secondary non-tertiary education or short-cycle tertiary education (ISCED 3A-5B), upper secondary education (ISCED 3C-3B) and pre-primary, primary and lower secondary (ISCED 0–2) respectively (70). Where indicated, educational status has been treated as a binary variable: no qualification vs. 'O' level and above.

2.2.3 Smoking

Smoking status at baseline was derived from two questions in the HLQ1: "Have you ever smoked as much as one cigarette a day for as long as a year?" and for those who answered "yes" to the first question, "Do you smoke cigarettes now?" The answers to these questions resulted in the following categorisation: never, former or current smoker. Similar questions at subsequent HLQs were used to update a participant's smoking status.

2.2.4 Physical activity

Self-reported physical activity was derived from HLQ1, using information on both occupational and leisure activity. Occupational activity was assessed using a four-category question ("sedentary", "standing", "moderate physical work" and "heavy manual work") with examples such as office worker, hairdresser, nurse and construction worker respectively. Leisure activity in both summer and winter was assessed from the number of hours per week spent on activities such as cycling, attending keep fit classes or aerobics and swimming or jogging. The estimated average hours of weekly leisure activity were calculated as the mean of summer and winter activities and categorised using 0, > 0 - 3.5, 3.5 - 7 and > 7. A score, divided into four ordered categories, with individuals labelled as "inactive", "moderately inactive", "moderately active" and "active", was created, combining leisure and occupational components. The score has been validated using free-living heart rate monitoring and measurements of oxygen consumption, from which energy expenditure has been estimated (71). The physical activity score was positively associated with energy expenditure (p-trend <0.001)). The weighted kappa statistic for the comparison of the baseline physical activity index with that for the repeated measure after 18-21 months was 0.6, P<0:0001, indicating acceptable agreement.

2.2.5 Medical history including prevalent diseases

Participants were asked about their medical histories with the question ''Has the doctor ever told you that you have any of the following?'', followed by a list of conditions that included myocardial infarction, stroke, diabetes, cancer, asthma, arthritis, depression, osteoporosis and bronchitis (derived from HLQ1 and HLQ2). The answer categories have changed over time and varied from 'tick boxes', to 'yes/no/don't know'. Missing values and 'don't know' answers were treated as 'no'. Table 2 summarises how the self-report of certain conditions was used in my papers, either for the exclusion or inclusion of participants.

Table 2 Summary of how the self-report of certain conditions was used in the papers

	Participants excluded from analyses if the following conditions were self-reported at 1HE and/or 2HE	Self-reported conditions used in analyses
Chapter 3: Weight change and mortality risk	Cancer, myocardial infarction and stroke; asthma and bronchitis in sensitivity analyses	
Chapter 4: Changes in WC and mortality risk	Cancer, myocardial infarction and stroke.	
Chapter 6: DII® and prevalence of MLTCs		Myocardial infarction, stroke, diabetes, cancer, asthma, arthritis, depression, osteoporosis and hypertension*

*Participants were also classified as having hypertension if they had measured systolic blood pressure ≥ 140mmHg, measured diastolic blood pressure ≥ 90 mmHg, or reported taking anti-hypertensive medication

HLQ2 data was used to classify participants regarding recent weight loss, with the question ''If you have lost more than 5 kgs (10 lbs) in the last five years, how did this weight loss occur?'' Options available included diet, exercise and illness. These data were used in Chapter 3.

2.2.6 Menopausal status

A separate section in the HLQ asked about menopause and the use of hormone replacement therapy. Menopausal status was derived from these questions, evaluated against the participant's age. Four categories were identified: pre-menopausal, early peri-menopausal (5 years after last menstruation), late peri-menopausal (1-5 years after last menstruation) and postmenopausal (> 5 years after last menstruation).

2.3 Objectively measured data at the HEs

2.3.1 Anthropometry

Weight was measured on a digital scale (Salter, UK), to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using a free-standing stadiometer (Chasemores, UK). For these measurements, participants wore light clothing and no shoes. Overweight and obesity are most commonly defined in terms of BMI, which was calculated as measured weight in kilograms divided by the square of height in metres and is expressed in kg/m^2 (72). BMI is routinely classified into the following five main

categories: underweight (BMI < 18.5 kg/m^2); healthy weight (BMI between 18.5 and 24.9 kg/m^2); overweight (BMI between 25.0 and 29.9 kg/m^2; obese (BMI between 30.0 and 39.9 kg/m^2) and severely or morbidly obese (BMI ≥ 40 kg/m^2). Obesity may be further classified as follows: Class 1 (or mild) obesity is considered between a BMI of 30.0 and 34.9 kg/m^2, 35.0 to 39.9 kg/m^2 is class 2 (or moderate) obesity, and ≥40.0 kg/m^2 is classified as class 3 (or severe) obesity.

A D-loop non-stretch fibreglass tape was used to measure waist circumference (WC), which was measured at the smallest circumference between the ribs and iliac crest to the nearest 0.1 cm while the participant was standing erect, with the abdomen relaxed, the arms at the side, the feet together and at the end of a normal expiration, without the tape compressing the skin (73). The measurement was taken at the umbilicus level, where there was no natural waistline.

2.3.2 Indices of Fat-Free Mass

How these indices of fat-free mass (FFM) have been created has been described in my previous paper (74). Bioelectrical impedance analysis (BIA) was carried out at the 2HE, using a standardised technique (Tanita TBF-531, Bodystat, Isle of Man, UK), suitable for use in large field-based studies and shown to be both a valid (75) and reliable (76) measure of body composition. Body density (BD) was calculated from total weight (Wt) in kilograms, height (Ht) in centimetres, and impedance (Z) in ohms, using the following standard regression formulae:

$$BD\ in\ men = 1.100455 - 0.109766 \times Wt\ \times Z\ \div Ht^2\ + 0.000174\ \times Z$$

$$BD\ in\ women = 1.090343 - 0.108941 \times Wt\ \times Z\ \div Ht^2\ + 0.00013\ \times Z$$

FFM in kilograms was then calculated, which is an estimate of the non-fat mass content of the body, i.e., metabolic tissue, water, and bone:

$$FFM = Wt - ((4.57\ \div BD - 4.142)\ \times Wt)$$

To scale for differences in SMM with increasing body weight, FFM was divided by BMI, (thereby also standardised for height, according to the method suggested by Studenski) (77), referred to as FFM_{BMI}.

2.3.3 Bone Density Assessment

At the 2HE, quantitative ultrasound measurements of the calcaneus (heel bone) were taken, using a contact ultrasound bone analyser (CUBA) device (McCue Ultrasonics) following standardised protocols. Broadband ultrasound attenuation

(BUA) (dB/MHz) measurements were taken at least in duplicate for each of the participant's feet, and the mean of the left and right foot measures was used for analysis. Each CUBA device used in the study was calibrated daily with its physical phantom. Additionally, calibration between devices was checked monthly using a roving phantom (CV 3.5%). The CUBA method of bone density assessment has been shown to be capable of predicting fracture risk (78). A decrease of 20 dB/Mhz (approximately 1 SD in BUA) has been shown to be associated with a relative risk of fracture of 1·95 (95% CI 1·50–2·52, p<0·0001), after adjustment for sex, age, weight, height, smoking status and past history of fracture (78). Additionally, it is cheaper and simpler to conduct in general practices compared with the gold-standard of dual-energy X-ray absorptiometry (DEXA).

2.3.4 Blood pressure

A trained nurse took two measurements of systolic and diastolic blood pressures using an Accutorr sphygmomanometer (Datascope Medical, Huntingdon, UK), with participants in a seated position, after having rested for three minutes. The most appropriate cuff size was selected, to consider the arm circumference. The mean of each of the two blood pressure readings were used in the analyses.

2.3.5 Blood sample analyses

Trained nurses obtained non-fasting blood samples by venepuncture at baseline and at the 2HE into plain and citrate bottles. Plasma concentrations of vitamin A (retinol) and vitamin E were analysed at IARC, Lyon (France), using HPLC. Serum total cholesterol was determined in a Norfolk laboratory using a RA 1000 Diagnostics (Bayer, Basingstoke, UK) instrument.

Plasma vitamin E concentration, in the form of α-tocopherol, was adjusted for cholesterol, as this is perceived to be a more reliable marker for vitamin E nutritional status (79), as tocopherols are transported in lipoproteins. Plasma samples were analysed for β-carotene concentrations by reversed-phase HPLC (HPLC-1100 system, Hewlett Packard) at IARC, Lyon (France), using a method based on that of Steghens *et al.* (80). Plasma for vitamin C was stabilized in a standardized volume of metaphosphoric acid, which was then stored at −70°C. Plasma vitamin C

concentration was determined using a fluorometric assay within one week of sampling (81).

Serum magnesium concentration was measured using blood sampled by peripheral venipuncture. The samples were stored in liquid nitrogen at -196°C until analysed using an Olympus AU640 Chemistry Immuno Analyser (Quotient Bioresearch, Fordham, UK) to perform a xylidyl blue-based colorimetric assay (Beckman Coulter, USA).

The concentration of high-sensitivity CRP (hs-CRP) was analysed using the Olympus AU640 Chemistry Immuno Analyzer (Olympus Diagnostics, Watford, UK).

2.4 Dietary assessment in EPIC-Norfolk

Three paper-based instruments have been used to assess diet in the EPIC-Norfolk cohort study: a self-administered 24-hour diet recall (24hDR), a Food Frequency Questionnaire (FFQ) and a 7-day Diet Diary (7dDD). Data from both the 7dDD and the FFQ have been used in this thesis and these methods are therefore described in more detail below.

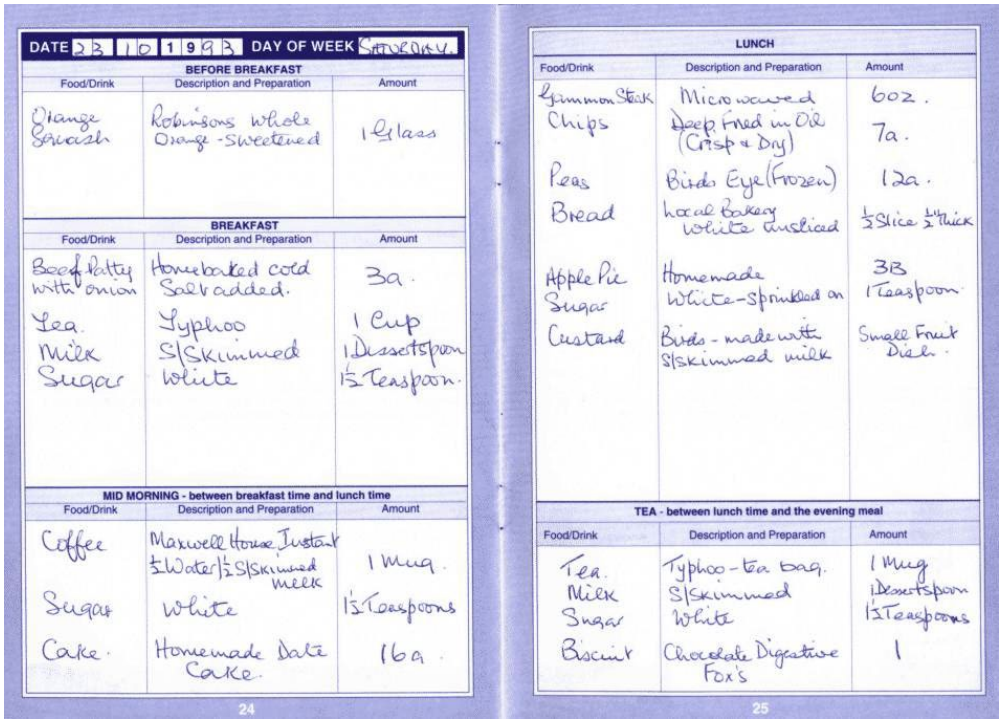
2.4.1 Dietary assessment using the 7dDD

Dietary intakes at the first health examination (1HE) were assessed using 7dDDs, which were completed by 25,507 participants, detailing all foods and drinks consumed, together with the portion sizes. Vitamin E intake, as calculated from the 7dDD, was used to assess associations with SMM, heel bone ultrasound attenuation and fracture risk (Chapter 5).

The 7dDD is a 45-page, A5 booklet, containing detailed instructions on how to describe and quantify foods and drinks consumed. Participants were asked to record at the time of consumption and were provided with suggestions on how to describe the foods and drinks consumed using household measures, standard units or weights from packaging. Participants could also choose from a series of seventeen colour photos, depicting three different portion sizes, representing small, medium and large portions, of commonly consumed foods. Meals were recorded in a pre-structured format: before breakfast, breakfast, mid-morning, lunch, tea, evening meal and

between meal snacks and drinks. See Figure 7 for an example of a partially completed day.

At the 1HE, trained nurses carried out a single 24hDR, recording the participant's food and drink intake as the first day of the 7dDD. This served the dual purpose of providing a detailed explanation of how to complete the 7dDD, as well as ensuring a minimum of one day of dietary intake data, as a carbon copy of each recall was kept and used for data entry if the 7dDD was not returned by the participant. The following six days of the 7dDD were to be completed at home, and the 7dDD returned by post in the prepaid envelope provided. After the last day of the 7dDD, there were general questions relating to the food and drink consumed last week, which covered cooking methods, addition of salt, type of fat used for spreading/baking/frying, bread, milk usually consumed etc. These questions were a useful source of information for a data enterer when the participant's description of their dietary intake lacked detail.



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 7 Extract from a completed 7dDD

Data Into Nutrients for Epidemiological Research (DINER) software was used by trained data enterers to enter the dietary information recorded in the 7dDDs (82),

which were then checked and processed by nutritionists to obtain nutrient data using DINERMO (83).

The nutrient data underlying DINER were McCance & Widdowson's 'The Composition of Foods', 5th edition and its associated supplements (84–93). A data enterer had to select from a list of approximately 11,000 foods and drinks, displayed in food groupings based on 'The Composition of Foods'. During data entry, data enterers had the ability to note any queries they might have in a free text field, relating to the suitability of the foods and drinks selected; these were later checked by a nutritionist and any necessary amendments carried out, including the creation of new foods or drinks in DINER, where deemed necessary. After the selection of an item, an appropriate quantity was chosen from a predefined list. Commercial products required additional information such as brand whereas homemade items required details of cooking fats used.

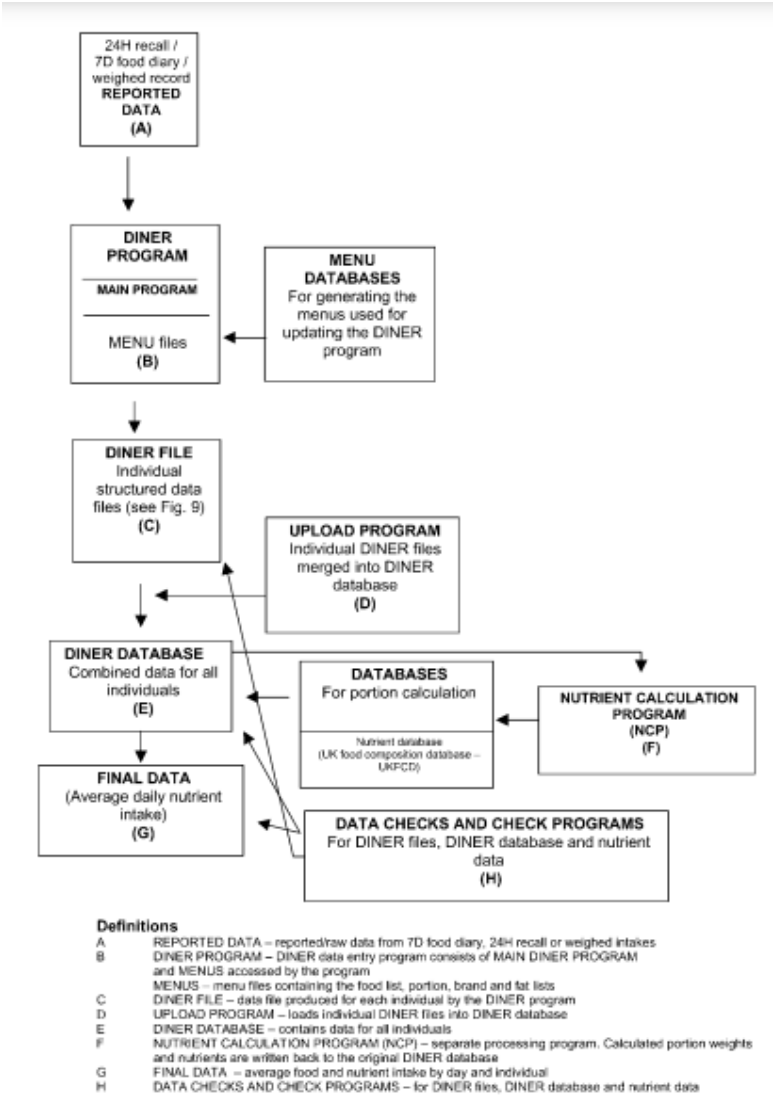


Figure 8 Schematic diagram of the DINER program with associated processes and definitions (82)

Figure 8 presents an overview of the DINER system and associated processes. I was heavily involved in the development of the DINER program, contributing to data entry aspects to create a tool that was logical and understandable for those who would enter the dietary data. In addition, I had responsibility for populating, maintaining and updating the numerous relational databases underlying it. These databases included food categories, food list, portion sizes, brands, and factors for density, edible part, and more complex situations such as when pasta, rice or meat were quantified in terms of the raw weight. The accuracy and level of detail that DINER could capture has enabled high-quality research into diet and disease associations and as of 23rd September 2024, it has 151 citations, according to Google Scholar.

The checking process of the entered diaries began with the data enterer who ran completeness checks, i.e. completion of all meal occasions in a day (whether something was consumed or not) and the entry of seven distinct dates. Nutritionists, including myself, created several checks to examine diaries for consistency and validity. Firstly, meal occasions were identified for food items that would be commonly consumed together, but for which inconsistent amounts had been entered; secondly, meals consisting of two or fewer items were checked for completeness; thirdly, days with less than three meal occasions were checked for completeness and validity.

DINERMO converted portion sizes into weights or volumes, the latter being converted to weights with the use of density measurements (83). Weights of raw foods, such as meats and pasta were converted to cooked weights. Weights were corrected for inedible part, where necessary, e.g. stones in fruits. All entered items were calculated in grams consumed. After the nutrients had been calculated, diaries were checked for outliers, both with respect to portion size and nutrient intake. I had a key role in identifying the most useful checks to effectively and efficiently spot potential errors in the entered data. If missing values in any of the underlying food, portion and/or conversion reference tables were discovered, these were updated, and a final nutrient calculation stage was carried out.

Traditionally, nutrient intakes from dietary intake data have been studied but food groups enable dietary data to be analysed in different ways. I and other nutritionists used EPIC- Norfolk dietary data to create several food groups to enable researchers to assess specific hypotheses involving food types and disease (83). These groups ranged from crude qualitative categories, where a food could only be categorised into one group, to more detailed quantitative categories, where the components of a product or dish could be disaggregated to provide intakes of food groups such as fruits, vegetables, red meat, white meat, processed meat, fatty fish and white fish.

2.4.2 Assessment of micronutrient intake from supplements

At the back of the 7dDD is a question relating to supplement use. Vitamin and mineral supplements recorded in the 7dDD were quantified using the Vitamin and

Mineral Supplement (ViMiS) database (94). Around the same time as DINER was being developed, it was realised that supplements were an important source of micronutrients. We therefore embarked on gathering information so that we could quantify the total nutrient intake of EPIC-Norfolk participants, from their diet and use of supplements. I was involved in the early collection of manufacturers' data on supplements, checking and collating conversion factors for the amount of vitamins and minerals in a compound and the data entry of supplement data into the database. Data on whether participants consumed supplements containing vitamin D, E and/or calcium and the amount obtained were used to assess "Associations of Dietary Intake and Plasma Concentrations of Vitamin E with Skeletal Muscle Mass, Heel Bone Ultrasound Attenuation and Fracture Risk" (Chapter 5).

2.4.3 Dietary assessment using the FFQ

FFQs were used at the 1HE to assess the dietary intake of the EPIC-Norfolk participants. The FFQ was posted to 25,639 participants in the EPIC-Norfolk cohort study (68) and was returned at the 1HE, where it was checked and completed, if required, by a trained nurse. In total, 25,351 participants returned a completed FFQ. The dietary data from the FFQ was used to create the (DII®), to study its associations with biomarkers of nutrients with antioxidant potential, biomarkers of inflammation and MLTCs (Chapter 6).

The EPIC-Norfolk FFQ was originally developed in 1988 and has been validated for the EPIC-Norfolk population (95–97). It is a semi-quantitative questionnaire, and its food list and portion sizes represent those of an adult population who are likely to have established eating habits and consume a traditional UK diet. The principles involved in the data collection and processing of the EPIC-Norfolk FFQ and the development of the CAFÉ (Compositional Analyses from Frequency Estimates) programme for calculating nutrient intakes have been published previously (98). However, the CAFÉ programme was not easily accessible to researchers and for this reason, I led on the development of a new, open source, cross-platform processing tool (FETA—FFQ EPIC Tool for Analysis) (99), based on and building upon the earlier CAFÉ system (98). Rather than create an exact replica of the existing program, I ensured that FETA was designed so that it can be customised for different study

populations, by modifying the underlying data files in FETA. A researcher can delete/add foods and/or FFQ lines to suit their FFQ, as well as amend portion sizes where required. Existing nutrient values in the food composition table can be easily modified or updated in the tool. It is also possible for FETA to be used with other questionnaires containing a different number of frequency categories.

The FFQ is a 10-page A4 document, consisting of two parts. Part one contains a list of 130 foods and for each of these items in the list, participants are asked to tick their usual frequency of consumption over the last year, choosing from nine frequency categories. These categories range from “never or less than once/month” to “6+ times per day”. See Figure 9 for an extract from part one. The questionnaire lines are either individual foods, combinations of individual foods or food types. The serving size for each line is specified in terms of units or common portions (e.g. one banana, one slice of bread) or household measures (e.g. glass, cup, spoon) or medium servings. Where a medium serving was assigned to a line, this did not take age or sex into account.

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
BREAD AND SAVOURY BISCUITS (one slice or biscuit)	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
White bread and rolls						✓			
Brown bread and rolls				✓					
Wholemeal bread and rolls	✓								
Cream crackers, cheese biscuits		✓							
Crispbread, eg. Ryvita		✓							
CEREALS (one bowl)									
Porridge, Readybrek				✓					
Breakfast cereal such as cornflakes, muesli etc.					✓				

Figure 9 Extract from a completed section of part one of the FFQ

Each item in part one of the FFQ is mapped to between one and six food codes. Decisions regarding which food codes to select were based on data from UK government surveys and other UK population data (68,100,101), based on data for individuals aged 40–74 years (100). Portion weight data were sourced from UK population data and weighed records in 40–74-year-old study participants (100,102). The EPIC-Norfolk FFQ uses 290 foods from the UK food composition database, McCance and Widdowson’s ‘The Composition of Foods’ (5th edition) and its

associated supplements (84–93). Thirteen foods in the nutrient database were modified to enable the program to incorporate the contribution of different fat types used during food preparation (frying and baking, questions 6 and 7 respectively – see Figure 10).

Additional questions on the participant's diet over the last year are asked in part two, a number of which ask for detailed information that link back to food items in part one, as illustrated in Figure 10. Additional questions ask for information on the type and brand of breakfast cereal, the type and quantity of milk consumed, the type of fat used in cooking and the amount of visible fat on meat consumed. The questions relating to fat are linked to relevant items in part one and help to estimate the consumption of total fat and fatty acids. The capture of more detailed information on breakfast cereals and fats used in food preparation enable more accurate intake data of a number of the DII® components, including monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids, (PUFAs), n-3 fatty acids, n-6 fatty acids and fibre, as well as vitamins and minerals used in fortification.

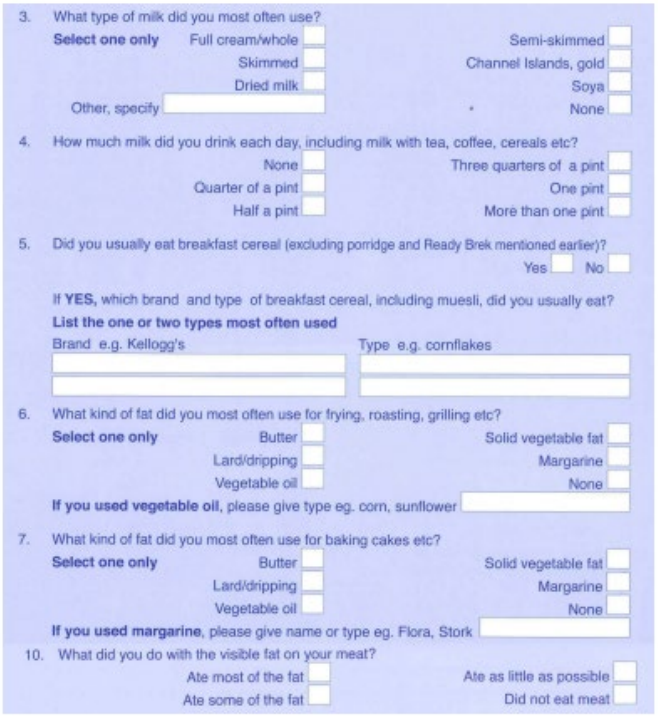

3. What type of milk did you most often use?
Select one only Full cream/whole ☐ Semi-skimmed ☐
Skimmed ☐ Channel Islands, gold ☐
Dried milk ☐ Soya ☐
Other, specify ☐ None ☐
4. How much milk did you drink each day, including milk with tea, coffee, cereals etc?
None ☐ Three quarters of a pint ☐
Quarter of a pint ☐ One pint ☐
Half a pint ☐ More than one pint ☐
5. Did you usually eat breakfast cereal (excluding porridge and Ready Brek mentioned earlier)?
Yes ☐ No ☐
If **YES,** which brand and type of breakfast cereal, including muesli, did you usually eat?
List the one or two types most often used
Brand e.g. Kellogg's ☐ Type e.g. cornflakes ☐
6. What kind of fat did you most often use for frying, roasting, grilling etc?
Select one only Butter ☐ Solid vegetable fat ☐
Lard/dripping ☐ Margarine ☐
Vegetable oil ☐ None ☐
If you used vegetable oil, please give type eg. corn, sunflower ☐
7. What kind of fat did you most often use for baking cakes etc?
Select one only Butter ☐ Solid vegetable fat ☐
Lard/dripping ☐ Margarine ☐
Vegetable oil ☐ None ☐
If you used margarine, please give name or type eg. Flora, Stork ☐
10. What did you do with the visible fat on your meat?
Ate most of the fat ☐ Ate as little as possible ☐
Ate some of the fat ☐ Did not eat meat ☐

Figure 10 Questions from part two of the FFQ, used by FETA

Participants who answered 'Yes' to the following question in part two of the FFQ were classified as supplement users: 'Have you taken any vitamins, minerals, fish oils,

fibre or other food supplements during the past year?'. The answers on the use of supplements were used to categorise participants as supplement users or non-supplement users in Chapter 6 which investigated the DII® and its associations with biomarkers of nutrients with antioxidant potential, biomarkers of inflammation and MLTCs.

2.4.4 Creation of the DII®

Figure 11 illustrates the steps involved in the creation of the DII® score, as stipulated by Shivappa *et al*. (66) but when I initially created the DII®, I only had data for 29 of the 45 parameters specified by the authors (66). However, I was able to obtain data for a further eight anti-inflammatory components, including isoflavones, flavan-3-ols, flavonols, flavones and anthocyanidins, as I had previously led on the updating of the nutrient database for the isoflavone content of foods and beverages (103), and advised on the updating of the nutrient database for flavan-3-ols, flavonols, flavones and anthocyanidins (104,105), enabling the inclusion of these parameters in the DII® score for my research.

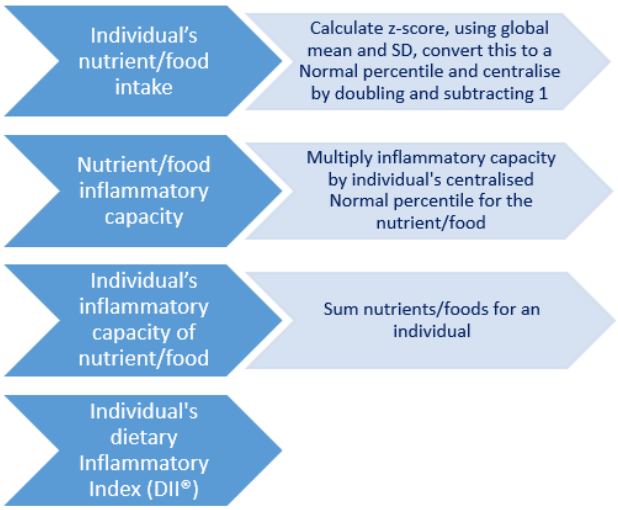


Figure 11 Steps in the creation of the DII score by Shivappa et al. (66)

(Figure taken from Mulligan et al. (106))

In both scenarios, the same number of participants were assigned to sex-specific quintiles. Table 3 and Table 4 below illustrate the number of men and women respectively, who remained in the same quintile or moved quintile, when additional parameters were added to the DII®.

Table 3 Numbers of men who remained in the same quintile or moved quintile when more parameters were included in the DII®

		Quintiles based on 37 parameters				
		Q1	Q2	Q3	Q4	Q5
Quintiles based on 29 parameters	Q1	1,846	368	9	0	0
	Q2	358	1,390	442	33	0
	Q3	19	447	1,281	447	28
	Q4	0	17	474	1,322	410
	Q5	0	1	16	421	1,784

Table 4 Numbers of women who remained in the same quintile or moved quintile when more parameters were included in the DII®

		Quintiles based on 37 parameters				
		Q1	Q2	Q3	Q4	Q5
Quintiles based on 29 parameters	Q1	2,149	523	9	1	0
	Q2	499	1,606	554	23	0
	Q3	34	504	1,568	560	15
	Q4	0	49	536	1,714	383
	Q5	0	0	14	384	2,283

These data show that approximately 80 – 85% of both men and women remained in the extreme quintiles, Q1 and Q5, whereas only 58% remained in Q3, when an additional eight anti-inflammatory parameters were added to the score, illustrating that the number of parameters included in the DII® does affect the ranking of participants and subsequently may therefore affect diet-disease associations.

Chapter 3. Weight change and 15 year mortality: results from the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) cohort study

3.1 Key points

What is already known on this subject?

- Overweight and obesity are major risks for deaths worldwide and are estimated to contribute to the burden of diabetes, ischaemic heart disease and certain cancers.
- In middle-aged and older adults, a higher mortality risk has been shown to be associated with weight loss, compared to maintaining a stable weight. Gaining weight has also been associated with an increased mortality risk.

I therefore chose to investigate associations between changes in weight in a middle- and older-aged population and mortality risk.

What this study adds.

- Objectively measured weight loss (> 2.5 kg) in middle-aged and older men and women, over approximately 4 years, was significantly associated with higher mortality over 15 years of follow-up.
- This association was also evident in subgroups of the population, after stratification for age, smoking, BMI, physical activity and the exclusion of individuals who said they had lost weight due to illness and deaths within the first 5 years of follow-up, as well as in dieters who reported to have lost more than 5 kg.
- Associations for CVD mortality in men who lost weight were stronger than those for all-cause mortality.
- After the exclusion of those who self-reported having asthma or bronchitis at either time-point, losing more than 5 kg was significantly associated with a higher hazard of death from respiratory causes in men.

- Results for weight gain were inconclusive, potentially affected by weight cycling.

3.2 Published journal article

Mulligan, A.A., Lentjes, M.A.H., Luben, R.N., Wareham, N.J., Khaw, K-T. Weight change and 15 year mortality: results from the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) cohort study. Eur J Epidemiol 33, 37–53 (2018). https://doi.org/10.1007/s10654-017-0343-y

European Journal of Epidemiology (2018) 33:37–53
https://doi.org/10.1007/s10654-017-0343-y

MORTALITY

Weight change and 15 year mortality: results from the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) cohort study

Angela A. Mulligan[1] · Marleen A. H. Lentjes[1] · Robert N. Luben[1] · Nicholas J. Wareham[3] · Kay-Tee Khaw[1,2]

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Abstract

Studies have reported a higher mortality risk associated with weight loss, particularly in middle-aged and older adults, although some of these studies did find that gaining weight was also associated with an increased mortality risk. We examined changes in weight in relation to mortality in a prospective population-based cohort study of men and women, resident in Norfolk, UK. Participants were assessed at baseline (1993–1997) and at a second examination (1998–2000), as part of the European Prospective Investigation into Cancer and Nutrition (EPIC-Norfolk) study, and followed up to 2015 for mortality. Participants with a self-reported history of cancer or cardiovascular disease, body mass index < 18.5 kg/m^2 or missing data on adjustment variables, at either time-point were excluded, leaving 12,580 participants, aged 39–78 in 1993–1997, eligible for analyses. Cox proportional hazards models were used to determine Hazard Ratios (HRs) for all-cause (2603 deaths), cardiovascular (749 deaths), cancer (981 deaths), respiratory (226 deaths) and other causes of mortality (647 deaths) by categories of weight change. After multivariate adjustment, the HRs (95% CIs) for all-cause mortality for men and women who lost more than 5 kg were 1.85 (1.48–2.31) and 1.64 (1.31–2.05) respectively. Higher hazards were also found for specific causes of mortality and weight loss > 5 kg. Similar associations were observed after excluding deaths in the first 5 years of follow-up. Results for weight gain were inconclusive. We conclude that objectively measured weight loss, but not weight gain, was associated with subsequent higher mortality risk in this population-based study of middle-aged and elderly men and women. However, undiagnosed, pre-existing disease and the inability to account for weight cycling need to be remembered when interpreting these results. Unravelling the causal pathways underlying this association will require more detailed studies, including that of changes in body composition.

Keywords Weight change · Weight loss · All-cause mortality · CVD mortality · Cancer mortality · EPIC-Norfolk

Abbreviations

EPIC European Prospective Investigation into Cancer and Nutrition
BMI Body mass index
CVD Cardio-vascular disease

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✉ Angela A. Mulligan
angela.mulligan@phpc.cam.ac.uk

[1] Strangeways Research Laboratory, European Prospective Investigation into Cancer and Nutrition, Department of Public Health and Primary Care, University of Cambridge, Worts Causeway, Cambridge, UK

[2] EPIC, Department of Gerontology, Addenbrooke's Hospital, School of Clinical Medicine, University of Cambridge, Cambridge, UK

[3] MRC Epidemiology Unit, Cambridge, School of Clinical Medicine, Institute of Metabolic Science, University of Cambridge, Cambridge Biomedical Campus, Cambridge, UK

Introduction

Overweight and obesity are major risks for deaths worldwide [1–4] and are estimated to contribute to 44% of the diabetes burden, 23% of the ischaemic heart disease burden and 7–41% of certain cancer burdens [5]. In 2012, it was estimated that 37% of adults (aged 16 and over) were overweight (body mass index (BMI) $\geq$ 25 to $<$ 30 kg/m^2) and 25% were classified as obese (BMI $\geq$ 30 kg/m^2), based on Health Survey for England data [6]. Data collected between 1993 and 2012 show that the percentage of English adults with a BMI $\geq$ 18.5 to $<$ 25 kg/m^2 has decreased from 41 to 32% among men and from 50 to 41% among women [7].

A recent NICE guideline makes recommendations on the provision of weight management services for overweight or obese adults [8]. It recommends that GP practices and other health care professionals who give advice about or refer people to lifestyle weight management programmes should be aware that there should be no upper BMI or upper age limit for funded referrals. However, a number of studies have reported a higher mortality risk associated with weight loss, compared to maintaining a stable weight, particularly in middle-aged and older adults [9–18], although some of these studies did find that gaining weight was also associated with an increased mortality risk [10, 13]. It has been proposed that the observed effects of a higher mortality risk with weight loss may be a balance between the consequences of the loss of potentially harmful abdominal and ectopic fat mass and the loss of potentially beneficial peripheral subcutaneous fat mass and lean body mass [19].

The main objective of this article was to investigate long term mortality from all causes, as well as specifically from cardiovascular disease, cancer and respiratory causes, in relation to measured weight change over an average period of 3.7 years, in 12,580 community-dwelling men and women.

Methods

EPIC-Norfolk study design

The Norfolk cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Norfolk) is part of the Europe-wide EPIC study, which involves over half a million people in ten countries [20] and was initially planned as a diet and cancer cohort. However, the study in Norfolk broadened its scope from the outset, to investigate the causes of disability and death in middle and later life and to include other lifestyle exposures such as physical activity and psychosocial factors [21]. Participants, aged between 39 and 79 years, were recruited from General Practitioners' surgeries, based in rural areas of Norfolk and market towns as well as the city of Norwich, from 1993 to 1997. Since virtually all the population of the UK are registered with a general practice through the National Health Service, general practice age sex registers act as a population sampling frame. This cohort at baseline was comparable to the UK national population with regard to many characteristics, including age, sex and anthropometry measurements but it had a lower proportion of current smokers [22].

The study was approved by the Norfolk District Health Authority Ethics Committee and all participants gave written, informed consent.

Main exposure: weight change

Of the 30,445 men and women, aged 39–79 years, who consented to participate in the study (39% response rate), 25,639 attended a baseline health examination (1HE) between 1993 and 1997 and 15,786 attended a second health examination (2HE) between 1998 and 2000.

At both health examinations, a trained nurse measured weight (to the nearest 0.1 kg) and height (to the nearest 0.1 cm), with participants wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight divided by the square of height (kg/m^2).

Absolute weight change was calculated as weight (kg) measured at 2HE minus weight (kg) measured at 1HE. Participants were assigned to one of 6 weight change categories: $>$ 5 kg loss, $>$ 2.5–5 kg loss, within 2.5 kg loss or gain ('maintenance', considered the reference category), $>$ 2.5–5 kg gain, $>$ 5–10 kg gain, $>$ 10 kg gain.

Annual weight and BMI changes were calculated from the absolute differences in weight and BMI respectively, divided by the participants' time lapse between the health examinations (kg/year and kg/m^2/year respectively).

Participant selection

Participants were eligible for inclusion if they had weight and height measurements at both time-points. Participants were excluded from analyses if they had a BMI $<$ 18.5 or who self-reported cancer or cardio-vascular disease (CVD), as were those with missing data on adjustment variables (smoking, social class, educational level and physical activity), in an attempt to address reverse causality. This left 12,580 participants for analyses, out of a maximum of 15,000 for whom we had a weight measurement at both 1HE and 2HE, in order to be able to calculate weight change (Fig. 1).

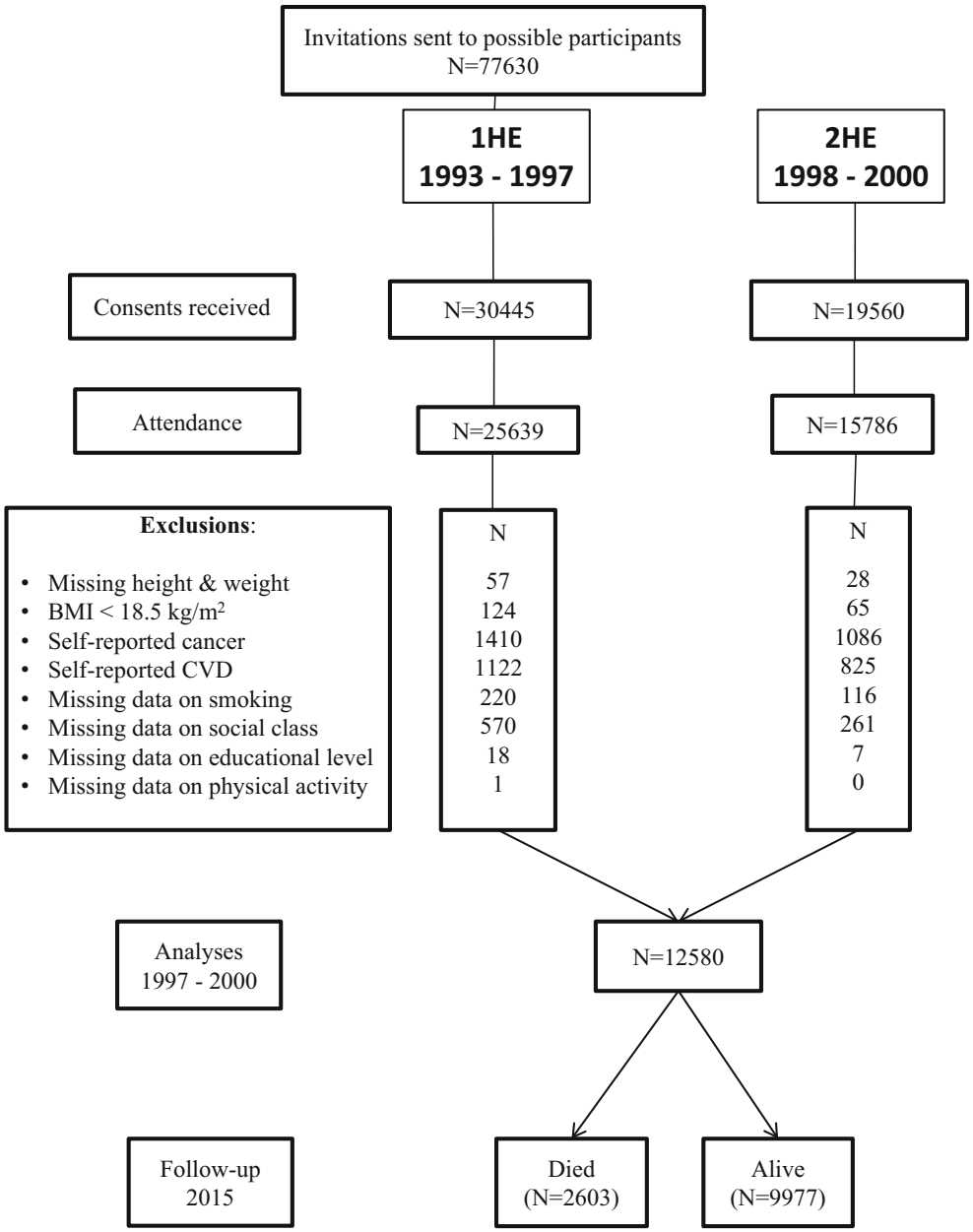

Fig. 1 Study population and sample size eligible for mortality analyses

Adjustment variables

Data collected via two self-administered Health and Lifestyle Questionnaires (HLQ1 and HLQ2), before the 1HE and 2HE respectively, were used to establish classification of a number of variables. Smoking status (derived from HLQ1 and HLQ2) (never, former, current) was derived from yes and no responses to the following questions "Have you ever smoked as much as one cigarette a day for as long as a year?" and "Do you smoke cigarettes now?". Self-reported physical activity (derived from HLQ1) was assessed using both occupational and leisure activity and individuals were assigned to one of four categories: inactive, moderately inactive, moderately active and active [23, 24]. Occupational social class at 1HE was defined according to the Registrar General's classification. Non-manual occupations were represented by codes I, (professional) II, (managerial and technical), IIIa (non-manual skilled) occupations while manual occupations were represented by codes IIIb (manual skilled), IV (partly skilled) and V (unskilled) occupations [25]. Educational level at 1HE was based on the highest qualification attained and was categorised into four groups: degree or equivalent, A level or equivalent, O level or equivalent and less than O

level or no qualifications. These four categories correspond to the International Standard Classification of Education (ISCED) 1997 [26] of bachelor/master/doctoral or equivalent (ISCED 5A-6), post-secondary non-tertiary education or short-cycle tertiary education (ISCED 3A-5B), upper secondary education (ISCED 3C-3B) and pre-primary, primary and lower secondary (ISCED 0–2) respectively. In this paper, those with an educational level of O level and above were combined into one category. Participants were asked about their medical histories with the question "Has the doctor ever told you that you have any of the following?" followed by a list of conditions that included heart attack, stroke, cancer, asthma and bronchitis (derived from HLQ1 and HLQ2).

HLQ2 data was used to classify participants regarding recent weight loss, with the question "If you have lost more than 5 kgs (10 lbs) in the last five years, how did this weight loss occur?" Options available included diet, exercise and illness.

Endpoints

All participants were flagged for death certification up until the end of March 2015, at the Office of National Statistics, United Kingdom. Death certificates were coded by nosologists according to the International Classification of Diseases (ICD). An underlying cause of death was defined by using ICD codes as follows: cancer death (ICD9, 140-208 or ICD10 C00-C97), cardiovascular death (ICD9 400-438 or ICD10 I10-I79), or respiratory disease (ICD9 460-519 or ICD10 J00-J99). Deaths that were not attributable to the three aforementioned causes were classified as deaths from other causes and included deaths from dementia, Parkinson's disease, bladder and renal diseases.

Statistical analyses

All adjustment variables were those measured at the first health examination in 1993–1997. The follow-up time was the underlying time variable; median (IQR) follow-up time was 15.4 (14.8, 16.2) years and began at the 2HE. The censor date was the date of death or end of the administrative follow-up (31st March 2015). Characteristics of the study population were summarised by weight change category, using means and SDs for continuous variables and frequencies and percentages for categorical variables. To ascertain whether variables should be included as predictors (with total mortality as the outcome), we performed the log-rank test of equality across strata for all the categorical variables and Cox's univariate proportional hazards regression for all the continuous variables. The predictors used in the final model were all variables for which the P value was < 0.20 in the univariate analyses and for which we also observed an association between the possible confounder and weight change categories. The Cox proportional hazards model was used to determine Hazard Ratios (HR) of all-cause and cause-specific mortality by weight change category for men and women separately, using a series of cumulative adjustment models: age (continuous variable), (model 1); including smoking (categorical variable), (model 2); including BMI (continuous variable), physical activity (4 categories), social class (manual vs. non-manual) and educational level (no qualifications vs. O level and above) (model 3). The interaction between sex and continuous BMI was not found to be significant ($P = 0.7606$). We tested for the proportional hazards assumption by including time interaction variables in the Cox regression models. Age was found to violate our test of the proportional hazards assumption ($P < 0.0001$). However, when we included the time interaction for age, only minimal changes to the hazard ratios of our main exposure (weight change) were observed. The category of greatest weight loss was also found to violate our test of the proportional hazards assumption ($P < 0.01$) and will be discussed later in the manuscript. In sensitivity analyses, we also examined HRs by weight change category, stratified by age and sex, in those who said they had lost weight as a result of dieting and after the exclusion of individuals who died within 3 or 5 years after the second health examination or those who said they had lost weight because of illness, as well as after excluding participants who self-reported asthma or bronchitis at either time-point. The data were analysed using Stata 12 (STATA Corp., Texas, USA).

Results

Cohort description

After exclusion, there were 12,580 men and women for analyses (80% of those who had attended 2HE), aged 42–82 years at the 2HE. The mean weight change over the average 4 years between 1HE and 2HE was a gain of 1.29 kg (SD 3.62) in men and 1.39 kg (SD 4.13) in women. Men were in general slightly older than women with a mean age of 62.1 and 61.0 years at 2HE respectively. Men also had a higher mean BMI than women at both health examinations, with 55.4% classified as overweight and 14.5% as obese at 2HE; in women these percentages were 40.4 and 18.4%.

Minimal differences were observed in the baseline characteristics of those who attended both 1HE and 2HE, before and after exclusions were applied (Supplementary Table 1). However, the prevalence of self-reported CVD, cancer, asthma and bronchitis was lower in those who also

Table 1 Characteristics of 5479 men and 7101 women by measured weight change category from 1HE (1993–1997) to 2HE (1998–2000)

	Weight change categories					
	Loss > 5 kg	Loss > 2.5 and ≤ 5 kg	Loss or gain ≤ 2.5 kg	Gain > 2.5 and ≤ 5 kg	Gain > 5 and ≤ 10 kg	Gain > 10 kg
MEN, N (row%)	215 (3.9)	423 (7.7)	2983 (54.4)	1206 (22.0)	577 (10.5)	75 (1.4)
Weight (1HE), kg	86.5 (10.7)	81.7 (11.3)	79.1 (10.3)	79.8 (10.5)	82.8 (12.3)	86.3 (13.9)
Weight (2HE), kg	79.0 (10.4)	78.1 (11.2)	79.4 (10.3)	83.4 (10.6)	89.4 (12.4)	99.1 (14.3)
Annual weight change, kg/year	− 2.1 (0.8)	− 1.0 (0.3)	0.1 (0.4)	1.0 (0.3)	1.9 (0.5)	3.5 (1.1)
Age (1HE), years	60.5 (9.2)	60.8 (9.0)	59.6 (8.9)	57.6 (8.8)	56.8 (8.4)	55.9 (8.7)
Age (2HE), years	63.7 (9.2)	64.2 (9.1)	62.7 (9.0)	60.8 (8.9)	60.1 (8.5)	59.2 (8.7)
BMI (1HE), kg/m^2	28.1 (3.2)	27.0 (3.2)	26.1 (2.9)	26.1 (3.0)	26.8 (3.5)	28.1 (4.6)
BMI (2HE), kg/m^2	25.8 (3.1)	25.9 (3.2)	26.3 (3.0)	27.3 (3.0)	29.0 (3.6)	32.1 (4.6)
Annual BMI change, kg/m^2/year	− 0.6 (0.3)	− 0.3 (0.1)	0.1 (0.1)	0.4 (0.1)	0.6 (0.2)	1.1 (0.6)
Smoking status (1HE)						
Current (509, 9.3%)	27 (12.6)	45 (10.6)	228 (7.6)	119 (9.9)	79 (13.7)	11 (14.7)
Former (2902, 53.0%)	106 (49.3)	229 (54.1)	1608 (53.9)	611 (50.7)	307 (53.2)	41 (54.7)
Never (2068, 37.7%)	82 (38.1)	149 (35.2)	1147 (38.4)	476 (39.5)	191 (33.1)	23 (30.7)
Smoking status (2HE)						
Current (440, 8.0%)	28 (13.0)	47 (11.1)	225 (7.5)	93 (7.7)	41 (7.1)	6 (8.0)
Former (2974, 54.3%)	105 (48.8)	227 (53.7)	1613 (54.1)	637 (52.8)	346 (60.0)	46 (61.3)
Never (2065, 37.7%)	82 (38.1)	149 (35.2)	1145 (38.4)	476 (39.5)	190 (32.9)	23 (30.7)
Physical activity (1HE)						
Inactive (1446, 26.4%)	86 (40.0)	128 (30.3)	770 (25.8)	288 (23.9)	154 (26.7)	20 (26.7)
Moderately inactive (1356, 24.8%)	51 (23.7)	105 (24.8)	751 (25.2)	315 (26.1)	120 (20.8)	14 (18.7)
Moderately active (1381, 25.2%)	43 (20.0)	95 (22.5)	746 (25.0)	300 (24.9)	174 (30.2)	23 (30.7)
Active (1296, 23.6%)	35 (16.3)	95 (22.5)	716 (24.0)	303 (25.1)	129 (22.4)	18 (24.0)
Social class (1HE)						
Non-manual (3401, 62.1%)	139 (64.6)	247 (58.4)	1862 (62.4)	766 (63.5)	345 (59.8)	42 (56.0)
Manual (2078, 37.9%)	76 (35.4)	176 (41.6)	1121 (37.6)	440 (36.5)	232 (40.2)	33 (44.0)
Educational level (1HE)						
No qualifications (1447, 26.4%)	60 (27.9)	127 (30.0)	772 (25.9)	302 (25.0)	166 (28.8)	20 (26.7)
O level and above (4032, 73.6%)	155 (72.1)	296 (70.0)	2211 (74.1)	904 (75.0)	411 (71.2)	55 (73.3)
Lost weight in last 5 years (2HE)						
Diet (267, 4.9%)	54 (20.1)	40 (15.3)	102 (38.1)	33 (12.3)	28 (10.4)	10 (3.7)
Illness (130, 2.4%)	22 (16.9)	23 (17.7)	54 (41.5)	19 (14.6)	8 (6.2)	4 (3.1)
WOMEN, N (row %)	362 (5.1)	517 (7.3)	3690 (52.0)	1540 (21.7)	841 (11.8)	151 (2.1)
Weight (1HE), kg	77.2 (13.3)	69.3 (11.0)	65.8 (10.4)	66.8 (10.4)	70.0 (11.2)	74.1 (12.4)
Weight (2HE), kg	69.0 (12.4)	65.7 (11.1)	66.2 (10.4)	70.4 (10.4)	76.7 (11.4)	87.6 (13.1)
Annual weight change, kg	− 2.4 (1.3)	− 1.0 (0.3)	0.1 (0.4)	1.0 (0.3)	1.9 (0.6)	3.7 (1.5)
Age (1HE), years	58.4 (9.1)	59.5 (9.1)	58.4 (8.9)	57.0 (8.7)	55.8 (8.1)	54.8 (7.1)
Age (2HE), years	61.5 (9.3)	62.6 (9.2)	61.6 (9.0)	60.1 (8.9)	59.1 (8.2)	58.2 (7.3)
BMI (1HE), kg/m^2	29.3 (4.9)	26.6 (3.9)	25.3 (3.8)	25.6 (3.8)	26.7 (4.1)	27.9 (4.4)
BMI (2HE), kg/m^2	26.4 (4.6)	25.4 (3.9)	25.6 (3.9)	27.1 (3.8)	29.4 (4.2)	33.2 (4.9)
Annual BMI change, kg/m^2/year	− 0.8 (0.5)	− 0.3 (0.1)	0.1 (0.2)	0.4 (0.1)	0.7 (0.2)	1.4 (0.6)
Smoking status (1HE)						
Current (641, 9.0%)	35 (9.7)	53 (10.2)	296 (8.0)	142 (9.2)	96 (11.4)	19 (12.6)
Former (2239, 31.5%)	132 (36.5)	166 (32.1)	1129 (30.6)	478 (31.0)	282 (33.5)	52 (34.4)
Never (4221, 59.4%)	195 (53.9)	298 (57.6)	2265 (61.4)	920 (59.7)	463 (55.0)	80 (53.0)
Smoking status (2HE)						
Current (569, 8.0%)	32 (8.8)	56 (10.8)	280 (7.6)	117 (7.6)	74 (8.8)	10 (6.6)

Table 1 (continued)

	Weight change categories					
	Loss > 5 kg	Loss > 2.5 and ≤ 5 kg	Loss or gain ≤ 2.5 kg	Gain > 2.5 and ≤ 5 kg	Gain > 5 and ≤ 10 kg	Gain > 10 kg
Former (2317, 32.6%)	135 (37.3)	163 (31.5)	1150 (31.2)	504 (32.7)	304 (36.2)	61 (40.4)
Never (4215, 59.4%)	195 (53.9)	298 (57.6)	2260 (61.2)	919 (59.7)	463 (55.0)	80 (53.0)
Physical activity (1HE)						
Inactive (1761, 24.8%)	115 (31.8)	145 (28.0)	906 (24.6)	354 (23.0)	201 (23.9)	40 (26.5)
Moderately inactive (2360, 33.2%)	119 (32.9)	168 (32.5)	1228 (33.3)	518 (33.6)	275 (32.7)	52 (34.4)
Moderately active (1722, 24.2%)	82 (22.6)	117 (22.6)	896 (24.3)	392 (25.4)	202 (24.0)	33 (21.8)
Active (1258, 17.7%)	46 (12.7)	87 (16.8)	660 (17.9)	276 (17.9)	163 (19.4)	26 (17.2)
Social class (1HE)						
Non-manual (4502, 63.4%)	222 (61.3)	339 (65.6)	2395 (64.9)	933 (60.6)	532 (63.3)	81 (53.6)
Manual (2599, 36.6%)	140 (38.7)	178 (34.4)	1295 (35.1)	607 (39.4)	309 (36.7)	70 (46.4)
Educational level (1HE)						
No qualifications (2598, 36.6%)	142 (39.2)	183 (35.4)	1356 (36.8)	554 (36.0)	296 (35.2)	67 (44.4)
O level and above (4503, 63.4%)	220 (60.8)	334 (64.6)	2334 (63.2)	986 (64.0)	545 (64.8)	84 (55.6)
Lost weight in last 5 years (2HE)						
Diet (777, 10.9%)	129 (16.6)	118 (15.5)	296 (37.9)	115 (14.8)	83 (10.5)	36 (4.6)
Illness (175, 2.5%)	31 (18.2)	23 (12.7)	67 (38.7)	30 (17.1)	17 (9.4)	7 (3.9)

Continuous variables are Mean (SD) and categorical variables are n (%)

1HE 1st health examination, *2HE* 2nd health examination, *BMI* body mass index

attended 2HE. Additionally, the percentage of deaths that occurred was lower in both men and women, after exclusion criteria were applied, and the percentage of participants who maintained their weight was slightly higher, which may be indicative of healthy volunteer bias. Nevertheless, the cohort still represents a diverse population with a wide socio-economic distribution and range of lifestyle factors, including physical activity, smoking status and weight.

Table 1 displays descriptive characteristics of men and women, by weight change category. Weight maintenance, which corresponded to a mean annual weight increase of 0.10 kg/year (SD 0.39) in men and 0.11 kg/year (SD 0.40), was observed in 54% of men and 52% of women. Participants with the highest weight gain or loss compared to weight maintenance were those with the highest weight and BMI at 1HE. Current smokers at 2HE were more likely to have lost weight whereas former smokers were more likely to have gained weight. Those who lost weight were more likely to be physically inactive; physically active participants were least likely to have lost weight. Manual workers were more likely to have gained more than 10 kg whereas non-manual workers were more likely to have lost more than 5 kg, compared to maintaining their weight. Women with no qualifications were more likely to have gained more than 10 kg, compared to weight maintenance A higher proportion of women than men said that they had lost weight as a result of dieting (10.9 vs. 4.9% respectively). However, approximately 38% of these participants who said that they had dieted were within 2.5 kg of their baseline weight, while 35% of men and 32% of women had lost more than 2.5 kg and 27% of men and 30% of women had gained more than 2.5 kg from the baseline assessment. Similar percentages of men and women stated that illness was the cause of their weight loss (2.4 vs. 2.5% respectively).

Main analyses: all-cause and cause-specific mortality

Over a median follow-up period of 15 years, 1421 deaths in men were recorded (401 deaths from CVD, 539 cancer-related deaths, 135 deaths from respiratory diseases and 346 deaths from other causes).

Total and cause-specific mortality HRs by weight change category for men are shown in Table 2. Men who lost weight had a statistically significant higher hazard of all-cause mortality than those who maintained their weight (HR 1.83 (CI 1.47–2.29) in those who lost more than 10 kg and 1.29 (CI 1.09–1.54) in those who lost between 2.5 and 5 kg); those who gained more than 10 kg also had a higher hazard but this was not significant. The findings for CVD mortality in men were stronger than for all-cause mortality. In model 3, adjusting for age, smoking, BMI, physical

Table 2 Total and cause-specific mortality in 5479 men by weight change category

	Weight change categories					
	Loss > 5 kg	Loss > 2.5 and ≤ 5 kg	Loss or gain ≤ 2.5 kg	Gain > 2.5 and ≤ 5 kg	Gain > 5 and ≤ 10 kg	Gain > 10 kg
Men, N	215	423	2983	1206	577	75
All cause mortality						
Number of events (%)	91 (41.9)	154 (36.2)	801 (26.5)	259 (21.1)	128 (21.9)	20 (26.3)
Model 1	*** 1.96 (1.57–2.44)	** 1.36 (1.14–1.62)	Ref	0.96 (0.83–1.11)	1.09 (0.90–1.32)	* 1.66 (1.06–2.59)
Model 2	*** 1.93 (1.55–2.40)	** 1.31 (1.10–1.56)	Ref	0.94 (0.81–1.08)	1.02 (0.84–1.23)	1.49 (0.95–2.32)
Model 3	*** 1.83 (1.46–2.29)	** 1.29 (1.09–1.54)	Ref	0.94 (0.81–1.08)	1.01 (0.84–1.23)	1.49 (0.95–2.33)
CVD mortality						
Number of events (%)	31 (14.3)	47 (11.0)	218 (7.2)	77 (6.3)	34 (5.8)	7 (9.2)
Model 1	*** 2.46 (1.68–3.61)	* 1.52 (1.10–2.09)	Ref	1.07 (0.82–1.39)	1.14 (0.80–1.65)	* 2.26 (1.06–4.80)
Model 2	*** 2.44 (1.66–3.58)	* 1.47 (1.07–2.02)	Ref	1.04 (0.80–1.36)	1.07 (0.74–1.54)	2.02 (0.95–4.31)
Model 3	*** 2.09 (1.41–3.09)	* 1.41 (1.02–1.94)	Ref	1.06 (0.81–1.38)	1.05 (0.73–1.51)	1.90 (0.89–4.07)
Cancer mortality						
Number of events (%)	28 (12.9)	53 (12.4)	310 (10.2)	112 (9.1)	39 (6.7)	8 (10.5)
Model 1	* 1.52 (1.03–2.24)	1.19 (0.88–1.60)	Ref	1.02 (0.82–1.27)	0.75 (0.53–1.06)	1.53 (0.76–3.09)
Model 2	* 1.50 (1.02–2.21)	1.15 (0.86–1.54)	Ref	1.00 (0.80–1.24)	* 0.70 (0.50–0.99)	1.37 (0.68–2.76)
Model 3	1.45 (0.98–2.15)	1.14 (0.84–1.52)	Ref	1.01 (0.80–1.25)	* 0.70 (0.49–0.99)	1.34 (0.66–2.72)
Respiratory mortality						
Number of events (%)	15 (6.9)	16 (3.8)	66 (2.2)	18 (1.5)	18 (3.1)	4 (5.3)
Model 1	*** 4.35 (2.48–7.64)	* 1.74 (1.01–3.02)	Ref	0.85 (0.50–1.46)	** 2.14 (1.26–3.61)	** 4.61 (1.68–12.68)
Model 2	*** 4.22 (2.40–7.43)	1.59 (0.92–2.75)	Ref	0.80 (0.47–1.38)	* 1.80 (1.06–3.05)	* 3.56 (1.29–9.82)
Model 3	*** 4.50 (2.51–8.08)	1.62 (0.93–2.82)	Ref	0.81 (0.47–1.38)	** 1.82 (1.08–3.10)	** 3.97 (1.43–11.01)
Other cause mortality						
Number of events (%)	17 (7.8)	38 (8.9)	207 (6.8)	52 (4.2)	37 (6.3)	1 (1.3)
Model 1	1.42 (0.85–2.36)	1.33 (0.94–2.36)	Ref	0.77 (0.57–1.05)	1.29 (0.90–1.83)	0.34 (0.05–2.44)
Model 2	1.40 (0.84–2.32)	1.30 (0.92–1.84)	Ref	0.76 (0.56–1.04)	1.24 (0.87–1.77)	0.33 (0.04–2.34)
Model 3	1.41 (0.84–2.36)	1.30 (0.92–1.85)	Ref	0.75 (0.55–1.02)	1.25 (0.88–1.78)	0.34 (0.05–2.39)

Associations were assessed using Cox proportional hazards regression with a median follow-up from 2HE of 15 years. Results are hazard ratios and 95% confidence intervals, HR (95% CI)

Model 1: adjusted for age (continuous)

Model 2: Model 1 + further adjusted for smoking (categorical)

Model 3: Model 2 + further adjusted for BMI (continuous), physical activity (categorical), social class (categorical) and educational level (categorical)

2HE 2nd health examination *CVD* cardiovascular disease, *BMI* body mass index

Significance of HRs: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

activity, social class and educational level, men who had a weight loss greater than 5 kg had more than double the hazard of CVD mortality, compared to those who maintained their weight [HR 2.09 (CI 1.41–3.09)]. Borderline significant findings for cancer mortality in men were found in those who lost the greatest amount of weight [HR 1.45 (CI 0.98–2.15)] whereas those who gained between 5 and 10 kg had a significantly lower hazard of 0.70 (CI 0.49–0.99). Gaining or losing more than 5 kg was significantly associated with a higher hazard of death from respiratory causes. In model 3, men who lost more than 5 kg had a HR of dying from respiratory causes of 4.50 (CI 2.51–8.08) whereas those who gained more than 10 kg had a HR of 3.97 (CI 1.43–11.01). After the exclusion of participants who self-reported having asthma or bronchitis at either time-point, the HR in men who lost more than 5 kg minimally attenuated to 4.08 (CI 2.00–8.33); in those who gained more than 10 kg, the HR was 2.71 (0.65–11.24) (data not shown). No significant findings were found with regard to weight change and dying from other causes in men. In general, the addition of BMI, physical activity, social class and educational level to the models had minimal effects on the HRs. Adjusting for categories of BMI, rather than as a continuous variable minimally changed the weight change-mortality associations.

In women, 1182 deaths were recorded over a median follow-up period of 15 years, (348 deaths from CVD, 442 cancer-related deaths, 91 deaths from respiratory causes

Table 3 Total and cause-specific mortality in 7101 women by weight change category

	Weight change categories					
	Loss > 5 kg	Loss > 2.5 and ≤ 5 kg	Loss or gain ≤ 2.5 kg	Gain > 2.5 and ≤ 5 kg	Gain > 5 and ≤ 10 kg	Gain > 10 kg
Women, N	362	517	3690	1540	841	151
All cause mortality						
Number of events (%)	94 (25.4)	128 (24.3)	651 (17.3)	223 (14.2)	115 (13.5)	15 (9.8)
Model 1	*** 1.77 (1.42–2.20)	** 1.34 (1.11–1.63)	Ref	0.94 (0.81–1.10)	1.14 (0.93–1.39)	1.06 (0.63–1.77)
Model 2	*** 1.72 (1.38–2.15)	** 1.32 (1.08–1.59)	Ref	0.94 (0.80–1.09)	1.12 (0.91–1.37)	1.02 (0.61–1.70)
Model 3	*** 1.68 (1.34–2.10)	** 1.32 (1.09–1.60)	Ref	0.93 (0.80–1.09)	1.11 (0.90–1.36)	0.98 (0.58–1.64)
CVD mortality						
Number of events (%)	25 (6.8)	33 (6.3)	196 (5.2)	70 (4.5)	33 (3.9)	6 (3.9)
Model 1	* 1.56 (1.02–2.39)	1.09 (0.75–1.59)	Ref	0.97 (0.73–1.28)	1.24 (0.86–1.80)	1.84 (0.81–4.17)
Model 2	* 1.55 (1.01–2.37)	1.08 (0.74–1.57)	Ref	0.96 (0.72–1.28)	1.23 (0.85–1.79)	1.83 (0.81–4.16)
Model 3	1.54 (1.00–2.37)	1.10 (0.75–1.60)	Ref	0.97 (0.73–1.29)	1.24 (0.85–1.80)	1.75 (0.77–4.00)
Cancer mortality						
Number of events (%)	30 (8.1)	47 (8.9)	234 (6.2)	90 (5.7)	48 (5.6)	5 (3.3)
Model 1	* 1.53 (1.04–2.23)	1.38 (1.00–1.91)	Ref	1.01 (0.79–1.29)	1.14 (0.83–1.56)	0.72 (0.30–1.76)
Model 2	* 1.48 (1.01–2.16)	1.35 (0.98–1.86)	Ref	0.99 (0.78–1.27)	1.10 (0.81–1.51)	0.68 (0.28–1.66)
Model 3	1.36 (0.92–2.01)	1.33 (0.96–1.83)	Ref	0.98 (0.76–1.25)	1.07 (0.78–1.47)	0.64 (0.26–1.55)
Respiratory mortality						
Number of events (%)	11 (3.0)	12 (2.3)	50 (1.3)	13 (0.8)	8 (0.9)	1 (0.6)
Model 1	* 2.34 (1.15–4.77)	1.56 (0.83–2.94)	Ref	0.74 (0.40–1.37)	1.04 (0.47–2.31)	1.26 (0.17–9.22)
Model 2	* 2.28 (1.18–4.65)	1.52 (0.81–2.85)	Ref	0.74 (0.40–1.36)	1.02 (0.46–2.27)	1.22 (0.17–8.98)
Model 3	* 2.30 (1.11–4.74)	1.55 (0.82–2.91)	Ref	0.75 (0.40–1.38)	1.04 (0.47–2.31)	1.25 (0.17–9.24)
Other cause mortality						
Number of events (%)	28 (7.6)	36 (6.8)	171 (4.6)	50 (3.2)	26 (3.1)	3 (2.0)
Model 1	*** 2.21 (1.48–3.31)	* 1.49 (1.04–2.14)	Ref	0.87 (0.63–1.19)	1.06 (0.69–1.61)	0.98 (0.31–3.09)
Model 2	*** 2.17 (1.45–3.25)	* 1.47 (1.02–2.11)	Ref	0.86 (0.63–1.18)	1.04 (0.68–1.59)	0.95 (0.30–3.00)
Model 3	*** 2.17 (1.44–3.27)	* 1.48 (1.03–2.12)	Ref	0.86 (0.63–1.19)	1.04 (0.68–1.60)	0.95 (0.30–3.00)

Associations were assessed using Cox proportional hazards regression with a median follow-up from 2HE of 15 years. Results are hazard ratios and 95% confidence intervals, HR (95% CI)

Model 1: adjusted for age (continuous)

Model 2: Model 1 + further adjusted for smoking (categorical)

Model 3: Model 2 + further adjusted for BMI (continuous), physical activity (categorical), social class (categorical) and educational level (categorical)

2HE 2nd health examination *CVD* cardiovascular disease, *BMI* body mass index

Significance of HRs: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

and 301 deaths from other causes). Table 3 presents data on total and cause-specific mortality HRs for women, by weight change category. Women who lost more than 5 kg had a significantly higher hazard of all-cause mortality of 1.68 (CI 1.34–2.10) compared to those who maintained their weight, whilst those who lost between 2.5 and 5 kg had a HR of 1.32 (CI 1.09–1.60). Losing weight and gaining more than 5 kg was associated with a higher hazard for CVD mortality, but these findings were not significant, although a weight loss of more than 5 kg was borderline significant [HR 1.54 (CI 1.00–2.37)]. Regarding cause-specific mortality in women who lost more than 5 kg, only respiratory deaths and other causes of deaths were significant in model 3. After the exclusion of participants who self-reported having asthma or bronchitis at either time-point, this HR in those who lost more than 5 kg was no longer significant [HR 1.78, (CI 0.68–4.69)]. In those who lost more than 5 kg, the hazard for deaths from other causes was 2.17 (CI 1.44–3.27). In general, the addition of BMI, physical activity, social class and educational level to the models had minimal effects on the HRs. Adjusting for categories of BMI instead of using BMI as a continuous variable minimally changed the observed associations between weight change and mortality. See

Supplementary Table 2 for the full coefficient tables of model 3 for all-cause mortality in men and women.

Sensitivity analyses

HRs for all-cause mortality, adjusted for age, sex, BMI, physical activity, smoking status, social class and educational level, and by stratified variables per weight change category are shown in Table 4.

Similar higher total mortality HRs were found for both men and women who lost weight. Compared to a stable weight, women who gained more than 10 kg had a HR of 0.98 (CI 0.58–1.64) whereas in men, the hazard was higher [1.49 (CI 0.95–2.33)]. We observed similar associations among participants who were younger or older than 65 years of age, whether categorised using 1HE or 2HE data in all weight change categories, with the exception of the greatest weight gain category using 2HE data [the latter probably due to the low number of events and/or participants in respective strata (11/173 and 24/53), which resulted in wide confidence intervals]. Higher hazards were observed in all BMI classifications at both 1HE and 2HE in those who lost weight compared to weight maintenance. In all four physical activity categories, HRs for total mortality were all of a similar direction for weight loss.

HRs for all-cause mortality and weight loss were generally consistent in the three categories of smoking status. However, there was no significant higher risk of mortality in either current or never smokers who lost < 5 kg. Data on the number of cigarettes smoked was available for 1013 of the 1150 current smokers at 1HE and additionally adjusting for this did not modify the association among smokers. We further examined the association of all-cause mortality with weight change, taking into account changes in smoking status (Supplementary Table 3). The greatest weight gain was observed among those participants who reported to have stopped smoking between 1HE and 2HE. Exclusion of recent smokers from the current smokers at 2HE strengthened the all-cause mortality HRs in the greatest weight loss category (HR 1.41 CI 0.92–2.18) (data not shown). Minimal changes were observed in the HRs of former smokers, after the exclusion of those who had recently stopped smoking.

We observed similar associations among participants regarding social class and educational level, in all weight change categories, with those who lost more than 5 kg having significantly higher HRs for all-cause mortality.

Even after excluding participants who died within 3 or 5 years of the 2HE and those who said they had lost weight because of illness, participants who lost weight had significantly higher HRs for all-cause mortality than those who maintained their weight. The observed higher HRs for all-cause mortality in those who had lost weight remained consistent after excluding participants who self-reported having asthma and/or bronchitis at either time-point. We then ran this analysis for respiratory mortality, rather than all-cause mortality, and found that those who lost more than 5 kg had a HR of 2.89 (CI 1.64–5.12), compared to those who maintained their weight; those who lost between 2.5 and 5 kg had a HR of 1.40 (CI 0.84–2.36).

In Supplementary Table 4, we further examined models 2 and 3 for all-cause mortality in both men and women, replacing smoking status at 1HE with smoking history, as categorised in Supplementary Table 3, and observed minimal changes in the HRs.

Time-varying analysis

When we included a time-interaction variable with weight change, the HR in those who lost more than 5 kg increased from 1.7 to 3.25 ($P = 0.001$) and the HR for follow-up time was 0.73, i.e., participants had a threefold hazard compared to participants who maintained their weight within a year from 2HE; however, this hazard decreased by 27% with every year of follow-up. This decrease in the hazard during follow-up might be explained by misclassification over time of participants with regard to exposure. It is also plausible that some participants lost weight because they were (acutely) ill and therefore had a higher hazard of dying at the start of follow-up.

Discussion

Summary of main findings

Findings from this population-based cohort study of 12,580 middle-aged and elderly men and women suggest that weight loss, over the previous 4 years or so, is associated with higher mortality over the next 15 years of follow-up. This result was observed after excluding those who were underweight or who self-reported cancer or CVD, at either time-point. This association was also evident in subgroups of the population, after stratification for age, smoking, BMI, physical activity and the exclusion of individuals who said they had lost weight due to illness and deaths within the first 5 years of follow-up, as well as in dieters who reported to have lost more than 5 kg. Results for weight gain were inconclusive.

Strengths and limitations

The major strengths of our study include its prospective design, its large population of free-living, middle-aged and elderly men and women, long follow-up time and the availability of information on a large number of factors

Table 4 Cox multivariable-adjusted HRs[a] after 15 years of follow-up for all-cause mortality in 12,580 men and women

				Weight change categories					
				Loss > 5 kg	Loss > 2.5 and ≤ 5 kg	Loss or gain ≤ 2.5 kg	Gain > 2.5 and ≤ 5 kg	Gain > 5 and ≤ 10 kg	Gain > 10 kg
Events (n)/N				180/577	277/940	1407/6673	466/2746	238/1418	35/226
	N	%	Events	HR (95% CI)	HR (95% CI)		HR (95% CI)	HR (95% CI)	HR (95% CI)
All	12,580	100.0	2603	*** 1.74 (1.48–2.03)	*** 1.31 (1.15–1.49)	Ref	0.93 (0.84–1.04)	1.05 (0.91–1.21)	1.21 (0.86–1.69)
By sex									
Men	5479	43.6	1421	*** 1.83 (1.46–2.29)	** 1.29 (1.09–1.54)	Ref	0.94 (0.81–1.08)	1.01 (0.84–1.23)	1.49 (0.95–2.33)
Women	7101	56.4	1182	*** 1.68 (1.34–2.10)	** 1.32 (1.09–1.60)	Ref	0.93 (0.80–1.09)	1.11 (0.90–1.36)	0.98 (0.58–1.64)
By age (1HE)									
<65 years old	9251	73.5	982	*** 1.64 (1.26–2.15)	** 1.36 (1.08–1.70)	Ref	0.91 (0.77–1.08)	1.07 (0.88–1.30)	1.09 (0.70–1.72)
≥65 years old	3329	26.5	1621	*** 1.80 (1.48–2.19)	** 1.28 (1.10–1.51)	Ref	0.95 (0.83–1.09)	1.02 (0.84–1.24)	1.37 (0.82–2.29)
By age (2HE)									
<65 years old	7734	61.5	619	** 1.67 (1.20–2.32)	1.26 (0.94–1.69)	Ref	0.90 (0.73–1.10)	1.04 (0.81–1.33)	0.76 (0.42–1.40)
≥65 years old	4846	38.5	1984	*** 1.73 (1.45–2.08)	*** 1.29 (1.12–1.50)	Ref	0.94 (0.84–1.07)	1.03 (0.87–1.22)	* 1.51 (1.01–2.27)
By smoking status (1HE)									
Current	1150	9.1	318	* 1.67 (1.10–2.54)	1.25 (0.89–1.76)	Ref	0.73 (0.54–1.00)	0.82 (0.57–1.19)	0.80 (0.33–1.98)
Former	5141	40.9	1293	** 1.54 (1.20–1.96)	*** 1.41 (1.17–1.68)	Ref	0.95 (0.82–1.10)	1.06 (0.87–1.29)	1.23 (0.78–1.95)
Never	6289	50.0	992	*** 1.98 (1.56–2.53)	1.19 (0.95–1.48)	Ref	0.98 (0.83–1.16)	1.14 (0.90–1.43)	1.44 (0.78–2.62)
By BMI (1HE)									
≥ 18.5 to < 25 kg/m^2	5288	42.0	973	** 1.76 (1.23–2.50)	** 1.43 (1.15–1.79)	Ref	0.93 (0.79–1.10)	0.96 (0.75–1.23)	1.07 (0.53–2.16)
≥ 25 to < 30 kg/m^2	5630	44.8	1226	*** 1.84 (1.47–2.30)	* 1.25 (1.04–1.50)	Ref	0.94 (0.81–1.10)	1.02 (0.83–1.25)	1.43 (0.88–2.32)
≥ 30 kg/m^2	1662	13.2	404	** 1.56 (1.15–2.12)	1.33 (0.96–1.86)	Ref	0.90 (0.67–1.22)	1.24 (0.92–1.67)	1.02 (0.54–1.95)
By BMI (2HE)									
≥ 18.5 to < 25 kg/m^2	4578	36.4	878	*** 1.82 (1.45–2.30)	* 1.28 (1.05–1.55)	Ref	0.92 (0.74–1.13)	0.72 (0.46–1.11)	4.84 (0.67–34.69)
≥ 25 to < 30 kg/m^2	5900	46.9	1246	*** 1.75 (1.36–2.24)	** 1.30 (1.07–1.58)	Ref	0.95 (0.82–1.10)	1.06 (0.87–1.30)	0.92 (0.43–1.94)
≥ 30 kg/m^2	2102	16.7	479	* 1.56 (1.02–2.40)	1.42 (0.98–2.06)	Ref	0.91 (0.72–1.16)	1.05 (0.82–1.35)	1.08 (0.71–1.65)
By physical activity (1HE)									
Inactive	3207	25.6	964	*** 1.82 (1.44–2.30)	*** 1.46 (1.18–1.79)	Ref	1.10 (0.92–1.30)	1.04 (0.82–1.32)	1.62 (0.96–2.72)
Moderately inactive	3716	29.5	723	*** 1.84 (1.35–2.51)	1.21 (0.94–1.56)	Ref	0.94 (0.78–1.15)	1.21 (0.93–1.58)	1.35 (0.72–2.55)
Moderately active	3103	24.7	520	* 1.48 (1.01–2.16)	1.28 (0.96–1.71)	Ref	* 0.75 (0.59–0.96)	0.89 (0.66–1.20)	0.80 (0.36–1.80)
Active	2554	20.3	396	* 1.72 (1.02–2.87)	1.18 (0.85–1.65)	Ref	0.82 (0.62–1.07)	1.04 (0.73–1.48)	0.82 (0.30–2.23)
By social class (1HE)									
Non-manual	7903	62.8	1610	*** 1.85 (1.52–2.25)	1.14 (0.96–1.36)	Ref	0.94 (0.83–1.08)	1.02 (0.85–1.21)	1.34 (0.87–2.07)
Manual	4677	37.2	993	** 1.55 (1.18–2.02)	*** 1.60 (1.32–1.95)	Ref	0.93 (0.78–1.10)	1.09 (0.87–1.36)	1.05 (0.61–1.79)

Table 4 (continued)

	N	%	Events	HR (95% CI)	HR (95% CI)		HR (95% CI)	HR (95% CI)	HR (95% CI)
By education level (1HE)									
No qualifications	4045	32.2	1089	*** 1.77 (1.39–2.26)	* 1.26 (1.02–1.54)	Ref	0.92 (0.79–1.08)	1.12 (0.91–1.38)	1.35 (0.83–2.20)
O level and above	8535	67.8	1514	*** 1.72 (1.40–2.12)	** 1.33 (1.13–1.58)	Ref	0.95 (0.82–1.09)	1.01 (0.84–1.21)	1.08 (0.67–1.73)
Excluding early deaths									
Events (n)/N				160/557	257/920	1302/6568	440/2720	221/1401	31/222
Excluding deaths < 3 years	12,388	98.5	2411	*** 1.70 (1.44–2.01)	*** 1.32 (1.16–1.51)	Ref	0.95 (0.86–1.06)	1.06 (0.91–1.22)	1.16 (0.81–1.67)
Events (n)/N				135/532	231/894	1190/6456	401/2681	202/1382	28/219
Excluding deaths < 5 years	12,164	96.7	2187	*** 1.61 (1.35–1.94)	*** 1.31 (1.14–1.51)	Ref	0.95 (0.85–1.06)	1.06 (0.91–1.23)	1.17 (0.80–1.70)
Events (n)/N				151/524	263/894	1364/6552	452/2697	232/1393	32/215
Excluding persons who said they had lost weight due to illness	12,275	97.6	2494	*** 1.61 (1.36–1.91)	*** 1.32 (1.15–1.50)	Ref	0.94 (0.84–1.04)	1.05 (0.92–1.21)	1.18 (0.83–1.67)
Events (n)/N				46/183	37/158	57/398	19/148	20/111	4/46
Persons who said they had lost weight due to dieting N (%)	1044	8.3	183	1.48 (0.99–2.22)	1.40 (0.92–2.13)	Ref	0.78 (0.46–1.33)	* 1.92 (1.14–3.23)	0.96 (0.35–2.67)
Events (n)/N				135/464	223/763	1084/5298	379/2266	176/1120	28/172
Excluding self-reported asthma and bronchitis at 1HE and 2HE N (%)	10,083	80.2	2025	*** 1.71 (1.42–2.05)	*** 1.35 (1.17–1.56)	Ref	0.95 (0.84–1.07)	1.04 (0.88–1.22)	1.32 (0.91–1.93)

Results are given for stratified variables by weight change category

1HE 1st health examination, *2HE* 2nd health examination, *BMI* body mass index

[a]Adjusted for age, sex, BMI, physical activity, smoking, social class and educational level (except where the categorical variable was used for stratification)

Significance of HRs: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

associated with weight change. In addition, height and weight were objectively measured rather than self-reported and were available at both time-points. In an effort to address reverse causality, we excluded participants with self-reported cancer or CVD, in addition to those who had a BMI < 18.5 kg/m^2, at either time-point. In our subgroup analyses, we excluded deaths within the first 3 and 5 years, in addition to those who said they had lost weight due to illness.

The main limitations of our cohort study include self-reported disease history, healthy volunteer bias and attrition. It is likely that some individuals in our study had an underlying disease condition that they did not report which may have resulted in weight loss and subsequent death. Whilst the more frail participants may not have returned for the 2HE and/or tended to have been excluded and therefore be under-represented in our analyses, it is plausible that selective attrition of the frailest participants, may have led to an under-estimation of our findings. However, it is also possible that participants in the weight loss categories were the more frail study participants, who were pre-frail at 2HE. The inability to take into account all changes in behaviours, including physical activity during follow-up time is also a limitation, which may have resulted in misclassification of individuals, with subsequent effects on observed associations. Our findings relate solely to changes in weight and not any other anthropometric measurements, such as height, waist circumference, waist–hip ratio, fat mass or muscle mass.

Comparison of cause-specific mortality with other studies

Regarding CVD mortality, statistically significant associations, after multi-variate adjustment, were found in men who lost weight, but not in women; statistical power was more limited due to the lower number of CVD deaths in women, although the HR in those who lost more than 5 kg suggests a higher hazard compared to stable weight. A prospective study in 5608 middle-aged men by Wannamethee et al. [12] found that sustained weight loss was associated with significantly higher total and CVD mortality, even after adjustment for lifestyle factors and pre-existing diseases and ill-health. Results from the Melbourne Collaborative Cohort Study [9] illustrate that weight loss in men and women, compared to minimal weight increase, was associated with a higher risk of all-cause and CVD mortality. Adams et al. [27] also found that weight loss was associated with a higher hazard ratio for CVD mortality in those aged 50–69 years [HR 1.51 (CI 1.35–1.69)].

We observed higher hazards of cancer mortality in both men and women who lost weight, although these did not quite reach significance. The Melbourne Collaborative Cohort Study [9] concluded that a change in body weight was not associated with obesity-related cancer mortality but the small number of cancer-related deaths in their study may explain why no association was observed. However, three prospective cohort studies found a positive association between cancer mortality and weight loss [28–30], although two of these studies were carried out in Japanese men and women [29, 30] and may not be generalizable to other populations as Japanese obesity rates differ substantially from those of Western populations [31] as do their cancer incidence and mortality rates and major cancer types [32].

In men, there was a significantly higher hazard of dying from respiratory causes in those who lost or gained more than 5 kg, whereas a higher hazard was only found in women who lost more than 5 kg. Attention must be drawn to the low numbers of deaths due to respiratory causes, particularly in men who gained more than 10 kg ($n = 4$). After the exclusion of participants who self-reported having asthma or bronchitis at either time-point, the HRs of dying from respiratory causes in those who gained more than 5 kg attenuated and were no longer significant. It is well-known that underweight individuals have an increased risk of dying from chronic respiratory disease [33–35]. To address reverse causation, we excluded all underweight participants (BMI < 18.5) at both time-points, in addition to deaths within the first 5 years. In our subgroup analyses, we showed that weight loss was still associated with higher hazards for all-cause mortality, even after excluding participants with prevalent respiratory disease at either time-point. However, it is possible that our exclusion period of deaths within 5 years is too short [35]. The Prospective Cohort Studies Collaboration of 900,000 adults found that each 5-unit lowering in BMI from 25 to 15 kg/m^2 was associated with a 1.7-fold increase in respiratory mortality [1].

Women who lost more than 2.5 kg had a higher risk of dying from other causes. We cannot be certain that all relevant confounders have been addressed nor that unmeasured confounding is not an issue. It is plausible that this may be explained by undiagnosed pre-existing diseases not explained by the exclusion factors applied. Further disaggregation of this miscellaneous category into more specific disease types may help clarify these results.

Explanatory factors of all-cause mortality compared to other studies

In our subgroup analyses, we found that both younger [$<$ 65 years at 1HE (73.5% of study population)] and older participants ($\geq$ 65 years) who lost weight had similar higher hazards for total mortality compared to weight

maintenance. These findings are in agreement with recent studies that have found higher total mortality risks with weight loss in middle-aged and elderly populations [9, 18].

We observed higher hazards in all BMI classifications at both 1HE and 2HE in those who lost weight compared to weight maintenance. In a stratified analysis, we observed that being obese at 1HE was associated with a higher hazard of death [1.16 (CI 1.03–1.30)] but not being overweight [0.94 (CI 0.86–1.02)] compared to normal weight (data not shown). Our results are therefore not different from the general assumption that being obese is associated with higher mortality [36, 37]. However, weight loss is an additional factor in this association and potentially an effect modifier. In this population-based cohort study, it seems that weight loss when obese is less hazardous than when overweight or normal weight. Controversy has, until recently, surrounded weight loss therapies in obese older adults [38, 39]. However, evidence from randomised controlled trials have reported positive outcomes on physical function, muscle quality and inflammatory status [40, 41].

When studying weight change, smoking is seen as an important source of confounding [42]. Previous studies investigating weight loss and mortality have therefore tended either to restrict analyses to never-smokers or have adjusted for smoking status. The rationale for this is that smokers tend to weigh less than non-smokers but have considerably higher mortality rates [43]. Some controversy surrounds the association between smoking and weight change; in our study, current smokers at 1HE had a greater mean increase in weight than either former or never smokers (1.59, 1.35 and 1.29 kg respectively), which is in agreement with recent studies [44, 45] but prospective investigations performed on three separate large US cohorts found that current non-obese smokers lost weight over a 4-year period [46].

In our analyses, we included smoking status in our multivariate-adjusted models but also stratified by smoking status. In the stratified analysis, we observed higher hazards for all-cause mortality with weight loss in never, former and current smokers, although the HRs were only significant in all three categories when weight loss was greater than 5 kg, suggesting that weight loss greater than 5 kg in this population, was positively associated with higher all-cause mortality, irrespective of smoking status.

There is a wealth of information on weight change and cessation of smoking [47, 48]. Whilst this study was not designed to investigate weight change in relation to changes in smoking status, we found that when we did so, that the greatest mean increase in weight was found in those who had recently stopped smoking (mean = 3.4 kg, SD = 4.8) and that long-term smokers had actually a smaller weight increase than long-term former smokers and never smokers (1.1, 1.4 and 1.3 kg respectively). Those who had recently started smoking had the smallest mean weight increase (0.2 kg). We have shown that our association of higher all-cause mortality with weight loss was strengthened in smokers (2HE) after the removal of recent smokers and that the exclusion of those who had recently stopped smoking only minimally affected the HRs in former smokers. We reran analyses for all-cause mortality, replacing smoking status at 2HE with smoking history, as classified in Supplementary Table 3 and observed minimal changes to the HR (Supplementary Table 4).

Associations between all-cause mortality and weight loss also remained when the data were investigated by categories of physical activity, with those who were less active tending to have slightly higher HRs than more active participants. The inactive category who gained weight had minimally higher mortality hazards but HRs were not significant. However, a large, European, prospective cohort study of 288,498 men and women, which included EPIC-Norfolk participants, found that baseline self-reported physical activity was not associated with a change in body weight in men or women, after adjustment for confounders and suggested that the association between lower physical activity and a gain in body weight may be restricted to younger and normal-weight individuals [49]. Only data on self-reported physical activity at baseline are included in this paper and any change in this behaviour during follow-up, or indeed random or systematic measurement error, may have led to misclassification and attenuated any observed associations.

Weight loss may be classified as intentional or unintentional. Participants may make conscious efforts to lose weight, through changes in diet and/or exercise. A recent meta-analysis of 15 randomised controlled trials in obese older adults found that intentional weight loss may be associated with approximately a 15% reduction in all-cause mortality [50], whilst others, in agreement with our findings, observed a higher mortality risk [51, 52]. Alternatively, weight loss may be due to illness or the diagnosis of a chronic disease. Recent data from the Longitudinal Aging Study Amsterdam show that unintentional weight loss in the past 6 months due to medical or unknown reasons or due to a change in eating pattern (unintentional or intentional) was associated with an increased 3-year mortality risk among community-dwelling men and women, aged $\geq$ 55 years [53]; this finding relating to unintentional weight loss is in agreement with previous studies [15, 54], including data from this study. A study of 4331 older men concluded that those who lost (− 5%) weight, total lean mass, or total fat mass over a 4.6 years period had a higher risk of mortality than those whose weight remained stable [17].

We also explored the association of weight change and all-cause mortality after excluding those who said that they

had lost more than 5 kg (10 lbs) in the last 5 years, due to illness and in a subgroup who said that they had lost weight due to dieting. Once again, we found that the association of a higher HR with weight loss was still evident. However, numerous studies over the last 20–30 years have suggested that adults who diet in order to lose weight are more likely to gain weight in the future and even become obese [55–57]. In this analysis, of those participants who said that they had lost more than 5 kg due to dieting during the 5 years before the 2HE, 29% of them had actually gained weight between health examinations. It is possible that during this time period, they did lose weight but then regained it, and possibly more, but we are unable to verify this. Additionally, recent reviews have shown that normal-weight individuals who diet to lose weight are more likely to gain weight in the future than non-dieters [58, 59] and that dieting in those of normal weight compared to those who are overweight or obese may be a stronger predictor of future weight gain [56]. Zheng et al. [60] found that of six BMI trajectories, those who were in the overweight stable trajectory had the lowest mortality risk whereas those of normal weight who lost weight had the second highest mortality risk in a study of 9538 adults aged 51–77 years from the US Health and Retirement Study. Results from a prospective, population-based cohort study of 1975 men and women, aged 70–79 years, found that, over a 5 years period, weight cycling was associated with higher mortality risk in women: HR 1.62 (CI 1.15–2.30) and in men: HR 1.50 (CI 1.08–2.08) [18]. Weight cycling was also found to be a risk factor for mortality in the Cardiovascular Health Study [16], after adjustment for demographic risk factors, height, self-reported health and comorbidities: HR 1.66 (CI 1.38–2.00). We expressed the mean absolute annual weight change for those who said that they had lost weight due to dieting by BMI category at 1HE (Supplementary Table 5). Women who have a normal weight at baseline (BMI $\geq$ 20 and $<$ 25 kg/m^2) had a mean annual weight increase of 0.47 kg/year (SD 1.33); men who said that they lost weight by dieting did have a mean weight loss in each of the three BMI categories. These data on dieting in normal-weight women in our study provide further evidence of a subsequent gain in weight, and highlight the importance of objective weight measurements.

Public health considerations

Weight or BMI do not simply reflect fat mass but also bone and lean body mass or muscle. Thus, weight loss may indicate not just fat loss but also loss in lean body mass, which may be particularly relevant in an ageing population, as weight loss and weight cycling in older adults are considered problematic because recovery of muscle mass is difficult [61–63]. Whereas, individuals who maintain body weight in later life may be those who are more likely to maintain bone mass and muscle compared to those who lose weight [40, 64]. Lee et al. [17] found that older men who lost (− 5%) weight, total lean mass, or total fat mass over a 4.6 years period had a higher risk of mortality than those who maintained their weight. Rapid weight loss and decreased muscle mass and strength are commonly associated with frailty, which is associated with mortality [65]. Some excess body weight in pre-frail and frail adults in later life may be beneficial, as may interventions to maintain or promote weight gain in frail older adults [66]. Weight management plans for obese, elderly individuals should therefore be specifically tailored in an effort to maintain or increase quality of life and physical function [67, 68]. A recent systematic review and meta-analysis found that weight-reducing diets for obese adults were associated with a 18% relative reduction in all-cause mortality but the authors also conclude that their findings support public health measures to prevent weight gain [69]. Yang et al. found an inverse relationship between lung cancer survival and weight loss at presentation and a potentially protective effect of obesity [36], in the form of greater physiological reserves, (excess fat and muscle), which may also be beneficial in other diseases displaying high catabolic states. The recent NICE guideline recommends that staff are trained to deliver multicomponent programmes that cover weight management, dietary habits, safe physical activity and behaviour-change strategies and that this should include the ability to adapt interventions to individual needs [8]. Given the wealth of evidence on the health consequences of obesity, efforts should perhaps be focussed on young adults [70] regarding the importance of lifestyle, including adequate nutrition and physical activity, and of achieving and maintaining a healthy weight in earlier adulthood.

Conclusion

In summary, weight loss of more than 2.5 kg over an interval of approximately 4 years is associated with a higher mortality over 15 years of follow-up in this population-based cohort study of 12,580 middle-aged and elderly men and women. However, the potential presence of undiagnosed pre-existing disease and the inability to take weight cycling into account need to be remembered when interpreting these results. Unravelling the causal pathways underlying the observed association between objectively measured weight loss and subsequent higher mortality risk in this population-based study will require more detailed studies, including that of changes in body composition, such as muscle mass.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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3.3 Supplementary material

(https://link.springer.com/article/10.1007/s10654-017-0343-y#Sec21)

Supplementary table 1 Baseline characteristics of EPIC-Norfolk men and women who attended 1HE, and those who attended both 1HE and 2HE, before and after exclusion criteria were applied. Continuous variables are Mean (SD) and categorical variables are n (%).

	Men			Women		
	1HE N=11607	1HE and 2HE N=6582	1HE and 2HE with exclusions[a] N=5479	1HE N=14032	1HE and 2HE N=8446	1HE and 2HEwith exclusions[a] N=7101
Weight, kg	80.4 (11.5)	80.1 (11.0)	80.2 (10.9)	67.9 (11.8)	67.4 (11.3)	67.5 (11.1)
Age, years	59.6 (9.3)	59.8 (8.9)	59.0 (8.9)	58.9 (9.3)	58.4 (8.9)	57.8 (8.8)
BMI, kg/m2	26.5 (3.3)	26.4 (3.2)	26.3 (3.1)	26.6 (4.4)	25.9 (4.1)	25.9 (4.1)
Smoking status						
Current	1405 (12.2)	616 (9.4)	509 (9.3)	1579 (11.4)	764 (9.1)	641 (9.0)
Former	6284 (54.5)	3563 (54.5)	2902 (53.0)	4477 (32.2)	2632 (31.4)	2239 (31.5)
Never	3837 (33.3)	2355 (36.0)	2068 (37.7)	7837 (56.4)	4977 (59.4)	4221 (59.4)
Physical activity						
Inactive	3586 (30.9)	1826 (27.7)	1446 (26.4)	4277 (30.5)	2226 (26.4)	1761 (24.8)
Moderately inactive	2858 (24.6)	1649 (25.1)	1356 (24.8)	4493 (32.0)	2760 (32.7)	2360 (33.2)
Moderately active	2660 (22.9)	1632 (24.8)	1381 (25.2)	3116 (22.2)	2018 (23.9)	1722 (24.2)
active	2502 (21.6)	1475 (22.4)	1296 (23.6)	2146 (15.3)	1442 (17.1)	1258 (17.7)
Social class						
Non-manual	6657 (58.4)	4034 (62.2)	3401 (62.1)	8394 (61.4)	5304 (64.0)	4502 (63.4)
Manual	4744 (41.6)	2451 (37.8)	2078 (37.9)	5274 (38.6)	2978 (36.0)	2599 (36.6)
Educational level						
No qualifications	3534 (30.5)	1801 (27.4)	1447 (26.4)	5920 (42.2)	3169 (37.5)	2598 (36.6)
O level and above	8064 (69.5)	4779 (72.6)	4032 (73.6)	8103 (57.8)	5272 (62.5)	4503 (63.4)
Self-reported diseases						
CVD (yes)	798 (6.9)	394 (6.0)		324 (2.3)	160 (1.9)	
Cancer (yes)	450 (3.9)	233 (3.5)		960 (6.8)	568 (6.7)	
Asthma (yes)	892 (7.7)	485 (7.4)		1271 (9.1)	752 (8.9)	
Bronchitis (yes)	1029 (8.9)	560 (8.5)		1337 (9.5)	788 (9.3)	
Deaths	3995 (34.4)	2028 (30.8)	1421 (25.9)	3432 (24.5)	1627 (19.3)	1182 (16.6)
Weight change categories						
loss >5 kg		275 (4.2)	215 (3.9)		454 (5.4)	362 (5.1)
loss >2.5 & ≤5 kg		505 (7.7)	423 (7.7)		634 (7.5)	517 (7.3)
Reference		3565 (54.2)	2983 (54.4)		4377 (51.8)	3690 (52.0)
gain >2.5 & ≤5 kg		1420 (21.6)	1206 (22.0)		1802 (21.3)	1540 (21.7)
gain >5 & ≤10 kg		713 (10.8)	577 (10.5)		979 (11.6)	841 (11.8)
gain >10 kg		104 (1.6)	75 (1.4)		200 (2.4)	151 (2.1)

[a] self-reported cancer and CVD, missing data on weight, height, smoking status, social class, educational level and BMI < 18.5 kg/m^2

BMI body mass index, *1HE* 1st health examination, *2HE* 2nd health examination

Supplementary table 2. Association between weight change and all-cause mortality, adjusted for all considered variables.

	HR	P>z	95% CI	
MEN (1421/5479)				
Weight change category				
loss >5 kg	1.83	0.001	1.46	2.29
loss >2.5 & ≤5 kg	1.29	0.001	1.09	1.54
loss or gain ≤ 2.5 kg	Ref			
gain >2.5 & ≤5 kg	0.94	0.382	0.81	1.08
gain >5 & ≤10 kg	1.01	0.893	0.84	1.23
gain >10 kg	1.49	0.080	0.95	2.33
Age, (1HE), per year	1.13	0.001	1.12	1.14
Smoking status (1HE)				
Never	Ref			
Current	2.55	0.001	2.13	3.06
Former	1.40	0.001	1.24	1.59
BMI (1HE), per kg/m^2	1.01	0.159	1.00	1.03
Physical activity (1HE)				
Inactive	Ref			
Moderately inactive	0.87	0.044	0.76	1.00
Moderately active	0.80	0.003	0.70	0.93
Active	0.86	0.046	0.74	1.00
Social class (1HE)				
Non-manual	Ref			
Manual	1.08	0.164	0.97	1.22
Educational level (1HE)				
No qualifications	Ref			
O level & above	0.98	0.800	0.88	1.11
WOMEN (1182/7101)				
Weight change category				
loss >5 kg	1.68	0.001	1.34	2.10
loss >2.5 & ≤5 kg	1.32	0.005	1.09	1.60
loss or gain ≤ 2.5 kg	Ref			
gain >2.5 & ≤5 kg	0.93	0.375	0.80	1.09
gain >5 & ≤10 kg	1.11	0.328	0.90	1.36
gain >10 kg	0.98	0.931	0.58	1.64
Age, (1HE), per year	1.13	0.001	1.12	1.14
Smoking status (1HE)				
Never	Ref			
Current	2.13	0.001	1.76	2.58
Former	1.16	0.022	1.02	1.31
BMI (1HE), per kg/m^2	1.00	0.488	0.99	1.02

Physical activity (1HE)				
Inactive	Ref			
Moderately inactive	0.82	0.006	0.72	0.95
Moderately active	0.80	0.007	0.68	0.94
Active	0.74	0.003	0.60	0.90
Social class (1HE)				
Non-manual	Ref			
Manual	0.98	0.779	0.87	1.11
Educational level (1HE)				
No qualifications	Ref			
O level & above	0.82	0.001	0.72	0.92

adjusted for 1HE values of age (continuous), smoking status (categorical), BMI (continuous), physical activity (categorical), social class (categorical) and educational level (categorical)
1HE 1st health examination, *BMI* body mass index

Supplementary table 3 Cox multivariable-adjusted HRs[a] for the association between weight change category and all-cause mortality in 12580 men and women, stratified by smoking history.

				Weight change categories					
				loss >5 kg (n=577)	loss >2.5 & ≤5 kg (n=940)	loss or gain ≤2.5 kg (n=6673)	gain >2.5 & ≤5 kg (n=2746)	gain >5 & ≤10 kg (n=1418)	gain >10 kg (n=226)
	Weight change, mean (SD), kg	N	Events	HR (95% CI)	HR (95% CI)		HR (95% CI)	HR (95% CI)	HR (95% CI)
Longterm smokers (current-current)	1.1 (4.0)	897	253	* 1.58 (1.01 - 2.46)	1.18 (0.83 - 1.70)	Ref	** 0.61 (0.42 - 0.88)	0.87 (0.53 - 1.43)	0.82 (0.26 - 2.63)
Recent smokers (never/former - current)	0.2 (3.6)	112	25	0.63 (0.08 - 5.10)	2.41 (0.60 – 9.64)	Ref	1.21 (0.31 – 4.70)	1.34 (0.29 - 6.24)	[b]
Longterm stoppers (former- former)	1.4 (3.9)	5038	1268	*** 1.57 (1.23 - 2.02)	*** 1.40 (1.17 - 1.69)	Ref	0.95 (0.82 - 1.11)	1.06 (0.87 - 1.30)	1.25 (0.79 - 1.97)
Recent stoppers (current - former)	3.4 (4.8)	253	65	1.71 (0.47 – 6.24)	2.29 (0.74 – 7.09)	Ref	1.34 (0.69 - 2.60)	1.02 (0.52 – 2.03)	1.21 (0.27 – 5.48)
Never smokers (never - never)	1.3 (3.8)	6280	992	*** 1.98 (1.56 - 2.53)	1.19 (0.95 - 1.48)	Ref	0.98 (0.83 - 1.16)	1.14 (0.90 - 1.44)	1.43 (0.78 - 2.62)

[a]Adjusted for age, sex, BMI, physical activity, social class and educational level
[b]Zero participants in category
Significance of HRs: ***P<0.001; ** P<0.01; * P<0.05
HR hazard ratio, *CI* confidence interval

Supplementary table 4 Association between weight change and all-cause mortality in 5479 men and 7101 women, assessed using Cox proportional hazards regression with a median follow-up from 2HE of 15 years. Results are hazard ratios and 95% confidence intervals, HR (95%CI).

	Weight change categories					
	loss >5 kg	**loss >2.5 & ≤5 kg**	**loss or gain ≤2.5 kg**	**gain >2.5 & ≤5 kg**	**gain >5 & ≤10 kg**	**gain >10 kg**
MEN, N	215	423	2983	1206	577	75
ALL CAUSE MORTALITY						
Number of events (%)	91 (41.9)	154 (36.2)	801 (26.5)	259 (21.1)	128 (21.9)	20 (26.3)
Model 2	*** 1.92 (1.54 - 2.40)	** 1.31 (1.10 - 1.56)	Ref	0.94 (0.81 - 1.08)	1.03 (0.85 - 1.25)	1.51 (0.97 - 2.36)
Model 3	*** 1.84 (1.47 - 2.30)	** 1.30 (1.09 - 1.54)	Ref	0.94 (0.82 - 1.09)	1.02 (0.84 - 1.24)	1.50 (0.96 - 2.34)
WOMEN, N (%)	362	517	3690	1540	841	151
ALL CAUSE MORTALITY						
Number of events (%)	94 (25.4)	128 (24.3)	651 (17.3)	223 (14.2)	115 (13.5)	15 (9.8)
Model 2	*** 1.71 (1.37 - 2.13)	** 1.31 (1.08 - 1.58)	Ref	0.94 (0.81 - 1.10)	1.14 (0.93 - 1.39)	1.05 (0.62 - 1.75)
Model 3	*** 1.65 (1.32 – 2.07)	** 1.31 (1.08 - 1.58)	Ref	0.93 (0.80 - 1.09)	1.12 (0.91 - 1.37)	1.00 (0.60 - 1.67)

Model 2: adjusted for age (continuous) and smoking history (categorical, as defined in Supplementary table 3)
Model 3: Model 2 and further adjusted for BMI (continuous), physical activity (categorical), social class (categorical) and educational level (categorical)
Significance of HRs: ***P<0.001; ** P<0.01; * P<0.05

Supplementary table 5 Mean annual weight changes in 1044 men and women who stated that they lost more than 5 kg in the last 5 years (HLQ2), due to dieting, by BMI category at 1HE.

	Men			**Women**		
		Weight change (kg/y)			Weight change (kg/y)	
	N	Mean	SD	N	Mean	SD
By BMI (1HE)						
≥ 18.5 & < 25 kg/m^2	25	-0.32	1.06	164	0.47	1.33
≥ 25 & < 30 kg/m^2	154	-0.21	1.47	380	0.06	1.65
≥ 30 kg/m^2	88	-0.10	1.83	233	-0.44	2.17

HLQ2 2nd Health and Lifestyle Questionnaire, *BMI* body mass index, *1HE* 1st health examination, *SD* standard deviation

Chapter 4. Changes in waist circumference and risk of all-cause and CVD mortality: results from the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) cohort study

4.1 Key points

What is already known on this subject?

- Obesity, usually defined using BMI, is a major risk factor for many chronic diseases, such as diabetes, CVD and certain cancer types, as well as mortality.
- Evidence is limited and conflicting, regarding the consequences of changes in body fat distribution.

Following on from my investigations into the associations between weight change and mortality risk, I proceeded to study the associations between changes in waist circumference and subsequent mortality risk. Additionally, I investigated the relationship between mortality risk and changes in weight and waist circumference simultaneously.

What this study adds.

- An objectively measured gain in waist circumference > 5 cm over approximately 4 years was associated with subsequent higher total mortality risk in men and women, and higher CVD mortality risk in men over 16 years of follow-up.
- Associations were not confounded by age, smoking, BMI, physical activity, educational level or social class, especially in men.
- In analyses of concurrent changes in waist circumference and weight, the greatest risk in men occurred with weight loss and gain in waist circumference for all-cause and CVD mortality. In women, the greatest risk for both all-cause and CVD mortality was observed in those with weight loss and maintenance of waist circumference.
- Associations for a loss in waist circumference and mortality were inconsistent.

4.2 Published journal article

Mulligan, A.A., Lentjes, M.A.H., Luben, R.N., Wareham, N.J, Khaw, K-T. Changes in waist circumference and risk of all-cause and CVD mortality: results from the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) cohort study. BMC Cardiovasc Disord 19, 238 (2019). https://doi.org/10.1186/s12872-019-1223-z

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RESEARCH ARTICLE Open Access

Changes in waist circumference and risk of all-cause and CVD mortality: results from the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) cohort study

Angela A. Mulligan[1,2*], Marleen A. H. Lentjes[1], Robert N. Luben[1], Nicholas J. Wareham[1,3] and Kay-Tee Khaw[1,4]

Abstract

Background: Measures of abdominal adiposity are strongly associated with all-cause mortality and cardiovascular disease (CVD). However, data are limited and conflicting regarding the consequences of changes in body fat distribution. The main aims of this paper are to investigate the association between changes in waist circumference (WC) and all-cause and CVD mortality and to examine these changes in relation to concurrent changes in weight.

Methods: The European Prospective Investigation into Cancer and Nutrition (EPIC-Norfolk) study recruited 25,639 participants between 1993 and 1997, aged 39–79, a number of whom also attended a second examination (1998–2000), and were followed up to 2016 for mortality. Participants were eligible for inclusion if they had WC, weight and height measurements at both time-points; those with a self-reported history of CVD or cancer, body mass index < 18.5 kg/m2 or missing data on covariates were excluded, leaving 12,337 participants for analyses. The median (IQR) follow-up time was 16.4 (15.7, 17.2) years. Hazard Ratios (HRs) for all-cause (2866 deaths) and CVD mortality (822 deaths), by categories of WC change, were determined using Cox proportional hazards analyses.

Results: After multivariable adjustment, the HRs (95% CIs) for all-cause mortality for men and women with a WC gain (WCG) > 5 cm were 1.51 (1.29–1.75) and 1.25 (1.06–1.46) respectively. For CVD mortality in men and women with a WCG > 5 cm, the HRs were 1.84 (1.39–2.43) and 1.15 (0.85–1.55) respectively. In analyses of concurrent changes in WC and weight, the greatest risk (HRs) (95% CIs) in men occurred with weight loss and WCG: 1.80 (1.13–2.86) for all-cause and 2.22 (1.03–4.82) for CVD mortality. In women, the greatest risk for both all-cause (HR 1.50 (1.16–1.95)) and CVD mortality (HR 1.81 (1.15–2.85)) was observed in those with weight loss and maintenance of WC (WCM).

Conclusions: Objectively measured WCG > 5 cm, was associated with subsequent higher total mortality risk and higher CVD mortality risk in men. Interventions focusing on preventing increase in central adiposity rather than lowering weight per se in later life may potentially have greater health benefits.

Keywords: Waist circumference change, Weight change, All-cause mortality, CVD mortality, EPIC-Norfolk

* Correspondence: angela.mulligan@phpc.cam.ac.uk; angela.mulligan@mrc-epid.cam.ac.uk
[1]European Prospective Investigation into Cancer and Nutrition, Department of Public Health and Primary Care, Strangeways Research Laboratory, University of Cambridge, Worts Causeway, Cambridge, UK
[2]European Prospective Investigation into Cancer and Nutrition, MRC Epidemiology Unit, Cambridge Biomedical Campus, University of Cambridge, Cambridge, UK
Full list of author information is available at the end of the article

Background

Obesity is a major risk factor for many chronic diseases, such as diabetes, cardiovascular disease (CVD) and certain cancer types, as well as mortality [1–3], and is commonly defined using body mass index (BMI) [4]. However, BMI does not take into account the distribution of the fat mass, which is of particular importance in older individuals, as the distribution of body fat changes with age [5]. Waist circumference (WC) strongly correlates with abdominal obesity and is a commonly used clinical measure of body fat distribution [6, 7]. Studies have shown that WC is associated with CVD risk [8–10] and CVD mortality [11]. WC has also been positively associated with all-cause mortality in a number of studies [12–18] with a few exceptions [19, 20]. The presence of central or abdominal obesity, as defined by a high WC, is one of five components used in the clinical diagnosis of the metabolic syndrome [21], which is associated with an increased risk of developing type 2 diabetes, CVD and subsequent mortality [22].

However, there are limited and conflicting data available regarding the consequences of changes in body fat distribution. A number of studies have found a gain in WC (WCG) to be predictive of subsequent all-cause and CVD mortality [23, 24] but a prospective cohort study found that WC loss (WCL) was associated with an increased risk of all-cause mortality, particularly for older adults [25]. However, no association was found between changes in WC and mortality in Swedish women [26], elderly Brazilians [27] or Iranian men [28].

Data pooled from 15 prospective studies that included 258,114 individuals, has found that a 1 cm increase in WC is associated with a 2% increased risk of future CVD [29]. In England, among women, the mean WC increased from 81.7 cm in 1993 to 88.1 cm in 2016, and the percentage with a very high WC (> 88 cm) rose from 26 to 46%; among men, the mean WC increased from 93.2 cm in 1993 to 97.0 cm in 2016, and the percentage with a very high WC (> 102 cm) increased from 20 to 34% [30].

We have recently reported that objectively measured weight loss (WTL), but not weight gain (WTG), was associated with subsequent higher mortality in healthy, middle-aged and elderly community-dwelling men and women, compared to those who maintained their weight (WTM) [31]. The main aim of this paper is therefore to investigate the association between changes in WC and all-cause and CVD mortality. A secondary aim is to examine these changes in relation to concurrent changes in weight as previous studies have tended to focus on either changes in WC or weight but not changes in both concurrently.

Methods

Study design

The European Prospective Investigation Into Cancer and Nutrition (EPIC) is large diet and cancer cohort [32]. The EPIC-Norfolk study participants, aged between 39 and 79 years, were recruited from 35 General Practitioners' surgeries in the Norfolk area of East Anglia, from 1993 to 1997 [33]. General practice age sex registers act as a population sampling frame as practically all of the UK the population are registered with a general practice through the National Health Service. Many of the characteristics of this cohort at baseline were comparable to the UK national population, including age, sex and anthropometry measurements but this cohort had a lower proportion of current smokers [34].

Ethical approval for the study was given by the Norwich District Health Authority Ethics Committee and all participants gave written, informed consent.

Main exposure: change in waist circumference

Of those who consented to take part in the study (N = 30,445), 25,639 attended a baseline health examination (1HE) (1993–1997) and 15,786 attended a second health examination (2HE) (1998–2000).

Trained nurses measured participants' weight and height, with participants wearing light clothing and without shoes. Weight was measured to the nearest 100 g using digital scales (Salter, UK). Height was measured to the nearest 0.1 cm using a free-standing stadiometer. A D-loop non-stretch fibreglass tape was used to measure WC, which was measured at the smallest circumference between the ribs and iliac crest to the nearest 0.1 cm while the participant was standing erect, with the abdomen relaxed, the arms at the side, the feet together and at the end of a normal expiration, without the tape compressing the skin. The measurement was taken at the level of the umbilicus where there was no natural waistline. Body mass index (BMI) is defined as the body mass (weight) divided by the square of the height, and is expressed in kg/m2, and is commonly used to categorise individuals as underweight (< 18.5 kg/m2), normal weight (≥18.5 to < 25 kg/m2), overweight (≥25 to 30 kg/m2), or obese (≥ 30 kg/m2) [4].

Absolute change in WC (cm) was calculated as WC measured at 2HE minus WC at 1HE. Absolute weight change (kg) was calculated as weight measured at 2HE minus weight measured at 1HE. Participants were assigned to one of five WC change categories: > 5 cm loss, > 2.5 to ≤5 cm loss, ≤ 2.5 cm loss or gain ('maintenance', including zero change; considered the reference category), > 2.5 to ≤5 cm gain, > 5 cm gain. Annual WC change was calculated from the absolute difference in WC, divided by the participants' time lapse between the health examinations (cm/y).

In order to study the possible association between changes in WC and changes in weight, participants were assigned to one of nine categories, based on a loss of > 2.5 cm or 2.5 kg, within 2.5 cm or 2.5 kg loss or gain

(maintenance), or a gain of > 2.5 cm or 2.5 kg respectively. The nine categories were as follows: WTM (weight maintenance) and WCM (WC maintenance) (reference category); WTL (weight loss) and WCL (WC loss); WTL and WCM; WTL and WCG (WC gain); WTM and WCL; WTM and WCG; WTG (weight gain) and WCL; WTG and WCM; and WTG and WCG.

Participant selection

Measurements of WC, weight and height measurements at both time-points were essential for participant inclusion ($N = 15{,}010$). Participants were excluded from analyses if they were categorised as underweight (BMI < 18.5 kg/m^2) or who self-reported CVD or cancer at either examination ($N = 2095$); those with missing data on adjustment variables (smoking, social class, educational level, physical activity, and menopausal status in women ($N = 529$) were also excluded. This left 12,337 participants for analyses (Fig. 1).

Adjustment variables

Data from two self-administered Health and Lifestyle Questionnaires (HLQ1 and HLQ2), at 1HE and 2HE respectively, were used to create categories of a number of variables. Current smokers were defined as those currently smoking cigarettes, former smoker as being a smoker previously and non-smokers were those who never smoked (derived from HLQ1 and HLQ2). Self-reported physical activity (derived from HLQ1) was classified into four levels, using both occupational and leisure activity: inactive, moderately inactive,

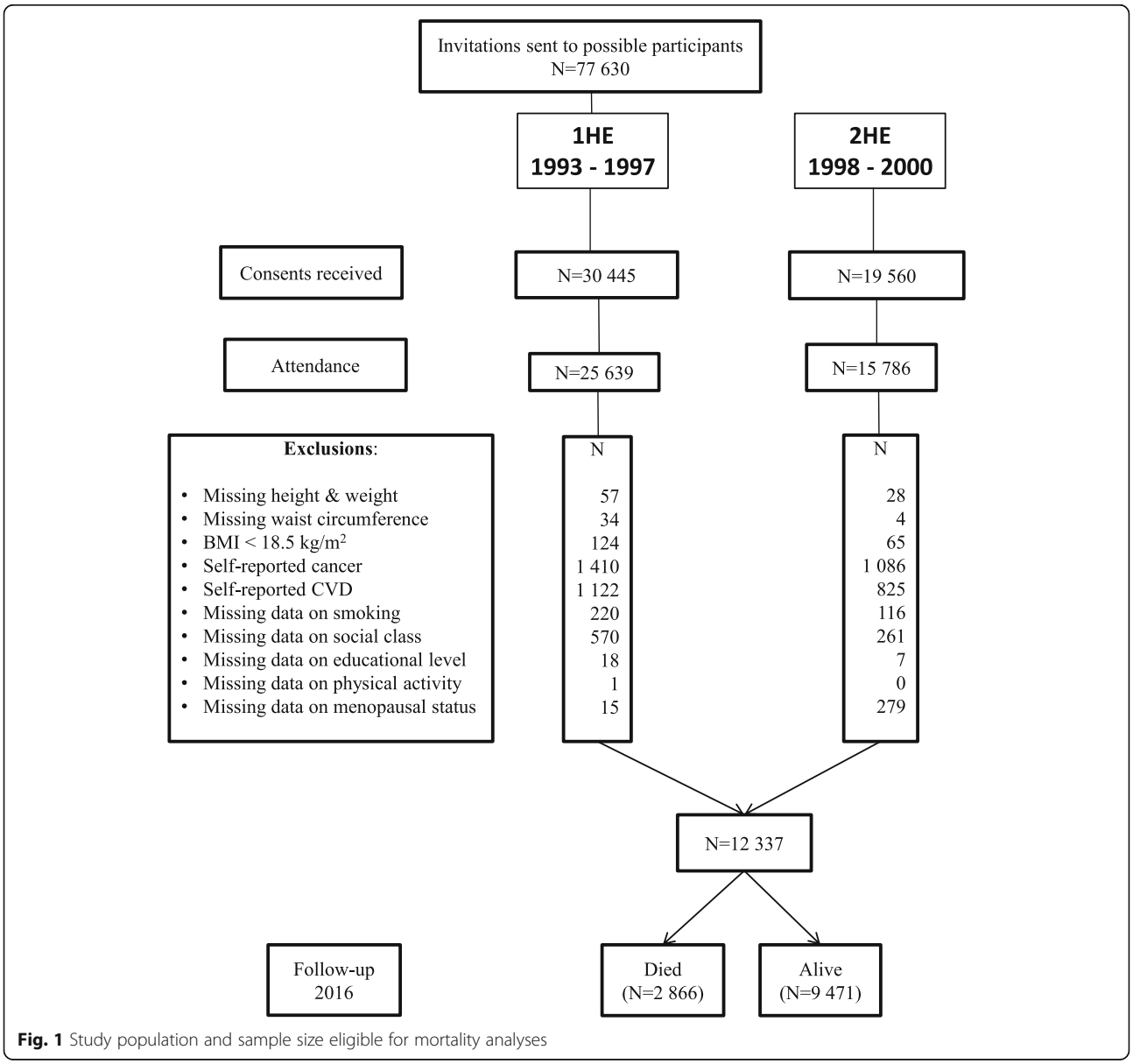

Fig. 1 Study population and sample size eligible for mortality analyses

moderately active and active [35, 36]. Social class at 1HE was defined according to the Registrar General's occupation-based classification scheme and categorised into the following six groups: professional, managerial and technical, non-manual skilled, manual skilled, partly skilled and unskilled [37]; for our analyses, these categories were combined to create 2 categories, non-manual (professional, managerial and technical, non-manual skilled) and manual (manual skilled, partly skilled and unskilled). Educational status at 1HE was categorised into four groups: degree or equivalent, A level or equivalent, O level or equivalent and less than O level or no qualification, corresponding to levels within the International Standard Classification of Education 1997 [38]. In our analyses, those with an educational level of O level and above were combined into one category. Menopausal status was categorised as premenopausal, early perimenopausal (< 1 year), late perimenopausal (1–5 years) or postmenopausal (> 5 years). Included adjustment variables were measured with HLQ1, with the exceptions of smoking status and menopausal status, which were measured with HLQ2. Participants were asked about their medical histories at both time-points. The diagnosis of chronic diseases such as heart attack, stroke and cancer were recorded as present when participants answered "yes" to question "Has a doctor ever told you that you have any of the following conditions?"

Endpoints

All fatal events occurring between 1998 and 31st March 2016 were captured by the Office of National Statistics, United Kingdom. Death certificates were coded by nosologists according to the International Classification of Diseases (ICD). An underlying cause of death from cancer or CVD was defined by using the following ICD codes: cancer death (ICD9, 140–208 or ICD10 C00-C97), or CVD death (ICD9 400–438 or ICD10 I10-I79).

Statistical analyses

Characteristics of the study population were summarised by WC change category, for continuous variables (mean and SD) and categorical variables (frequency and percentage). The follow-up time began at the 2HE and formed the underlying time variable; median (IQR) follow-up time was 16.4 (15.7, 17.2) years. Participants who died were censored at their date of death and those who did not die were censored at the end of follow-up (31st March 2016). Three Cox proportional hazards models were used to determine Hazard Ratios (HRs) for all-cause and CVD mortality by WC change category for men and women separately: age (continuous variable), (model 1); including BMI (continuous variable), WC (continuous variable), physical activity, social class, educational level, smoking (2HE) and menopausal status (2HE) in women (model 2); and including difference in weight (continuous variable) (model 3). The proportional hazards' assumption was tested by including time interaction variables in the Cox regression models and we found that age violated our test ($P < 0.0001$). However, when the time interaction for age was included, only minimal changes to the HRs of WC change were observed, so results are shown with age adjustment alone. We also examined HRs by WC change category, separately in men and women, in subgroups, stratified by age, WC, smoking status, BMI, physical activity, educational level, social class, menopausal status in women, and after the exclusion of individuals who died within 3 or 5 years of the 2HE. The data were analysed using Stata 14 (STATA Corp., Texas, USA).

Results

Description of the cohort

Over a 3.5 year period, men had a mean WC and weight increase of 0.83 cm (SD 5.19) and 1.29 kg (SD 3.61) respectively; similar mean increases of 0.81 cm (SD 5.60) in WC and 1.38 kg (SD 4.14) in weight were found in women. The baseline characteristics of those who attended both 1HE and 2HE, before and after exclusions were applied were similar (Additional file 1: Table S1). However, the prevalence of self-reported CVD and the percentages of current smokers and physically inactive participants were higher in those who attended the 1HE only, compared to those who also attended the 2HE. Also, the percentage of deaths that occurred was lower in both men and women, after exclusion criteria were applied, with the highest percentage found in those who only attended the 1HE.

Table 1 describes the characteristics of men and women, by WC change category. WCM was observed in 40% of men and 38% of women. Participants with the greatest WCL were, on average, older and had the largest WC, weight and BMI at 1HE. Men with the greatest WCG were more likely to have a smaller WC at 1HE, whereas women with the greatest WCG had a similar WC at 1HE, compared to those in the WCM category. Current smokers at 2HE were more likely to have a WCL whereas former smokers, a WCG. Male non-manual workers were more likely to have a WCL > 5 cm whereas male manual workers were more likely to have a WCG > 5 cm. Participants with no qualifications were more likely to have a WCG > 5 cm. A greater percentage of deaths occurred in those with a WCL > 5 cm.

Main analyses: total and CVD mortality

At the end of a median (IQR) follow-up time of 16.4 (15.7, 17.2) years, 1551 deaths in men and 1315 deaths in women were recorded, 440 and 382 deaths from CVD, respectively. Total and CVD mortality HRs for men and women by WC change category are shown in

Table 1 Characteristics at 1st and 2nd health examinations of 5469 men and 6868 women, stratified by categories of change in waist circumference

	Categories of change in waist circumference (WC) (cm)											
	Loss > 5 cm		Loss > 2.5 and ≤ 5 cm		Loss or gain ≤2.5 cm		Gain > 2.5 and ≤ 5 cm		Gain > 5 cm		All	
MEN, N (row %)	641 (11.7)		656 (12.0)		2211 (40.4)		926 (16.9)		1035 (18.9)		5469	100.0%
WC at 1HE (cm)	99.0	9.2	96.8	9.1	95.3	8.9	93.8	8.6	92.4	9.7	95.1	9.3
WC at 2HE (cm)	91.0	9.2	93.2	9.1	95.4	8.9	97.6	8.6	100.5	10.0	96.0	9.6
WC change (cm)	−8.0	3.1	−3.7	0.7	0.1	1.4	3.8	0.7	8.1	2.9	0.8	5.2
Annual WC change (cm)	−2.3	1.0	−1.1	0.3	0.0	0.4	1.1	0.3	2.2	0.9	0.2	1.5
Age at 1HE (years)	59.6	9.3	59.7	9.0	58.8	8.9	58.7	8.9	58.6	8.7	58.9	8.9
Age at 2HE (years)	62.8	9.4	62.8	9.1	61.9	9.0	61.9	9.0	61.8	8.7	62.1	9.0
Age change (years)	3.2	0.8	3.1	0.8	3.1	0.8	3.2	0.8	3.3	0.8	3.2	0.8
Weight at 1HE (kg)	81.7	10.7	80.4	10.9	80.0	10.8	79.8	10.2	80.2	11.7	80.2	10.9
Weight at 2HE (kg)	79.0	10.5	79.9	11.0	81.0	11.2	82.4	10.7	84.4	12.8	81.5	11.5
Weight change (kg)	−2.7	3.6	−0.5	2.9	1.1	2.6	2.6	2.7	4.2	3.5	1.3	3.6
BMI at 1HE (kg/m^2)	26.8	3.2	26.5	3.1	26.3	3.1	26.2	3.0	26.3	3.3	26.3	3.1
BMI at 2HE (kg/m^2)	26.0	3.1	26.4	3.2	26.7	3.2	27.1	3.1	27.8	3.6	26.9	3.3
BMI change (kg/m^2)	−0.8	1.2	−0.1	0.9	0.4	0.9	0.9	0.9	1.5	1.2	0.5	1.2
Education - 1HE												
None	172	26.8	164	25.0	573	25.9	248	26.8	287	27.7	1444	26.4%
O level and above	469	73.2	492	75.0	1638	74.1	678	73.2	748	72.3	4025	73.6%
Social class - 1HE												
Non-manual	412	64.3	414	63.1	1352	61.2	585	63.2	632	61.1	3395	62.1%
Manual	229	35.7	242	36.9	859	38.8	341	36.8	403	38.9	2074	37.9%
Smoking status - 1HE												
Current	66	10.3	60	9.2	185	8.4	86	9.3	110	10.6	507	9.3%
Former	321	50.1	341	52.0	1200	54.3	477	51.5	559	54.0	2898	53.0%
Never	254	39.6	255	38.9	826	37.4	363	39.2	366	35.4	2064	37.7%
Smoking status - 2HE												
Current	67	10.5	59	9.0	166	7.5	65	7.0	81	7.8	438	8.0%
Former	320	49.9	342	52.1	1221	55.2	498	53.8	589	56.9	2970	54.3%
Never	254	39.6	255	38.9	824	37.3	363	39.2	365	35.3	2061	37.7%
Physical activity - 1HE												
Inactive	193	30.1	160	24.4	601	27.2	233	25.2	257	24.8	1444	26.4%
Moderately inactive	163	25.4	196	29.9	529	23.9	216	23.3	249	24.1	1353	24.7%
Moderately active	156	24.3	159	24.2	541	24.5	249	26.9	272	26.3	1377	25.2%
Active	129	20.1	141	21.5	540	24.4	228	24.6	257	24.8	1295	23.7%
Deaths during follow-up	218	34.0	196	29.9	585	26.5	246	26.6	306	29.6	1551	28.4%
Lost weight in last 5 years (2HE)												
Diet	61	22.9	42	15.8	92	34.6	28	10.5	43	16.2	266	4.9%
Illness	28	21.7	7	5.4	51	39.5	17	13.2	26	20.2	129	2.4%
WOMEN, N (row %)	873 (12.7)		875 (12.7)		2596 (37.8)		1131 (16.5)		1393 (20.3)		6868	100.0%
WC at 1HE (cm)	87.2	11.4	82.6	10.5	80.0	9.5	79.1	9.7	79.6	9.2	81.0	10.2
WC at 2HE (cm)	78.8	10.7	78.9	10.5	80.1	9.6	82.8	9.7	88.0	10.0	81.9	10.5
WC change (cm)	−8.4	3.5	−3.7	0.8	0.1	1.4	3.7	0.7	8.4	3.3	0.8	5.6
Annual WC change (cm)	−2.4	1.2	−1.1	0.3	0.0	0.4	1.1	0.3	2.4	1.1	0.2	1.6
Age at 1HE (years)	58.7	9.0	58.5	9.2	58.0	8.9	57.4	8.6	57.5	8.5	58.0	8.8

Table 1 Characteristics at 1st and 2nd health examinations of 5469 men and 6868 women, stratified by categories of change in waist circumference *(Continued)*

	Categories of change in waist circumference (WC) (cm)											
	Loss > 5 cm		Loss > 2.5 and ≤ 5 cm		Loss or gain ≤2.5 cm		Gain > 2.5 and ≤ 5 cm		Gain > 5 cm		All	
Age at 2HE (years)	61.9	9.2	61.6	9.3	61.2	9.0	60.5	8.6	60.6	8.6	61.1	8.9
Age change (years)	3.1	0.8	3.2	0.8	3.1	0.8	3.1	0.8	3.2	0.8	3.2	0.8
Weight at 1HE (kg)	70.1	12.8	67.8	11.7	66.2	10.4	66.7	10.6	68.5	10.7	67.5	11.1
Weight at 2HE (kg)	67.7	12.1	67.3	11.7	67.3	10.8	69.2	11.2	73.0	12.0	68.9	11.6
Weight change (kg)	−2.4	4.8	−0.5	3.3	1.1	2.9	2.5	3.2	4.5	4.2	1.4	4.1
BMI at 1HE (kg/m^2)	26.9	4.6	26.0	4.3	25.5	3.8	25.6	3.9	26.3	3.9	25.9	4.0
BMI at 2HE (kg/m^2)	26.1	4.3	26.0	4.4	26.0	4.0	26.6	4.2	28.1	4.4	26.6	4.3
BMI change (kg/m^2)	−0.8	1.8	−0.1	1.2	0.5	1.1	1.0	1.2	1.9	1.6	0.7	1.6
Education - 1HE												
None	291	33.3	321	36.7	983	37.9	402	35.5	528	37.9	2525	36.8%
O level and above	582	66.7	554	63.3	1613	62.1	729	64.5	865	62.1	4343	63.2%
Social class - 1HE												
Non-manual	537	61.5	558	63.8	1659	63.9	727	64.3	877	63.0	4358	63.4%
Manual	336	38.5	317	36.2	937	36.1	404	35.7	516	37.0	2510	36.6%
Smoking status - 1HE												
Current	94	10.8	72	8.2	224	8.6	100	8.8	128	9.2	618	9.0%
Former	290	33.2	287	32.8	794	30.6	329	29.1	469	33.7	2169	31.6%
Never	489	56.0	516	59.0	1578	60.8	702	62.1	796	57.1	4081	59.4%
Smoking status - 2HE												
Current	88	10.1	70	8.0	206	7.9	76	6.7	105	7.5	545	7.9%
Former	297	34.0	289	33.0	814	31.4	353	31.2	494	35.5	2247	32.7%
Never	488	55.9	516	59.0	1576	60.7	702	62.1	794	57.0	4076	59.4%
Physical activity - 1HE												
Inactive	234	26.8	236	27.0	622	24.0	245	21.7	369	26.5	1706	24.8%
Moderately inactive	282	32.3	275	31.4	874	33.7	390	34.5	457	32.8	2278	33.2%
Moderately active	220	25.2	206	23.5	631	24.3	291	25.7	328	23.6	1676	24.4%
Active	137	15.7	158	18.1	469	18.1	205	18.1	239	17.2	1208	17.6%
Deaths during follow-up	212	24.3	181	20.7	487	18.8	176	15.6	259	18.6	1315	19.2%
Menopausal status (2HE)												
Pre-menopausal	51	5.8	55	6.3	170	6.6	67	5.9	86	6.2	429	6.3%
Early peri-menopausal	26	3.0	34	3.9	80	3.1	63	5.6	48	3.5	251	3.7%
Late peri-menopausal (1–5 y)	149	17.1	168	19.2	504	19.4	213	18.8	288	20.7	1322	19.3%
Post-menopausal (> 5 y)	647	74.1	618	70.6	1842	71.0	788	69.7	971	69.7	4866	70.9%
Lost weight in last 5 years (2HE)												
Diet	179	23.6	108	14.3	233	30.8	94	12.4	143	18.9	757	11.0%
Illness	33	19.5	25	14.8	62	36.7	26	15.4	23	13.6	169	2.5%

Values are mean and SD or frequency and percentage
1HE 1st health examination, *2HE* 2nd health examination, *BMI* body mass index

Table 2. The addition of change in weight to the model strengthened the positive WCG-mortality associations (model 3), especially in men, where the findings for CVD mortality were stronger than for all-cause mortality, in those with a WCG > 5 cm (HR 1.84 and 1.51 respectively). Women with a WCG > 5 cm had a significantly higher hazard of all-cause mortality of 1.25 (CI 1.06–1.46) compared to WCM. No significant associations were found regarding changes in WC and CVD mortality in women. No significant associations were

Table 2 Associations between total and CVD mortality in 5469 men and 6868 women and categories of change in waist circumference

	Categories of change in waist circumference (WC) (cm)				
	Loss > 5 cm	Loss > 2.5 and ≤ 5 cm	Loss or gain ≤2.5 cm	Gain > 2.5 and ≤ 5 cm	Gain > 5 cm
Men, N (%)	641 (11.7)	656 (12.0)	2211 (40.4)	926 (16.9)	1035 (18.9)
All-cause mortality					
Number of events	218 (34.0)	196 (29.9)	585 (26.5)	246 (26.6)	306 (29.5)
Model 1	** 1.28 (1.09–1.49)	1.07 (0.91–1.26)	Ref	1.04 (0.90–1.21)	* 1.19 (1.03–1.36)
Model 2	1.14 (0.97–1.34)	1.03 (0.87–1.21)	Ref	1.06 (0.91–1.23)	** 1.26 (1.10–1.46)
Model 3	0.93 (0.78–1.10)	0.95 (0.80–1.12)	Ref	1.15 (0.99–1.34)	*** 1.51 (1.29–1.75)
CVD mortality					
Number of events	64 (10.0)	62 (9.4)	153 (6.9)	66 (7.1)	95 (9.2)
Model 1	* 1.42 (1.06–1.90)	1.28 (0.95–1.72)	Ref	1.08 (0.81–1.44)	** 1.42 (1.10–1.84)
Model 2	1.22 (0.90–1.64)	1.21 (0.90–1.63)	Ref	1.09 (0.82–1.46)	** 1.53 (1.17–1.98)
Model 3	0.97 (0.70–1.34)	1.11 (0.82–1.49)	Ref	1.18 (0.88–1.58)	*** 1.84 (1.39–2.43)
Women, N (%)	873 (12.7)	875 (12.7)	2596 (37.8)	1131 (16.5)	1393 (20.3)
All-cause mortality					
Number of events	212 (24.3)	181 (20.7)	487 (18.8)	176 (15.6)	259 (18.6)
Model 1	** 1.26 (1.08–1.48)	1.03 (0.87–1.22)	Ref	0.86 (0.73–1.03)	1.09 (0.93–1.26)
Model 2	1.13 (0.95–1.33)	0.97 (0.82–1.16)	Ref	0.91 (0.76–1.08)	1.15 (0.98–1.34)
Model 3	1.03 (0.87–1.24)	0.93 (0.78–1.10)	Ref	0.95 (0.79–1.13)	** 1.25 (1.06–1.46)
CVD mortality					
Number of events	49 (5.6)	56 (6.4)	152 (5.8)	50 (4.4)	75 (5.4)
Model 1	0.91 (0.66–1.26)	0.97 (0.72–1.32)	Ref	0.81 (0.59–1.12)	1.04 (0.79–1.38)
Model 2	0.80 (0.57–1.11)	0.91 (0.67–1.24)	Ref	0.87 (0.63–1.20)	1.14 (0.86–1.52)
Model 3	0.77 (0.54–1.09)	0.89 (0.65–1.22)	Ref	0.87 (0.63–1.21)	1.15 (0.85–1.55)

***$p < 0.001$; **$p < 0.01$; *$p < 0.05$

Associations were assessed using Cox proportional hazards regression with a median follow-up from 2HE of 16 years. Results are hazard ratios and 95% confidence intervals, HR (95% CI)

Model 1: adjusted for age (continuous)

Model 2: Model 1 + further adjusted for BMI (continuous), waist circumference (continuous), smoking (categorical), physical activity (categorical), educational level (categorical) and social class (categorical), and menopausal status (categorical) in women

Model 3: Model 2 + further adjusted for weight change (continuous)

2HE 2nd health examination, *CVD* cardiovascular disease, *BMI* body mass index

found for WCL and total or CVD mortality in the multivariate adjusted models, in either men or women.

Subgroup analyses

HRs for all-cause mortality in men and women, adjusted as per model 3, grouped by confounder categories are shown in Tables 3 and 4 respectively. Participants with a WCG > 5 cm had a higher HR than those in the WCM category, irrespective of their WC categorisation at 1HE, although stronger associations were more evident in men. Men who had a WCG > 5 cm had significantly higher HRs, irrespective of whether they were < 65 years (HR 1.78 CI 1.40–2.26) or > 65 years (HR 1.38 (CI 1.13–1.68) of age at 1HE (Table 3) although a significant association was only found among women > 65 years (HR 1.35 (CI 1.10–1.67) (Table 4). Higher hazards were observed for participants with a WCG > 5 cm, irrespective of smoking status, with the exception of women who were former smokers, where a WCG between 2.5 and 5 cm had a significantly lower HR of 0.62 (CI 0.45–0.86) (Table 4). Irrespective of BMI, physical activity, educational level or social class categorisation at HE1, WCG > 5 cm was associated with a higher HR in both men and women (Tables 3 and 4 respectively). Almost 71% of women were classified as postmenopausal and a higher hazard was found in those with a WCG > 5 cm (Table 4). Even after excluding participants who died within 3 or 5 years of the 2HE, participants with a WCG > 5 cm had significantly higher HRs for all-cause mortality, than those in the WCM category.

As significant findings were evident for CVD mortality and men, but not women, in relation to changes in WC, we further explored these in subgroups, stratified by potential confounding characteristics, adjusted as per model 3 (Additional file 1: Table S2). Generally, a higher hazard was evident for all values of the confounding

Table 3 Cox multivariable-adjusted HRs after 16 years of follow-up for all-cause mortality in 5469 men. Results are given for stratified variables by WC change category

					Categories of change in waist circumference (WC) (cm)				
					loss > 5 cm	loss > 2.5 & ≤5 cm	loss or gain ≤2.5 cm	gain > 2.5 & ≤ 5 cm	gain > 5 cm
	N	%	Deaths	%	HR (95% CI)	HR (95% CI)		HR (95% CI)	HR (95% CI)
All	5469	100.0	1551	100.0	0.93 (0.78–1.10)	0.95 (0.80–1.12)	Ref	1.15 (0.99–1.34)	*** 1.51 (1.29–1.75)
By WC (1HE)									
< 94 cm	2515	46.0	604	38.9	0.93 (0.67–1.28)	1.20 (0.91–1.57)	Ref	1.04 (0.81–1.32)	* 1.28 (1.02–1.61)
≥ 94 & ≤ 102 cm	1854	33.9	520	33.5	0.93 (0.69–1.24)	0.79 (0.58–1.06)	Ref	* 1.34 (1.04–1.73)	*** 1.96 (1.50–2.55)
> 102 cm	1100	20.1	427	27.5	0.89 (0.66–1.20)	0.90 (0.67–1.21)	Ref	1.20 (0.88–1.65)	* 1.42 (1.02–1.99)
By age (1HE)									
< 65 y	3882	71.0	573	36.9	0.99 (0.73–1.34)	0.97 (0.73–1.29)	Ref	1.22 (0.95–1.57)	*** 1.78 (1.40–2.26)
≥ 65 y	1587	29.0	978	63.1	0.91 (0.74–1.13)	0.94 (0.77–1.15)	Ref	1.14 (0.94–1.38)	** 1.38 (1.13–1.68)
By smoking status (2HC)									
current	438	8.0	170	11.0	0.75 (0.45–1.23)	0.98 (0.60–1.58)	Ref	1.01 (0.61–1.68)	** 2.11 (1.32–3.36)
former	2970	54.3	983	63.4	0.91 (0.72–1.14)	0.89 (0.72–1.10)	Ref	1.15 (0.95–1.39)	*** 1.54 (1.28–1.87)
never	2061	37.7	398	25.7	1.17 (0.84–1.63)	1.08 (0.78–1.49)	Ref	1.26 (0.93–1.70)	1.22 (0.89–1.67)
By BMI (1HE)									
≥ 18.5 & < 25	1913	35.0	505	32.6	1.03 (0.75–1.41)	0.94 (0.70–1.26)	Ref	1.03 (0.79–1.34)	1.29 (0.99–1.69)
≥ 25 & < 30	2926	53.5	831	53.6	0.80 (0.63–1.02)	0.94 (0.75–1.17)	Ref	1.22 (0.99–1.50)	***1.69 (1.37–2.08)
≥ 30	630	11.5	215	13.9	1.26 (0.81–1.95)	0.89 (0.56–1.42)	Ref	1.38 (0.91–2.10)	1.34 (0.88–2.04)
By physical activity (1HC)									
inactive	1444	26.4	545	35.1	0.91 (0.68–1.21)	0.98 (0.74–1.30)	Ref	1.21 (0.93–1.57)	*** 1.72 (1.34–2.22)
mod inactive	1353	24.7	374	24.1	1.03 (0.72–1.49)	1.08 (0.79–1.47)	Ref	1.14 (0.82–1.58)	* 1.47 (1.07–2.03)
mod active	1377	25.2	336	21.7	0.86 (0.59–1.23	0.69 (0.47–1.01)	Ref	0.95 (0.69–1.30)	1.19 (0.86–1.65)
active	1295	23.7	296	19.1	0.88 (0.57–1.33)	1.01 (0.69–1.49)	Ref	1.22 (0.87–1.70)	* 1.50 (1.06–2.12)
By educational level (1HE)									
No qualifications	1444	26.4	537	34.6	0.90 (0.68–1.20)	1.03 (0.78–1.36)	Ref	0.90 (0.68–1.19)	*** 1.76 (1.36–2.28)
O level and above	4025	73.6	1014	65.4	0.96 (0.77–1.19)	0.90 (0.73–1.11)	Ref	** 1.29 (1.08–1.55)	** 1.38 (1.14–1.67)
By social class (1HE)									
Non-manual	3395	62.1	932	60.1	1.03 (0.82–1.29)	0.90 (0.73–1.11)	Ref	1.12 (0.92–1.37)	** 1.39 (1.14–1.69)
Manual	2074	37.9	619	39.9	0.81 (0.62–1.06)	1.04 (0.80–1.35)	Ref	1.17 (0.92–1.49)	*** 1.67 (1.31–2.13)

Table 3 Cox multivariable-adjusted HRs after 16 years of follow-up for all-cause mortality in 5469 men. Results are given for stratified variables by WC change category *(Continued)*

					Categories of change in waist circumference (WC) (cm)				
					loss > 5 cm	loss > 2.5 & ≤5 cm	loss or gain ≤2.5 cm	gain > 2.5 & ≤ 5 cm	gain > 5 cm
Excluding early deaths									
Excluding deaths < 3 y	5347	97.8	1429	92.1	0.90 (0.75–1.08)	0.94 (0.79–1.12)	Ref	1.16 (0.99–1.35)	*** 1.52 (1.29–1.78)
Excluding deaths < 5 y	5211	95.3	1293	83.4	0.94 (0.78–1.14)	0.94 (0.79–1.13)	Ref	1.13 (0.96–1.34)	*** 1.45 (1.23–1.72)

Adjusted for age, BMI, baseline WC, physical activity, smoking, educational level, social class and change in weight (except where the variable was used for stratification)
***$p < 0.001$; ** $p < 0.01$; * $p < 0.05$

variables in men with a WCG; findings relating to WCL were inconsistent. In men who had a WCG > 5 cm, the strongest associations were found in those who were younger, current smokers, overweight, physically active, had no qualifications, had a manual job and a normal WC. Even after excluding participants who died within 3 or 5 years of the 2HE, men with a WCG had significantly higher HRs for CVD mortality, than those in the WCM category.

Total and CVD mortality: WC changes in relation to weight changes

Weight change was strongly positively correlated with a change in WC in both men (r = 0.60) and women (r = 0.57) ($P < 0.001$). We also explored the association of category changes in WC and total mortality in combination with category changes in weight; the interaction between WC change categories and weight change categories was not found to be significant ($p = 0.29$ for men and 0.82 for women). No significant interaction was found between WC change categories and weight change categories with regard to CVD mortality (p = 0.71 for men and 0.52 for women).

Figure 2a illustrates the associations between category changes in WC and weight and total mortality, adjusted for age, BMI, baseline WC, physical activity, smoking, educational level, social class and change in weight, and menopausal status in women. Similar trends in both men and women were generally evident. In men, significantly higher HRs were observed for all WTL categories, irrespective of whether men were categorised as WCL, WCM or WCG, as well as for WCG and WTM (HR 1.37 (CI 1.17–1.61)), compared to those in the WTM and WCM category. In women, significantly higher HRs were observed for total mortality for 2 categories – WTL and WCL (HR 1.40 (CI 1.14–1.70)) and WTL and WCM (HR 1.50 (CI 1.16–1.95)), compared to the reference category of WTM and WCM.

Figure 2b illustrates the similar associations with CVD mortality, adjusted for age, BMI, baseline WC, physical activity, smoking, educational level, social class and change in weight, and menopausal status in women. In men, significantly higher HRs were found in the WTL and WCL (HR 1.81 (CI 1.32–2.49)), WTL and WCG (HR 2.22 (CI 1.03–4.82)), and WTM and WCG (HR 1.38 (CI 1.02–1.88)) categories, compared to the reference category of WTM and WCM. A significant association for CVD mortality in women was only found in the WTL and WCM category (HR 1.81 (CI 1.15–2.85)). In all analyses, the category with the lowest HR was WTG and WCL.

Discussion

Summary of main findings

In this study of 12,337 middle-aged and elderly men and women, a WCG of more than 5 cm over approximately 4 years was associated with higher total mortality in both men and women and higher CVD mortality in men over the next 16 years of follow-up. Particularly among men, the associations were also evident after stratification for age, smoking, BMI, physical activity, educational level, social class and after the exclusion of deaths within the first 5 years of follow-up. Associations for WCL and mortality were inconsistent.

Strengths and limitations

The major strengths of this study include its large population of free-living, middle-aged and elderly men and women, its prospective design, and the long follow-up time. Additionally, WC, weight and height were objectively measured, not self-reported, and information on a large number of factors associated with WC and weight was available. To minimize the possibility of reverse causality, that is, participants who were sub-clinically ill were more likely to lose weight and WC which would have resulted in a higher mortality risk; participants were excluded with self-reported cancer or CVD at both time-points, as were those who had a BMI < 18.5 kg/m^2. Those who died within the first 3 and 5 years were also excluded in our subgroup analyses. Nevertheless, we cannot completely rule out the possibility of some effect of reverse causality and/or residual confounding.

Table 4 Cox multivariable-adjusted HRs after 16 years of follow-up for all-cause mortality in 6868 women. Results are given for stratified variables by WC change category

					Categories of change in waist circumference (WC) (cm)				
					loss > 5 cm	loss > 2.5 & ≤5 cm	loss or gain ≤2.5 cm	gain > 2.5 & ≤ 5 cm	gain > 5 cm
	N	%	Deaths	%	HR (95% CI)	HR (95% CI)		HR (95% CI)	HR (95% CI)
All	6868	100.0	1315	100.0	1.03 (0.87–1.24)	0.93 (0.78–1.10)	Ref	0.95 (0.79–1.13)	** 1.25 (1.06–1.46)
By WC (1HE)									
< 80 cm	3510	51.1	516	39.2	1.14 (0.82–1.57)	1.00 (0.75–1.33)	Ref	0.95 (0.73–1.23)	1.07 (0.83–1.38)
≥ 80 & ≤ 88 cm	1868	27.2	392	29.8	1.26 (0.92–1.73)	1.11 (0.81–1.51)	Ref	0.78 (0.54–1.11)	1.28 (0.96–1.71)
> 88 cm	1490	21.7	407	31.0	0.80 (0.60–1.07)	0.76 (0.55–1.03)	Ref	1.06 (0.76–1.47)	** 1.53 (1.11–2.11)
By age (1HE)									
< 65 y	5153	75.0	518	39.4	1.23 (0.92–1.64)	1.07 (0.80–1.43)	Ref	1.04 (0.80–1.36)	1.10 (0.85–1.43)
≥ 65 y	1715	25.0	797	60.6	0.92 (0.74–1.15)	0.83 (0.66–1.03)	Ref	0.89 (0.70–1.12)	** 1.35 (1.10–1.67)
By smoking status (2HC)									
current	545	7.9	127	9.7	1.30 (0.78–2.15)	0.81 (0.44–1.51)	Ref	1.26 (0.71–2.24)	1.15 (0.64–2.07)
former	2247	32.7	479	36.4	0.80 (0.60–1.08)	0.79 (0.60–1.06)	Ref	** 0.62 (0.45–0.86)	1.14 (0.88–1.48)
never	4076	59.3	709	53.9	1.15 (0.90–1.48)	1.05 (0.83–1.33)	Ref	1.13 (0.90–1.42)	* 1.32 (1.06–1.65)
By BMI (1HE)									
≥ 18.5 & < 25	3258	47.4	548	41.7	1.09 (0.82–1.43)	0.91 (0.69–1.19)	Ref	0.93 (0.71–1.20)	1.27 (0.98–1.64)
≥ 25 & < 30	2614	38.1	540	41.1	1.04 (0.79–1.38)	1.00 (0.76–1.30)	Ref	0.84 (0.62–1.13)	1.23 (0.96–1.58)
≥ 30	996	14.5	227	17.3	0.86 (0.57–1.32)	0.82 (0.52–1.29)	Ref	1.17 (0.78–1.75)	1.31 (0.88–1.96)
By physical activity (1HC)									
inactive	1706	24.8	501	38.1	1.14 (0.86–1.51)	0.97 (0.74–1.27)	Ref	0.97 (0.72–1.30)	1.06 (0.81–1.39)
mod inactive	2278	33.2	420	31.9	1.15 (0.83–1.58)	1.09 (0.79–1.50)	Ref	1.11 (0.82–1.50)	** 1.51 (1.14–2.00)
mod active	1676	24.4	247	18.8	0.67 (0.43–1.03)	0.74 (0.49–1.12)	Ref	0.70 (0.47–1.03)	1.14 (0.79–1.65)
active	1208	17.6	147	11.2	1.15 (0.70–1.92)	0.73 (0.42–1.27)	Ref	0.92 (0.54–1.54)	1.41 (0.86–2.32)
By educational level (1HE)									
No qualifications	2525	36.8	670	51.0	1.07 (0.84–1.38)	0.88 (0.69–1.12)	Ref	0.88 (0.68–1.12)	1.20 (0.95–1.50)
O level and above	4343	63.2	645	49.0	1.00 (0.78–1.29)	0.99 (0.77–1.27)	Ref	1.02 (0.80–1.31)	* 1.29 (1.03–1.63)
By social class (1HE)									
Non-manual	4358	63.5	848	64.5	1.00 (0.80–1.26)	0.99 (0.80–1.22)	Ref	0.80 (0.64–1.01)	** 1.30 (1.07–1.58)
Manual	2510	36.5	467	35.5	1.08 (0.81–1.44)	0.81 (0.59–1.10)	Ref	1.21 (0.92–1.59)	1.14 (0.85–1.51)
Menopausal status (2HC)									
Pre-menopausal	429	6.2	9	0.7	0.00 (0.00 -)	5.16 (0.75–35.62)	Ref	0.00 (0.00 -)	3.91 (0.61–24.96)
Early peri-menopausal (< 1 y)	251	3.7	10	0.8	0.00 (0.00 -)	0.38 (0.04–4.00)	Ref	0.52 (0.09–2.90)	0.77 (0.11–5.21)
Late peri-menopausal (1–5 y)	1322	19.2	68	5.2	* 2.39 (1.00–5.69)	1.79 (0.81–3.98)	Ref	** 2.84 (1.40–5.77)	1.51 (0.69–3.32)
Post-menopausal (> 5 y)	4866	70.9	1228	93.4	1.01 (0.84–1.21)	0.89 (0.74–1.06)	Ref	0.88 (0.74–1.06)	* 1.23 (1.04–1.45)
Excluding early deaths									
Excluding deaths < 3 y	6800	99.0	1247	94.8	1.02 (0.85–1.22)	0.94 (0.78–1.12)	Ref	0.96 (0.80–1.15)	** 1.23 (1.04–1.33)
Excluding deaths < 5 y	6713	97.7	1160	88.2	1.03 (0.86–1.2)	0.94 (0.78–1.13)	Ref	0.98 (0.82–1.18)	* 1.24 (1.04–1.48)

Adjusted for age, BMI, baseline WC, physical activity, smoking, educational level, social class, menopausal status and change in weight (except where the variable was used for stratification)
***$p < 0.001$; ** $p < 0.01$; * $p < 0.05$

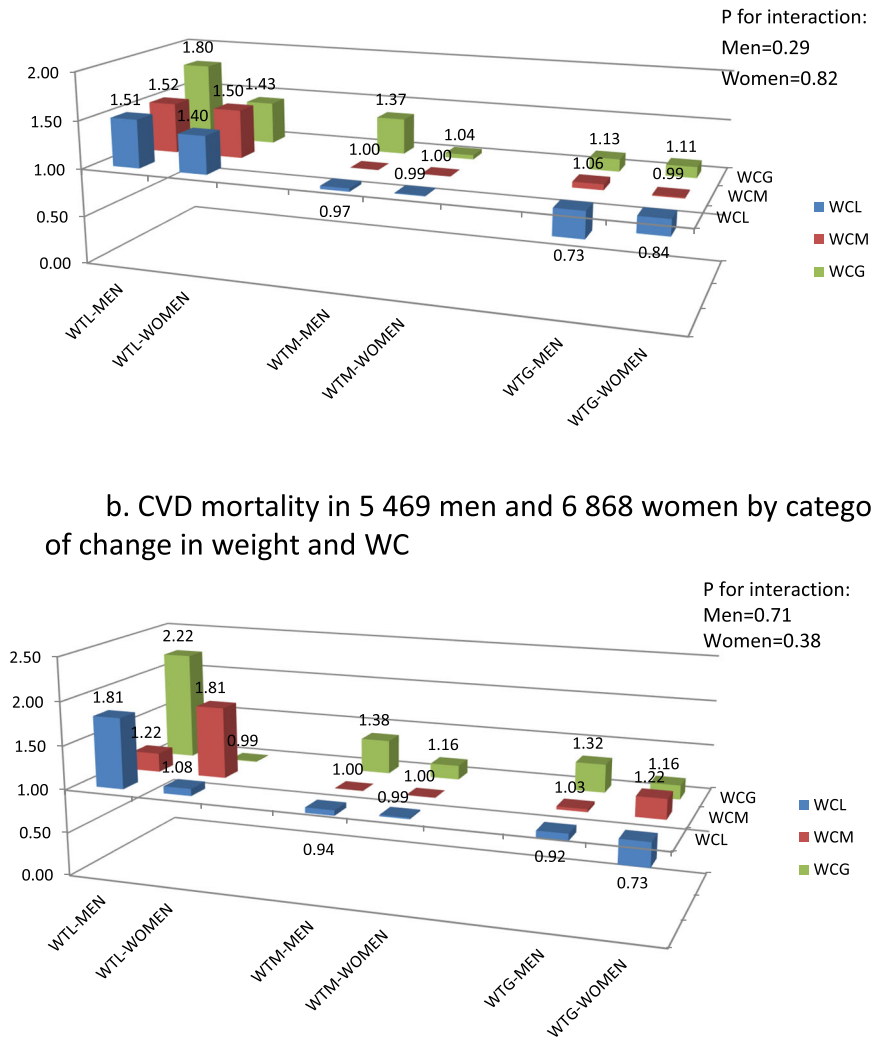

Fig 2 a Total mortality in 5 469 men and 6 868 women by categories of change in weight and WC, b. CVD mortality in 5 469 men and 6 868 women by categories of change in weight and WC

The main limitations of our study include self-reported disease history, healthy volunteer bias and attrition. The prevalence of self-reported CVD was lower in those who also attended the 2HE, as was the percentage of current smokers and physically inactive participants, which may be indicative of healthier individuals being more likely to return for a follow up assessment (Additional file 1: Table 1). Additionally, the percentage of deaths that occurred was lower in both men and women, after exclusion criteria were applied; the percentage of deaths was highest in those who only attended the 1HE. It is possible that some individuals in our study had undiagnosed/sub-clinical conditions such as digestive disorders, or various psychosocial diseases, such as depression, that they did not report, resulting in weight and potentially WC loss and subsequent death, and possibly an overestimation of the association [39]. The more frail participants may have been excluded and/or have not returned for the 2HC and therefore be under-represented in our analyses. However, it is also conceivable that some of the participants in the weight and WC loss categories were pre-frail at 2HE. A further limitation is the inability to account for all behaviour changes during follow-up, including physical activity, which may have had effects on the observed associations.

Comparison of total and CVD mortality with other studies

The highest HRs for both total and CVD mortality in men, which were statistically significant, were observed in the WTL and WCG category, although this was a small category, consisting of only 35 participants and 19 events.

In women, the highest HR was found in the WTL and WCM category, for both total and CVD mortality. The category with the lowest HR in both men and women, for both total and CVD mortality was the weight gain and WC loss category, although this was not statistically significant. The findings that WTL, in combination with changes in WC, was associated with the highest HRs, agrees with our previous study, which found that weight loss of more than 2.5 kg over approximately 4 years is associated with a higher mortality over 15 years of follow-up [31] in this middle-aged and elderly population.

Our findings that WCG is associated with higher all-cause mortality are in agreement with a number of previous publications. A recent pooled sample of 2492 Danish and Swedish women found that WCG over 6 years was associated with increased total and CVD mortality [23]. We did not find significant associations with WCG and CVD mortality in women; statistical power was more limited due to the lower number of CVD deaths in women in our study. In the study of Danish and Swedish women, associations were particularly strong for women with normal weight and for ever-smokers. In our analyses, we found that WCG > 5 cm had a similar HR for all BMI categories and only never smokers had a statistically higher HR. The differences in findings may be due to the characteristics of the 2 cohorts; our cohort of women was older, had a higher mean BMI and a lower percentage of ever-smokers. A prospective study of 26,625 healthy middle-aged men and women has also shown that increases in WC over a 5.3 year period were positively associated with mortality in men and women over 6.7 years of follow-up; the HR was 1.09 (1.02, 1,16) per 5 cm for both men and women combined, after adjusting for baseline BMI, WC and change in BMI [24].

However, results from the Melbourne Collaborative Cohort Study of 21,298 men and women aged 40–69 years at baseline found that WCL over approximately 12 years was associated with an increased risk of all-cause mortality (HR: 1.26; 95% CI: 1.09–1.47) over 7.7 years of follow-up, especially in older adults, compared to those who had minimal changes, but did not find an association with WCG [25]. It is possible that the shorter time period of less than 4 years between our WC measurements may partially explain our lack of associations with WCL and mortality. However, in a study of older adults aged 70–77 years, WCL over a 3 year period (≥3.1 cm) was significantly associated with an all-cause mortality risk over 20 years of follow-up of 1.52 (95% CI: 1.01–2.31), which was comparable to their weight loss finding, in agreement with our previous results [31]; no significant associations were found for WCG and all-cause mortality, or changes in WC and CVD mortality [40]. No significant associations were found with self-reported changes in WC over a 12 years and concurrent mortality, after 9 years of follow-up, in a large, prospective study among 23,254 Swedish women [26], although the cohort was relatively young (median age of 52 years at follow-up), with a relatively low number of deaths ($N = 570$) during follow-up, of which 79 were attributable to CVD. A smaller study of 1138 older adults, predominantly female, from the Bambuí (Brazil) Cohort Study of Aging [27] did not find any associations with WC change and mortality; the relatively short time interval of 3 years between measurements, in addition to the follow-up period of 8 years may have contributed to the lack of findings. No associations were found for WC change over 3.6 years and mortality in a study of 1805 Iranian men, aged ⩾30 years [28]; the limited number of deaths ($N = 88$) during the relatively short-term follow-up of 6.6 years resulted in limited power to assess the effect of changes in WC for mortality events.

Changes in WC and weight and plausible mechanisms

Weight, particularly in older populations, is an indicator encompassing not just fat but also bone and muscle mass and these different components may relate to health in different directions; thus, weight loss in later life may be an indicator of increasing frailty. Body fat distribution changes with age, with a reduction in subcutaneous fat and an increase in central adiposity [41]. The accumulation of body fat in the abdominal region has been shown to be associated with a number of adverse health outcomes such as diabetes, metabolic syndrome, CVD and all-cause mortality, independently of BMI [12, 42–44]. Adipose tissue has an important role to play in numerous metabolic and endocrine functions, including the expression and secretion of inflammatory cytokines, such as leptin, adiponectin, and interleukin 6 (IL-6), which are important regulatory factors of energy intake and inflammatory responses [45]. In a study of 20 abdominally obese, older women, hyperinsulinemia was positively associated with adipose IL-6 gene expression, but negatively associated with adipose adiponectin expression [46]. A 20-week weight loss program in obese, older women decreased leptin production in both gluteal and abdominal adipose tissue, but only increased adiponectin production from abdominal adipose tissue [47]. These results highlight the importance that regional adipose tissue hormone/cytokine production may play in mechanisms of metabolic complications associated with abdominal obesity and the importance of improving body fat distribution, through appropriate exercise and diet programs [48].

Public health considerations

Obesity is a complex condition, for which there are numerous risk factors, many of which are modifiable [49, 50]. Attention needs to be focussed on various levels of policy strategies for its prevention [51, 52]. Aa a number of studies

have shown that WC is increasing at a faster rate than BMI or body weight [53–55], current recommendations, based on observational cohort studies, including findings from this publication, should advocate prevention of WC gain into adulthood, and weight and WC stability from midlife onwards.

Conclusion

In summary, an increase in WC of more than 5 cm over approximately 4 years, with little weight gain, is significantly associated with higher total mortality in both men and women and higher CVD mortality in men over the next 16 years of follow-up in this population-based cohort study of 12,337 middle-aged and elderly men and women. These findings are in marked contrast to our earlier observation that weight loss is associated with increased mortality risk [31]. The apparently paradoxical observations suggest that WC may be a better indicator of the adverse health consequence of obesity in later life than weight. Interventions targeted at fat distribution and focusing on preventing increase in central adiposity rather than lowering weight per se in later life may be more likely to have health benefits.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12872-019-1223-z.

Additional file 1. Supplementary information. Baseline characteristics of EPIC-Norfolk men and women who attended 1HE, and those who attended both 1HE and 2HE, before and after exclusion criteria were applied. Cox multivariable-adjusted HRs after 16 years of follow-up for CVD mortality in 5469 men.

Abbreviations

1HE: Baseline health examination; 2HE: Second health examination; BMI: Body mass index; CVD: Cardiovascular disease; EPIC: European prospective investigation into Cancer and nutrition; WC: Waist circumference; WCG: Waist circumference gain; WCL: Waist circumference loss; WCM: Waist circumference maintenance; WTG: Weight gain; WTL: Weight loss; WTM: Weight maintenance

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Authors' contributions

AAM wrote the manuscript and analysed the data. KTK and MAHL advised on statistical analysis. RL manages the study data. KTK and NW are principal investigators who contributed to the conception and study design and guidance on the presentation and interpretation of these data. All authors contributed to data analysis and interpretation of the data, and all authors reviewed and approved the final manuscript.

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Availability of data and materials

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the Norfolk District Health Authority Ethics Committee and all participants gave written, informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]European Prospective Investigation into Cancer and Nutrition, Department of Public Health and Primary Care, Strangeways Research Laboratory, University of Cambridge, Worts Causeway, Cambridge, UK. [2]European Prospective Investigation into Cancer and Nutrition, MRC Epidemiology Unit, Cambridge Biomedical Campus, University of Cambridge, Cambridge, UK. [3]MRC Epidemiology Unit, Cambridge, Institute of Metabolic Science, Cambridge Biomedical Campus, University of Cambridge School of Clinical Medicine, Cambridge, UK. [4]EPIC, Department of Gerontology, Addenbrooke's Hospital, School of Clinical Medicine, University of Cambridge, Cambridge, UK.

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Publisher's Note

4.3 Supplementary material

(https://link.springer.com/article/10.1186/s12872-019-1223-z#Sec21)

Table S1 Baseline characteristics of EPIC-Norfolk men and women who attended 1HE, and those who attended both 1HE and 2HE, before and after exclusion criteria were applied. Continuous variables are Mean (SD) and categorical variables are N (%).

	Men			Women		
	1HE N=11 607	1HE and 2HE N=6 582	1HE and 2HE with exclusions[a] N=5 469	1HE N=14 032	1HE and 2HE N=8 446	1HE and 2HE with exclusions[a] N=6 868
Waist, cm	95.8 (9.8)	95.3 (9.4)	95.1 (9.3)	82.2 (10.9)	81.2 (10.4)	81.0 (10.2)
Weight, kg	80.4 (11.5)	80.1 (11.0)	80.2 (10.9)	67.9 (11.8)	67.4 (11.3)	67.5 (11.1)
Age, years	59.6 (9.3)	59.8 (8.9)	59.0 (8.9)	58.9 (9.3)	58.4 (8.9)	57.9 (8.8)
BMI, kg/m2	26.5 (3.3)	26.4 (3.2)	26.3 (3.1)	26.2 (4.4)	25.9 (4.1)	25.9 (4.0)
Smoking status						
Current	1 405 (12.2)	616 (9.4)	507 (9.3)	1 579 (11.4)	764 (9.1)	618 (9.0)
Former	6 284 (54.5)	3 563 (54.5)	2 898 (53.0)	4 477 (32.2)	2 632 (31.4)	2 169 (31.6)
Never	3 837 (33.3)	2 355 (36.0)	2 064 (37.7)	7 837 (56.4)	4 977 (59.4)	4 081 (59.4)
Physical activity						
Inactive	3 586 (30.9)	1 826 (27.7)	1 444 (26.4)	4 277 (30.5)	2 226 (26.4)	1 706 (24.8)
Moderately inactive	2 858 (24.6)	1 649 (25.1)	1 353 (24.7)	4 493 (32.0)	2 760 (32.7)	2 278 (33.2)
Moderately active	2 660 (22.9)	1 632 (24.8)	1 377 (25.2)	3 116 (22.2)	2 018 (23.9)	1 676 (24.4)
active	2 502 (21.6)	1 475 (22.4)	1 295 (23.7)	2 146 (15.3)	1 442 (17.1)	1 208 (17.6)
Social class						
Non-manual	6 657 (58.4)	4 034 (62.2)	3 395 (62.1)	8 394 (61.4)	5 304 (64.0)	4 358 (63.4)
Manual	4 744 (41.6)	2 451 (37.8)	2 074 (37.9)	5 274 (38.6)	2 978 (36.0)	2 510 (36.6)
Educational level						
No qualifications	3 534 (30.5)	1 801 (27.4)	1 444 (26.4)	5 920 (42.2)	3 169 (37.5)	2 525 (36.8)
O level and above	8 064 (69.5)	4 779 (72.6)	4 025 (73.6)	8 103 (57.8)	5 272 (62.5)	4 343 (63.2)
Self-reported diseases						
CVD (yes)	798 (6.9)	394 (6.0)		324 (2.3)	160 (1.9)	
Cancer (yes)	450 (3.9)	233 (3.5)		960 (6.8)	568 (6.7)	
Deaths	4 285 (36.9)	2 196 (33.4)	1 551 (28.4)	3 754 (26.8)	1 820 (21.6)	1 315 (19.2)
WC change categories						
loss >5 cm		774 (11.8)	641 (11.7)		1 116 (13.2)	873 (12.7)
loss >2.5 & ≤5 cm		788 (12.0)	656 (12.0)		1 085 (12.8)	875 (12.7)
Reference		2 662 (40.4)	2 211 (40.4)		3 152 (37.3)	2 596 (37.8)
gain >2.5 & ≤5 cm		1 089 (16.6)	926 (16.9)		1 383 (16.4)	1 131 (16.5)
gain >5 cm		1 269 (19.3)	1 035 (18.9)		1 710 (20.2)	1 393 (20.3)

[a] self-reported cancer and CVD, missing data on weight, height, waist, smoking status, social class, educational level, BMI < 18.5 kg/m^2 and menopausal status in women

BMI body mass index, *1HE* 1st health examination, *2HE* 2nd health examination

Table S2. Cox multivariable-adjusted HRs after 16 years of follow-up for CVD mortality in 5 469 men. Results are given for stratified variables by WC change category.

					Categories of change in waist circumference (WC) (cm)				
					loss > 5 cm	loss > 2.5 & ≤5 cm	loss or gain ≤ 2.5 cm	gain > 2.5 & ≤ 5 cm	gain > 5 cm
	N	%	Deaths	%	HR (95% CI)	HR (95% CI)		HR (95% CI)	HR (95% CI)
All	5 469	100.0	440	100.0	0.97 (0.70 - 1.34)	1.11 (0.82 - 1.49)	Ref	1.18 (0.88 - 1.58)	*** 1.84 (1.39 - 2.43)
By WC (1HE)									
< 94 cm	2515	46.0	158	35.9	0.80 (0.41 - 1.56)	1.48 (0.89 - 2.46)	Ref	1.28 (0.80 - 2.05)	* 1.57 (1.00 - 2.46)
≥ 94 & ≤ 102 cm	1854	33.9	138	31.4	0.94 (0.53 - 1.68)	0.94 (0.54 - 1.64)	Ref	1.31 (0.77 - 2.22)	*** 2.66 (1.62 – 4.35)
> 102 cm	1100	20.1	144	32.7	1.07 (0.64 - 1.78)	1.03 (0.62 - 1.72)	Ref	1.09 (0.62 - 1.91)	1.56 (0.90 – 2.70)
By age (1HE)									
<65 y	3882	71.0	134	30.5	1.39 (0.74 - 2.60)	* 1.80 (1.03 - 3.14)	Ref	* 1.89 (1.09 - 3.26)	*** 3.38 (2.03 - 5.64)
≥65 y	1587	29.0	306	69.5	0.89 (0.60 - 1.30)	0.93 (0.64 - 1.34)	Ref	1.02 (0.71 - 1.44)	* 1.46 (1.04 -2.05)
By smoking status (2HC)									
current	438	8.0	55	12.5	1.33 (0.56 - 3.18)	1.06 (0.42 - 2.67)	Ref	1.23 (0.49 - 3.09)	** 3.10 (1.39 - 6.94)
former	2970	54.3	273	62.0	0.82 (0.53 - 1.27)	1.03 (0.70 - 1.51)	Ref	1.28 (0.89 - 1.82)	*** 1.69 (1.18 - 2.43)
never	2061	37.7	112	25.5	1.32 (0.72 - 2.45)	1.36 (0.75- 2.47)	Ref	0.97 (0.51 - 1.84)	1.70 (0.98 - 2.94)
By BMI (1HE)									
≥ 18.5 & < 25	1913	35.0	137	31.1	1.09 (0.60 - 1.99)	1.15 (0.67 - 1.99)	Ref	1.19 (0.71 - 2.01)	1.60 (0.96 - 2.66)
≥ 25 & < 30	2926	53.5	220	50.0	0.80 (0.50 - 1.30)	1.09 (0.71 - 1.67)	Ref	1.24 (0.81 - 1.90)	***2.37 (1.60 - 3.51)
≥ 30	630	11.5	83	18.9	1.20 (0.59 - 2.44)	1.17 (0.58 - 2.36)	Ref	1.22 (0.62 - 2.41)	1.28 (0.63 - 2.59)
By physical activity (1HC)									
inactive	1444	26.4	160	36.4	0.65 (0.38 - 1.14)	0.97 (0.59 - 1.60)	Ref	0.87 (0.53 - 1.45)	1.56 (0.98 - 2.47)
mod inactive	1353	24.7	101	23.0	1.83 (0.97 - 3.44)	1.28 (0.71 - 2.31)	Ref	1.08 (0.56 - 2.11)	1.48 (0.78 - 2.82)
mod active	1377	25.2	93	21.1	0.84 (0.39 - 1.81	1.12 (0.56 - 2.22)	Ref	1.80 (0.98 - 3.28)	* 2.16 (1.16 - 4.00)

active	1295	23.7	86	19.5	1.08 (0.51 - 2.30)	1.07 (0.51 - 2.26)	Ref	1.35 (0.70 - 2.63)	** 2.86 (1.57 - 5.23)
By educational level (1HE)									
No qualifications	1444	26.4	150	34.1	0.74 (0.41 - 1.32)	1.21 (0.73 - 2.01)	Ref	1.09 (0.65 - 1.83)	** 2.01 (1.25 - 3.25)
O level and above	4025	73.6	290	65.9	1.13 (0.77 - 1.68)	1.06 (0.72 - 1.54)	Ref	1.23 (0.86 - 1.77)	** 1.74 (1.23 - 2.46)
By social class (1HE)									
Non-manual	3395	62.1	270	61.4	1.12 (0.75 - 1.69)	0.95 (0.64 - 1.40)	Ref	0.92 (0.62 - 1.36)	* 1.47 (1.03 - 2.09)
Manual	2074	37.9	170	38.6	0.78 (0.45 - 1.35)	1.44 (0.88 - 2.33)	Ref	* 1.70 (1.08 - 2.69)	*** 2.56 (1.62 - 4.06)
Excluding early deaths									
Excluding deaths < 3y	5347	97.8	405	92.0	0.98 (0.69 - 1.37)	1.14 (0.83 - 1.55)	Ref	1.20 (0.88 - 1.62)	*** 1.80 (1.34 - 2.41)
Excluding deaths < 5y	5211	95.3	357	81.1	1.05 (0.73 - 1.50)	1.08 (0.78 - 1.52)	Ref	1.08 (0.78 - 1.50)	** 1.64 (1.20 - 2.25)

Adjusted for age, BMI, baseline WC, physical activity, smoking, educational level, social class and change in weight (except where the variable was used for stratification)
***p<0.001; ** p<0.01; * p<0.05

Chapter 5. Positive Associations of Dietary Intake and Plasma Concentrations of Vitamin E with Skeletal Muscle Mass, Heel Bone Ultrasound Attenuation and Fracture Risk in the EPIC-Norfolk Cohort

5.1 Key points

What is already known on this subject?

- The prevalence of sarcopenia (low levels of muscle strength, muscle quantity/quality and physical function), frailty and fractures is increasing in our ageing society.
- A paucity of research exists, mainly in older adults, which suggests that vitamin E, a lipid-soluble, antioxidant vitamin, may be protective with respect to muscle mass, bone health and fractures.

After my investigations into changes in weight and WC and subsequent mortality risk, I continued to study body composition, in the form of indices of FFM. In this paper, I used both dietary data and circulating concentrations of vitamin E to explore their potential associations with measures of SMM and bone density status, and subsequent fracture risk.

What this study adds.

- Significant positive associations were observed between both dietary vitamin E intake and plasma concentrations of both serum cholesterol-adjusted α- and γ-tocopherol and fat-free mass (FFM) and broadband ultrasound attenuation (BUA), and generally significant positive protective associations for fracture risk.
- The associations found with vitamin E for bone density status and fracture risk were independent of vitamin D and calcium intake, which are known to be important for bone health.
- Findings from this study indicate protection for musculoskeletal health with higher intakes and blood concentrations of vitamin E.

5.2 Published journal article

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antioxidants

MDPI

Article

Positive Associations of Dietary Intake and Plasma Concentrations of Vitamin E with Skeletal Muscle Mass, Heel Bone Ultrasound Attenuation and Fracture Risk in the EPIC-Norfolk Cohort

Angela A. Mulligan [1,2,*], Richard P. G. Hayhoe [3], Robert N. Luben [1] and Ailsa A. Welch [3]

[1] Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge, Cambridgeshire CB1 8RN, UK; robert.luben@phpc.cam.ac.uk
[2] NIHR BRC Diet, Anthropometry and Physical Activity Group, MRC Epidemiology Unit, University of Cambridge, Cambridgeshire CB2 0AH, UK
[3] Department of Epidemiology and Public Health, Norwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich NR4 7TJ, UK; R.Hayhoe@uea.ac.uk (R.P.G.H.); A.Welch@uea.ac.uk (A.A.W.)
* Correspondence: angela.mulligan@phpc.cam.ac.uk or angela.mulligan@mrc-epid.cam.ac.uk

Abstract: The prevalence of sarcopenia, frailty and fractures is increasing. Prevention options are limited, but dietary factors including vitamin E have the potential to confer some protection. This study investigated cross-sectional associations between dietary and plasma concentrations of vitamin E with indices of skeletal muscle mass (SMM) (n = 14,179 and 4283, respectively) and bone density (n = 14,694 and 4457, respectively) and longitudinal fracture risk (n = 25,223 and 7291, respectively) in European Prospective Investigation Into Cancer and Nutrition (EPIC)-Norfolk participants, aged 39–79 years at baseline. Participants completed a health and lifestyle questionnaire, a 7-day diet diary (7dDD) and had anthropometric measurements taken. Fat-free mass (as a SMM proxy) was measured using bioimpedance and bone density was measured using calcaneal broadband ultrasound attenuation (BUA) and incident fractures over 18.5 years of follow-up. Associations between indices of SMM, BUA and fracture risk were investigated by quintiles of dietary vitamin E intake or plasma concentrations. Positive trends in SMM indices and BUA were apparent across dietary quintiles for both sexes, with interquintile differences of 0.88–1.91% ($p < 0.001$), and protective trends for total and hip fracture risk. Circulating plasma α- and γ-tocopherol results matched the overall dietary findings. Dietary vitamin E may be important for musculoskeletal health but further investigation is required to fully understand the relationships of plasma tocopherols.

Keywords: sarcopenia; frailty; skeletal muscle; bone density status; fracture risk; vitamin E

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1. Introduction

The prevalence of sarcopenia (low levels of muscle strength, muscle quantity/quality and physical function [1]), frailty and fractures is increasing in our aging society. In the UK, the number of older people is growing; in 2018, there were 1.6 million people aged 85 years and over; by mid 2043, this is projected to nearly double to 3 million [2]. In parallel, the number of sarcopenic patients will dramatically increase, adding to the already considerable resultant public health issues [3]. A recent prospective study by Sousa et al. [4] found that sarcopenia was independently associated with hospitalisation costs and with an estimated increase of 34% for patients aged ≥65 years. Approximately 520,000 fragility fractures occurred in the UK in 2017, with fracture-related costs of GBP 4.5 billion; these numbers are estimated to increase by 26.2% and 30.2%, respectively, by 2030 [5]. Losses in bone density and skeletal muscle mass and strength occur gradually from the age 30 years, with increasing rates of loss in those over the age of 60 years [6,7]. These conditions are

currently difficult to treat and, therefore, maintaining skeletal muscle and bone health during aging is important.

It has long been established that a close physiological relationship exists between muscle and bone, which changes with aging, but more recently it has become apparent that this is not solely related to mechanical function [8,9]. Factors, such as myokines, that are secreted by muscle, including insulin-like growth factor 1 and fibroblast growth factor 2, have paracrine and endocrine effects which can affect bone repair and metabolism [10,11]. Additionally, bone secretes factors such as osteocalcin and connexin 43, that have direct effects on muscle [12,13]. Studies have shown that both sarcopenia and frailty are risk factors for fractures and falls [14–18].

Known determinants of muscle and bone aging [19] include modifiable lifestyle risk factors, such as cigarette smoking, low physical activity and poor diet [20]. Limited research exists, mainly in older adults, which suggests that vitamin E, a lipid-soluble, antioxidant vitamin, may also be protective with respect to muscle mass and frailty [21–26], as skeletal muscle is the organ with the highest consumption of oxygen in the body. Positive associations of vitamin E intake with bone mineral density (BMD) and fracture risk have also been reported in both men and women [27–30]. The major forms of vitamin E in food are the α- and γ-tocopherols, and thus these are found in greater abundance than other tocopherols and tocotrienols in tissues. The predominant form of vitamin E in the body is α-tocopherol, which has tended to be the focus of research, although some research has been carried out on other tocopherols, in particular, γ-tocopherol [31,32]. A number of mechanisms have been suggested as to how vitamin E may slow down aging of skeletal muscle [33–35]. Reduction in oxidative stress is thought to be a mechanism by which vitamin E homologues protect bone but it has been reported that α- and γ-tocopherol have opposing inflammatory functions and may uncouple bone turnover, such as by increasing bone resorption without affecting bone formation [36,37].

The current study therefore aimed to investigate the potential associations of reported dietary vitamin E intake (α-tocopherol equivalents), as well as plasma concentrations of both α- and γ-tocopherol and the ratio of α:γ-tocopherol, with measures of skeletal muscle mass (SMM) and bone density status concurrently, in a large cohort general population cohort of middle-aged and elderly men and women. Additionally, both dietary and plasma concentrations of vitamin E were examined in relation to fracture risk during 18.5 years of follow-up.

2. Materials and Methods

2.1. EPIC-Norfolk Study Design

The Norfolk cohort of the European Prospective Investigation Into Cancer and Nutrition (EPIC-Norfolk) is part of the Europe-wide EPIC study, which involves over half a million people in ten countries [38] and was initially designed to investigate diet and the risk of developing cancer. Details of cohort recruitment, data collection and participant characteristics have been published previously [39]. In brief, participants aged between 39 and 79 years were recruited from General Practitioners' surgeries, based in the rural areas of Norfolk and market towns as well as the city of Norwich, from 1993 to 1997. Since virtually all the population of the UK are registered with a general practice through the National Health Service, general practice age sex registers act as a population sampling frame. This cohort at baseline was comparable to the UK national population with regard to many characteristics, including age, sex and anthropometry measurements, but it had a lower proportion of current smokers [40]. The study was approved by the Norfolk District Health Authority Ethics Committee (98CN01) and all participants gave written informed consent, according to the Declaration of Helsinki. Of the 30,445 men and women who consented to participate in the study (39% response rate), 25,639 attended a baseline health examination (1HE) between 1993 and 1997. Of these, 15,028 attended a second health examination (2HE) between 1998 and 2000.

2.2. Measurements of Body Composition

2.2.1. Height, Weight and BMI

At both health examinations, a trained nurse measured weight (to the nearest 0.1 kg) and height (to the nearest 0.1 cm), with participants wearing light clothing and no shoes. Body mass index (BMI) was calculated from these measurements as weight in kilograms divided by height squared in metres (kg/m^2).

2.2.2. Indices of Fat-Free Mass (FFM)

Bioelectrical impedance analysis (BIA) was carried out at 2HE, using a standardised protocol (Bodystat, Isle of Man, UK), suitable for use in large field-based studies and shown to be a valid [41] and reliable [42] measure of body composition. The TANITA Body Fat Monitor/Scale TBF-531 BIA analyser (Tanita UK Ltd., Middlesex, UK) calculated body density (BD) from total weight (Wt) in kilograms, height (Ht) in centimeters, and impedance (Z) in ohms, using the following standard regression formulae: BD in men = $1.100455 - 0.109766 \times Wt \times Z \div Ht^2 + 0.000174 \times Z$; BD in women = $1.090343 - 0.108941 \times Wt \times Z \div Ht2 + 0.00013 \times Z$. Fat-free mass (FFM) in kilograms was then calculated: $FFM = Wt - ((4.57 \div BD - 4.142) \times Wt)$, which is an estimate of the total mass of nonfat compartments of the body—i.e., metabolic tissue, water, and bone. In an effort to scale for differences in skeletal muscle mass with increasing body weight, scaled for height, FFM standardised by BMI (FFM_{BMI}) was calculated as FFM divided by BMI, according to the method suggested by Studenski [43].

2.2.3. Bone Density Assessment

Quantitative ultrasound measurements of the calcaneus (heel bone) were taken at 2HE, using a contact ultrasound bone analyser (CUBA) device (McCue Ultrasonics) following standard protocols. Broadband ultrasound attenuation (BUA) (dB/MHz) measurements were taken at least in duplicate for each foot of the participant, and the mean of the left and right foot measures was used for analysis. Each of the five CUBA devices used in the study was calibrated daily with its physical phantom. In addition, calibration between devices was checked monthly using a roving phantom (CV 3.5%). The CUBA method of bone density assessment has been shown to be capable of predicting fracture risk [44,45], and is cheaper and simpler to conduct in general practice settings compared with the gold-standard of dual-energy X-ray absorptiometry (DEXA).

2.2.4. Fracture Incidence

Self-reported fracture was recorded using questionnaires at both 1HE and 2HE, and incident fracture was ascertained using linkage to the East Norfolk Health Authority database (ENCORE) of hospital attendances by Norfolk residents [46]. Incidence of all osteoporotic fractures in the cohort, up to the end of March 2018, was determined by retrieving data using each participant's National Health Service (NHS) number and searching for events logged using the International Classification of Diseases (ICD) 9 and 10 diagnostic codes for osteoporotic hip, spine or wrist fractures.

2.3. Measurement of Vitamin E Intake

Dietary intakes at 1HE were assessed using 7-day diet diaries (7dDDs), which were completed by 25,507 participants, detailing all food and drink consumed, together with the portion sizes. Data Into Nutrients for Epidemiological Research (DINER) software was used to enter the dietary information provided by the 7dDDs [47], which was then checked and processed by nutritionists to obtain nutrient data using DINERMO [48]. Vitamin and mineral supplements recorded in the 7dDD were quantified using the Vitamin and Mineral Supplement (ViMiS) database [49].

2.4. Blood Analysis

At 1HE, a 42 mL sample of blood was collected in citrated and plain monovettes and stored in a refrigerator. The next day, blood samples were processed and stored at −196 °C as plasma and serum. Serum cholesterol was determined for the full cohort in a Norfolk laboratory using a RA 1000 Diagnostics (Bayer, Basingstoke, UK) instrument, and cohort concentrations ranged from 2.10 to 12.40 mmol/L. The vitamins α- and γ-tocopherol were analysed on a cohort subset that consisted of a series of previous case-control studies, where cases were defined by incident cardiovascular disease or cancer and four matched, disease-free controls. Plasma concentrations were analysed at IARC, Lyon (France), using high-performance liquid chromatography for the vitamins. In our analyses, concentrations for α-tocopherol ranged from 0.71 the 106.54 μmol/L and from 0.03 to 9.85 μmol/L for γ-tocopherol; we excluded one participant for whom the ratio of α-tocopherol to γ-tocopherol was greater than 1000). Plasma α- and γ-tocopherol concentrations were adjusted for cholesterol, as this is seen as a more reliable marker for vitamin E nutritional status [50] since tocopherols are transported via circulation through lipoproteins; adjusted concentrations are presented in μmol/mmol, calculated by dividing the plasma tocopherol concentrations (μmol/L) by total cholesterol (mmol/L).

2.5. Measurement of Confounding Variables

Data collected via two self-administered health and lifestyle questionnaires (HLQ1 and HLQ2), before the 1HE and 2HE, respectively, were used to establish classification of a number of variables. Family history of osteoporosis was categorised as yes or no; menopausal status (women only (2HE)) was categorised as premenopausal, perimenopausal (<1 year), perimenopausal (1–5 years) or postmenopausal; hormone replacement therapy (HRT) status (women only (2HE)) was categorised as current, former or never users. The use of statins and steroids at 2HE were categorised as yes or no. Smoking status (derived from HLQ2) (never, former, current) was derived from yes and no responses to the following questions "Have you ever smoked as much as one cigarette a day for as long as a year?" and "Do you smoke cigarettes now?". Self-reported physical activity (derived from HLQ1) was assessed using both occupational and leisure activities and individuals were assigned to one of four categories: inactive, moderately inactive, moderately active and active [51,52]. Occupational social class at 1HE was defined according to the Registrar General's classification. Nonmanual occupations were represented by codes I, (professional) II, (managerial and technical), and IIIa (nonmanual skilled) occupations while manual occupations were represented by codes IIIb (manual skilled), IV (partly skilled) and V (unskilled) occupations [53].

2.6. Statistical Analysis

All analyses were stratified by sex as significant differences in body composition, SMM and age-related changes in bone existing [44] between men and women. $p < 0.05$ was considered to be statistically significant in individual analyses. To minimise missing data exclusions, some missing values were recoded as follows: missing menopausal status data (2.8%) as premenopausal if age <50 years and never-user of HRT, or as postmenopausal if age >55 years or a current or former HRT user. Participants missing data for other variables in the multivariable model were excluded. Participants were excluded from analyses if they had missing or extreme BIA impedance values (<300 or >1000 ohms [54]), FFM < 25, or for participants with extremes of BMI (<14 or ≥36 kg/m^2), since bioelectrical impedance measures are considered unreliable at these levels [55].

2.7. Cross-sectional Analyses

Cross-sectional analyses were carried out using data from the 2HE, using dietary or plasma data from the 1HE; 14,179 participants had complete data for diet and muscle analyses, and 4283 had complete data for plasma and muscle analyses; the figures for BUA analyses are 14,694 and 4457, respectively (Figure 1).

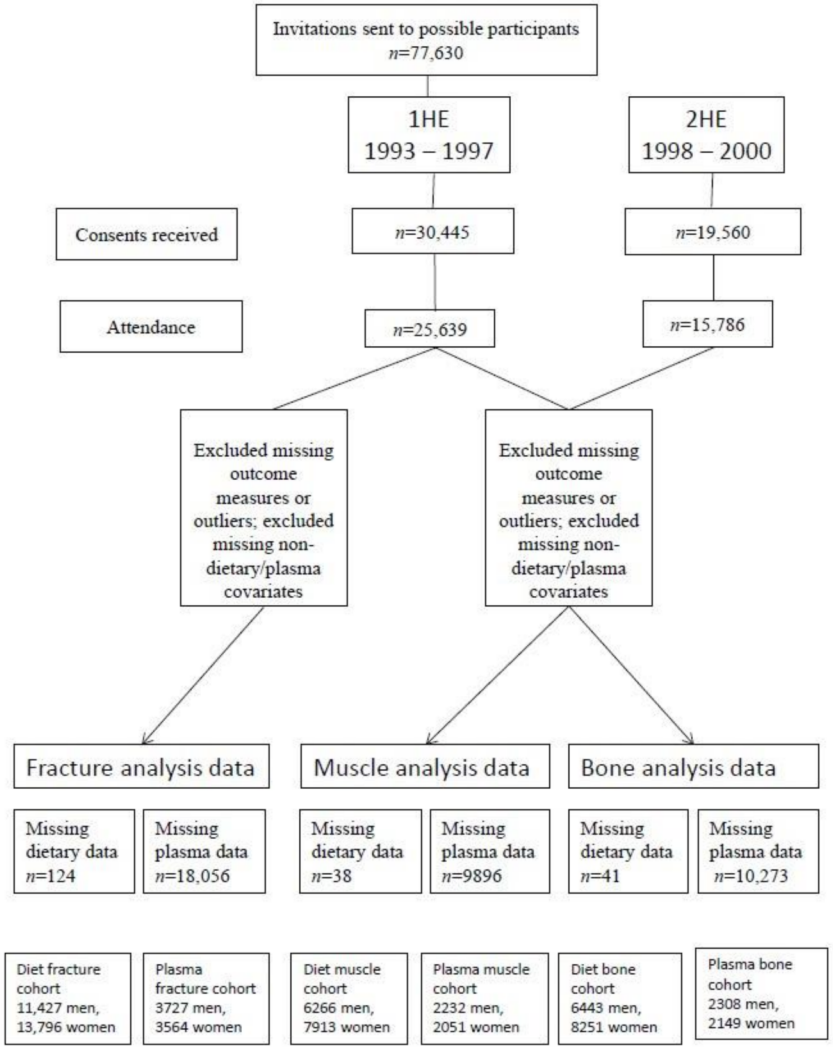

Figure 1. Flowchart of participants included in the analyses.

Multivariable adjusted regression with ANCOVA was used to investigate differences in indices of SMM and calcaneal BUA across sex-specific dietary intake quintiles of vitamin E intake (mg α-tocopherol equivalents). Trend testing was achieved by treating the median values for quintiles as a continuous variable [56]. Each model was adjusted for important physiological, lifestyle, and dietary factors, known to influence risk in this population. For SMM, these included age, smoking status, physical activity, social class, energy intake, percentage energy from protein, corticosteroid and statin use, menopausal and HRT status in women; for BUA, these included age, BMI, family history of osteoporosis, menopausal and HRT status in women, corticosteroid use, smoking status, physical activity, Ca intake, total energy intake, and Ca- and vitamin D-containing supplement use. The data were also analysed to take the amount of vitamin E from supplements into consideration, as excluding supplements may underestimate total nutrient intake [57]. In separate analyses, indices of SMM and calcaneal BUA were investigated across sex-specific plasma concentration quintiles of α-tocopherol, γ-tocopherol and the ratio of α:γ-tocopherol, with the covariates described above, but excluding dietary intake data.

2.8. Longitudinal Analyses

Longitudinal analyses used data from the 1HE together with incident hospital-recorded fractures for the participants (all hip, spine and wrist fracture cases up to 31 March 2018); the mean follow-up time was 18.5 years (467,077 total person years), and was calculated as the time between an individual's 1HE and this cut-off date, or death if earlier. Data

for diet and fracture analyses were available for 25,223 participants; data for plasma and fracture analyses were available for 7291 participants (Figure 1). Prentice-weighted Cox regression was used to investigate associations between incidence of fractures and sex-specific quintiles of dietary vitamin E intake (mg α-tocopherol equivalents), or plasma concentrations, using the same adjustments as for the BUA models. Missing values were treated in the same way as in the BUA models. Total risk for hip, spine or wrist fracture was calculated as the risk for the first occurrence of one of these fractures; this does not consider multiple fractures, and therefore the sum of the specific-site fracture incidences does not sum to the total.

3. Results

3.1. Characteristics of the Study Population

Selected characteristics are summarised in Table 1, stratified by dietary analysis group and sex. Mean dietary and supplement-derived intakes of α-tocopherol equivalents (mg/day) are shown for the different study groups. In the dietary model analyses, numbers of men and women are similar for the SMM and BUA measures and intakes of dietary and supplement α-tocopherol equivalents are also similar. Dietary intakes of α-tocopherol equivalents are slightly lower in the fracture dietary analyses groups, as is the percentage of participants taking vitamin E-containing supplements and the amount of α-tocopherol equivalents obtained from these supplements. No UK Reference Nutrient Intake value [58] has been defined for vitamin E, although safe intakes of α-tocopherol equivalents have been set at 4 mg for men and 3 mg for women. Of the 11,427 men in the fracture dietary analysis group, 1.7% had an intake <4 mg (n = 194); 1.0% of the 13,796 women had an intake <3 mg (n = 132). However, the European Food Safety Agency (EFSA) Panel on Dietetic Products, Nutrition and Allergies (NDA) set Adequate Intakes (AIs) of α-tocopherol for adults as 13 mg/day for men and 11 mg/d for women [59]; it was felt that Average Requirements (ARs) and Population Reference Intakes (PRIs) could not be set for α-tocopherol. Only 30.7% of the men and 25.8% of the women in the fracture cohort met these AIs. The fracture dietary analyses groups had a higher percentage of current smokers, manual workers and physically inactive participants.

Table 2 presents selected characteristics, stratified by plasma analysis group and sex. Mean dietary and supplement-derived intakes of α-tocopherol equivalents (mg/day) are shown for the different study groups, in addition to plasma tocopherol concentrations, unadjusted and adjusted for cholesterol. In the plasma model analyses, numbers of men and women are similar for the SMM and BUA measures and concentrations of the plasma tocopherols and intakes of dietary and supplement α-tocopherol equivalents are also similar. Concentrations of the cholesterol-adjusted plasma tocopherols are also similar in the fracture plasma analysis groups, although the ratio is slightly lower in both men and women. A plasma tocopherol concentration of at least 11.6 μmol/L, or a minimum tocopherol:cholesterol ratio of 2.25 μmol/mmol is considered to be the lowest satisfactory value; the dietary requirement of vitamin E is that which is necessary to keep the ratio above this level [58], although in a recent publication by EFSA, it was considered that they were insufficient data on markers of α-tocopherol intake/status/function (e.g., plasma/serum α-tocopherol concentration, markers of oxidative damage) to calculate the requirement for α-tocopherol [59]. Of the participants in the SMM plasma analysis group, only 4 men and 8 women had values <11.6 μmol/L; one of these woman had a tocopherol:cholesterol ratio <2.25 μmol/mmol. Eight men and 13 women had tocopherol:cholesterol ratios <2.25 μmol/mmol. Once again, the larger fracture plasma analysis groups had a higher percentage of current smokers, manual workers and physically inactive participants.

Table 1. Selected characteristics of the European Prospective Investigation Into Cancer and Nutrition (EPIC)-Norfolk cohort population, stratified by sex and diet analysis group.

	Diet Analysis Cohort *				**Diet Analysis Cohort ***				**Diet Analysis Cohort †**			
	(SMM)				**(BUA)**				**(Fractures)**			
	Men (*n* = 6266)		**Women (*n* = 7913)**		**Men (*n* = 6443)**		**Women (*n* = 8251)**		**Men (*n* = 11,427)**		**Women (*n* = 13,796)**	
Age (years)	62.8	9.0	61.5	9.0	62.9	9.0	61.5	9.0	59.6	9.3	58.9	9.3
BMI (kg/m^2)	26.7	3.0	26.1	3.7	26.9	3.3	26.5	4.4	26.5	3.3	26.2	4.4
Dietary α-tocopherol equivalents intake (mg/day)	11.90	5.00	9.52	3.73	11.91	5.04	9.50	3.74	11.63	5.24	9.29	3.78
Energy intake (kcal/day)	2287	501	1736	378	2285	502	1732	380	2240	528	1695	395
Protein intake (% energy)	14.8	2.4	15.5	2.8								
Calcium intake (mg/day)					942	289	785	243	919	297	767	248
Vitamin D intake (μg/day)					3.88	2.82	2.99	1.94	3.73	2.75	2.94	2.08
Vitamin E-containing supplement use, n (%)	1000	16.0	2077	26.2	1024	15.9	2153	26.1	1587	13.9	3244	23.5
Supplemental α-tocopherol equivalents (mg/day)	37.27	91.86	35.99	77.52	37.73	93.61	35.12	75.72	35.10	85.16	35.29	78.73
Vitamin D-containing supplement use, n (%)					1612	25.0	2752	33.4	2551	22.3	4232	30.7
Supplemental vitamin D (μg/day)					4.34	2.84	4.17	2.57	4.25	2.75	4.18	2.66
Calcium-containing supplement use, n (%)					102	1.6	504	6.1	165	1.4	742	5.4
Supplemental calcium (mg/day)					200	195	347	269	196	190	342	268
FFM (kg)	61.6	5.8	40.6	4.5								
FFM_{BMI}	2.32	0.26	1.59	0.26								
BUA (dB/MHz)					90.06	17.51	72.09	16.46				
Total incident fractures, n (%)									877	7.7	2092	15.2
Incident hip fractures, n (%)									356	3.1	971	7.0
Incident spine fractures, n (%)									223	2.0	357	2.6
Incident wrist fractures, n (%)									155	1.4	504	3.6
Social class, n (%)												
Professional	516	8.2	544	6.9	523	8.12	565	6.85	860	7.5	863	6.3
Managerial	2566	41.0	2919	36.9	2630	40.82	3016	36.55	4299	37.6	4722	34.2
Skilled nonmanual	782	12.5	1545	19.5	804	12.48	1600	19.39	1404	12.3	2684	19.4
Skilled manual	1401	22.4	1565	19.8	1440	22.35	1636	19.83	2840	24.8	2847	20.6
Semiskilled	771	12.3	937	11.8	803	12.46	1001	12.13	1501	13.1	1805	13.1
Nonskilled	145	2.3	262	3.3	153	2.37	282	3.42	334	2.9	536	3.9
Missing	85	1.4	141	1.8	90	1.4	151	1.83	189	1.6	339	2.5

Table 1. *Cont.*

Smoking status, n (%)												
Current	490	7.8	638	8.1	509	7.9	660	8.0	1391	12.2	1560	11.3
Former	3495	55.8	2547	32.2	3609	56.01	2697	32.69	6232	54.5	4446	32.2
Never	2281	36.4	4728	59.8	2325	36.09	4894	59.31	3804	33.3	7790	56.5
Physical activity, n (%)												
Inactive	1712	27.3	2043	25.8	1779	27.61	2165	26.24	3516	30.8	4174	30.3
Moderately inactive	1576	25.2	2575	32.5	1615	25.07	2695	32.66	2810	24.6	4429	32.1
Moderately active	1567	25.0	1920	24.3	1601	24.85	1980	24.00	2635	23.1	3074	22.3
Active	1411	22.5	1375	17.4	1448	22.47	1411	17.10	2466	21.6	2119	15.4
Family history of osteoporosis, n (%)												
No	6098	97.4	7413	93.7	6267	97.3	7731	93.7	11,121	97.3	12,988	94.1
Yes	166	2.6	500	6.3	176	2.7	520	6.3	306	2.7	808	5.9
Corticosteroid use, n (%)												
Current or former (>3 months)	260	4.2	402	5.1	270	4.2	422	5.1	349	3.0	478	3.5
Never (<3 months)	6006	95.8	7511	94.9	6173	95.8	7829	94.9	11,078	97.0	13,318	96.5
Statin use, n (%)												
No	5922	94.5	7623	96.3								
Yes	344	5.5	290	3.7								
Menopausal status, n (%)												
Premenopausal			472	6.0			482	5.8			233	16.9
Perimenopausal (<1 y)			265	3.4			272	3.3			750	5.4
Perimenopausal (1–5 y)			1389	17.6			1455	17.6			2473	17.9
Postmenopausal			5787	73.1			6042	73.2			8239	59.7
HRT, n (%)												
Current			1693	21.4			1757	21.3			2802	20.3
Former			1417	17.9			1482	18.0			1570	11.4
Never			4803	60.7			5012	60.7			9424	68.3

Values are mean ± SD or frequency (percentage). SMM = skeletal muscle mass; BUA = broadband ultrasound attenuation; FFM = fat-free mass; HRT = hormone replacement therapy. * SMM and BUA group characteristics at second health examination (2HE) (time of ultrasound), unless only available at 1HE. † Fracture group characteristics at 1HE or time of consent. SMM: men = 6264 for family history of osteoporosis.

Table 2. Selected characteristics of the EPIC-Norfolk cohort population, stratified by sex and plasma analysis group.

	Serum Analysis—SMM *				**Serum Analysis—BUA ***				**Serum Analysis—Fractures †**			
	Men (*n* = 2232)		**Women (*n* = 2051)**		**Men (*n* = 2308)**		**Women (*n* = 2149)**		**Men (*n* = 3727)**		**Women (*n* = 3564)**	
Age (years)	67.0	7.5	64.5	8.5	67.0	7.5	64.5	8.5	64.39	7.87	62.02	8.72
BMI (kg/m^2)	26.8	3.0	26.3	3.7	27.0	3.4	26.8	4.3	26.74	3.33	26.39	4.20
Dietary α-tocopherol equivalents intake (mg/day)	11.35	4.70	9.30	3.56	11.38	4.72	9.28	3.59	11.02	4.76	9.12	3.69
Serum α-tocopherol (μmol/L)	26.44	7.74	28.39	8.35	26.47	7.88	28.42	8.34	26.36	7.90	28.53	8.50
Serum chol-adjusted α-tocopherol (μmol/mmol)	4.35	1.06	4.43	1.05	4.37	1.08	4.43	1.05	4.34	1.07	4.42	1.06
Serum γ-tocopherol (μmol/L)	1.83	0.90	1.83	0.91	1.83	0.90	1.84	0.93	1.86	0.90	1.88	0.94
Serum chol-adjusted γ-tocopherol (μmol/mmol)	0.30	0.14	0.28	0.13	0.30	0.14	0.29	0.13	0.31	0.14	0.29	0.14
Serum α-tocopherol:γ-tocopherol ratio (chol-adjusted)	17.97	16.27	20.61	29.31	17.96	16.53	20.41	28.73	17.55	15.29	19.92	25.35
FFM (kg)	61.31	5.80	40.45	4.66								
FFM_{BMI}	2.30	0.25	1.56	0.25								
BUA (dB/MHz)					89.45	17.75	69.83	16.16				
Total incident fractures, n (%)									308	8.3	643	18.0
Incident hip fractures, n (%)									129	3.5	313	8.8
Incident spine fractures, n (%)									89	2.4	124	3.5
Incident wrist fractures, n (%)									40	1.1	152	4.3
Energy intake (kcal/day)	2217	488	1715	374	2218	489	1709	377	2161	502	1675	383
Calcium intake (mg/day)					924	283	780	250	900	283	764	249
Vitamin D intake (μg/day)					3.95	2.72	3.04	1.97	3.85	2.71	3.02	2.21
Vitamin E-containing supplement use, n (%)	350	15.7	512	25.0	360	15.6	534	24.8	503	13.5	806	22.6
Supplemental α-tocopherol equivalents (mg/day)	36.05	92.50	39.84	83.53	36.13	92.35	39.12	82.00	34.54	85.44	38.10	80.96
Vitamin D-containing supplement use, n (%)	596	26.7	657	32.0	607	26.3	682	31.7	893	24.0	1075	30.2
Supplemental vitamin D (μg/day)	4.35	2.78	4.30	2.71	4.35	2.77	4.35	2.74	4.27	2.70	4.25	2.80
Calcium-containing supplement use, n (%)	38	1.7	109	5.3	38	1.6	113	5.3	53	1.4	174	4.9
Supplemental calcium (mg/day)	195	193	334	250	195	193	338	248	230	219	314	249
Social class, n (%)												
Professional	179	8.02	114	5.56	181	7.84	120	5.58	258	6.92	192	5.39
Managerial	905	40.55	731	35.64	934	40.47	762	35.46	1382	37.08	1195	33.53
Skilled nonmanual	293	13.13	422	20.58	302	13.08	436	20.29	475	12.74	708	19.87
Skilled manual	493	22.09	403	19.65	509	22.05	422	19.64	926	24.85	711	19.95
Semiskilled	280	12.54	262	12.77	294	12.74	280	13.03	504	13.52	502	14.09
Nonskilled	48	2.15	68	3.32	51	2.21	76	3.54	115	3.09	145	4.07
Missing	34	1.52	51	2.49	37	1.60	53	2.47	67	1.8	111	3.11
Smoking status, n (%)												
Current	165	7.39	145	7.07	168	7.28	148	6.89	410	11.00	388	10.89
Former	1337	59.90	668	32.57	1394	60.40	711	33.09	2240	60.10	1171	32.86
Never	730	32.71	1238	60.36	746	32.32	1290	60.03	1077	28.90	2005	56.26
Physical activity, n (%)												
Inactive	705	31.59	581	28.33	740	32.06	614	28.57	1363	36.57	1212	34.01
Moderately inactive	537	24.06	678	33.06	545	23.61	714	33.22	883	23.69	1121	31.45
Moderately active	525	23.52	485	23.65	539	23.35	502	23.36	790	21.20	769	21.58
Active	465	20.83	307	14.97	484	20.97	319	14.84	691	18.54	462	12.96

Table 2. *Cont.*

	Serum Analysis—SMM *				Serum Analysis—BUA *				Serum Analysis—Fractures †			
	Men (*n* = 2232)		Women (*n* = 2051)		Men (*n* = 2308)		Women (*n* = 2149)		Men (*n* = 3727)		Women (*n* = 3564)	
Family history of osteoporosis, n (%)												
No	2177	97.58	1929	94.05	2249	97.44	2025	94.23	3645	97.8	3381	94.87
Yes	54	2.42	122	5.95	59	2.56	124	5.77	82	2.2	183	5.13
Corticosteroid use, n (%)												
Current or former (>3 months)	123	5.51	116	5.66	130	5.63	123	5.72	150	4.02	142	3.98
Never (<3 months)	2109	94.49	1935	94.34	2178	94.37	2026	94.28	3577	95.98	3422	96.02
Menopausal status, n (%)												
Premenopausal			31	1.51			33	1.54			287	8.05
Perimenopausal (<1 y)			44	2.15			44	2.05			155	4.35
Perimenopausal (1–5 y)			268	13.07			283	13.17			534	14.98
Postmenopausal			1708	83.28			1789	83.25			2588	72.62
HRT, n (%)												
Current			396	19.31			410	19.08			660	18.52
Former			348	16.97			370	17.22			390	10.94
Never			1307	63.73			1369	63.70			2514	70.54

Values are mean ± SD or frequency (percentage). SMM = skeletal muscle mass; BUA = broadband ultrasound attenuation; FFM = fat-free mass; HRT = hormone replacement therapy. Serum BUA: Men = 2300 for vitamin E, vitamin D, calcium and energy intakes; women = 2143 for vitamin E, vitamin D, calcium and energy intakes. Serum fracture: men = 3707 for vitamin E, vitamin D, calcium and energy intakes; women = 3551 for vitamin E, vitamin D, calcium and energy intakes. SMM: men = 2231 for family history of osteoporosis; women = 2045 for body mass index (BMI). * SMM and BUA group characteristics at 2HE (time of ultrasound), unless only available at 1HE. † Fracture group characteristics at 1HE or time of consent.

3.2. Food Sources of α- and γ-Tocopherols

Good sources of vitamin E include plant oils—such as rapeseed, sunflower, soya, corn and olive oil—nuts, seeds and wheatgerm. The main sources of γ-tocopherol include oils [60] (especially soybean and corn oils, which are used extensively in processed foods), nuts and seeds [61] (especially walnuts, pecans and pistachios, as well as sesame, flax and pumpkin seeds), as well as spinach, carrots, avocado, dark green leafy vegetables and wheatgerm. Figure 2 shows the main food group sources for men and women. Generally, the main food groups contributing to vitamin E intake in men and women were similar, with butters, spreads and margarines being the main contributors. Foods in the grains and cereal-based products groups include both sweet and savoury biscuits, cakes, pies and quiches.

3.3. Correlations between Dietary Vitamin E Intake and Plasma Concentrations

A number of weak but significant correlations were found between the dietary intake of α-tocopherol equivalents and plasma concentrations of α-tocopherol. Dietary intake of α-tocopherol equivalents was significantly correlated with plasma concentration of α-tocopherol in the SMM cohort in men ($r = 0.079$, $p < 0.001$, $n = 2232$), but not in women ($r = 0.038$, $p = 0.084$, $n = 2051$). In the BUA cohort, significant correlations were found in both men ($r = 0.083$, $p < 0.001$, $n = 2300$) and women ($r = 0.044$, $p < 0.05$, $n = 2143$). In the fracture cohort, dietary intake of α-tocopherol equivalents was significantly correlated with plasma concentration of α-tocopherol in both men ($r = 0.105$, $p < 0.001$, $n = 3707$) and women ($r = 0.08$, $p < 0.001$, $n = 3551$).

When the plasma concentration of α-tocopherol was adjusted for total cholesterol, the correlations with dietary intake were found to be slightly stronger. Dietary intake of α-tocopherol equivalents was significantly correlated with plasma concentration of α-tocopherol in the SMM cohort in both men ($r = 0.136$, $p < 0.001$, $n = 2232$) and women ($r = 0.1203$, $p < 0.001$, $n = 2051$). In the BUA cohort, significant correlations were found in both men ($r = 0.131$, $p < 0.001$, $n = 2300$) and women ($r = 0.125$, $p < 0.001$, $n = 2143$). In the fracture cohort, dietary intake of α-tocopherol equivalents was significantly correlated

with plasma concentration of α-tocopherol in both men ($r = 0.1565$, $p < 0.001$, $n = 3707$) and women ($r = 0.153$, $p < 0.001$, $n = 3551$).

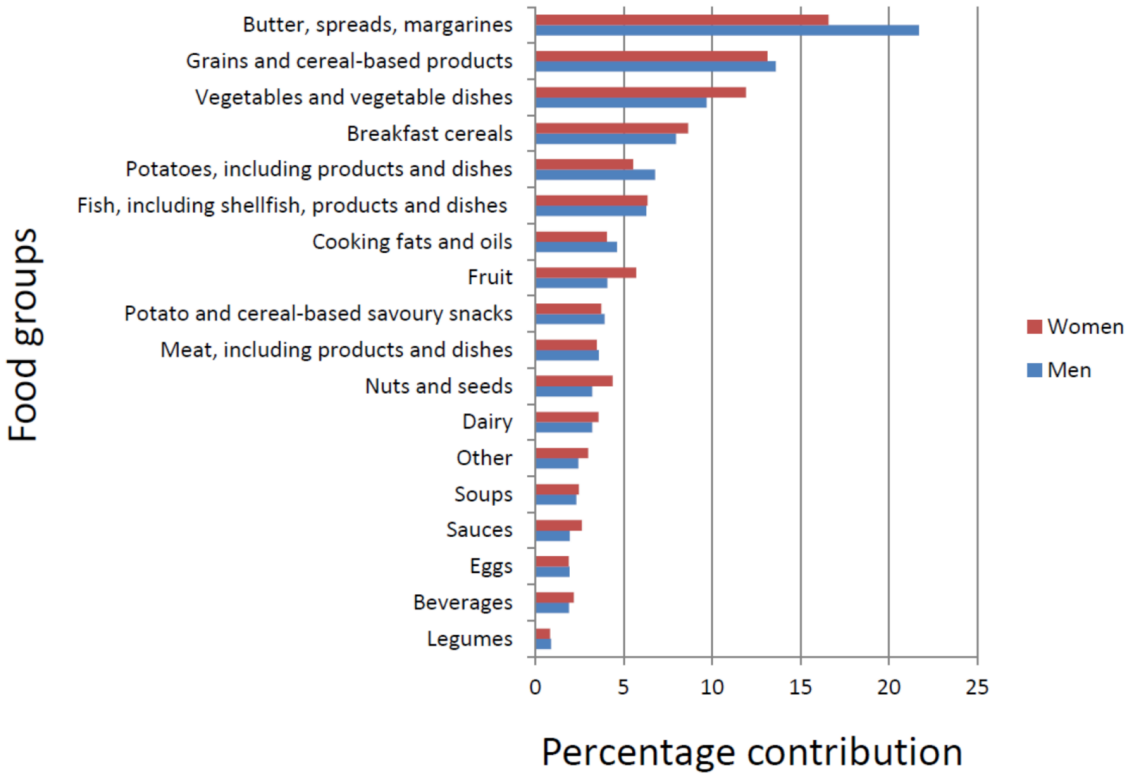

Figure 2. Percentage composition of food groups for vitamin E intake of men and women in the EPIC-Norfolk cohort. "Butters, spreads and margarines" include reduced fat types. "Grains and cereal-based products" include rice and rice-based dishes, pasta, sweet and savoury flans, pies and quiches, biscuits, cakes, breads and bread rolls. "Vegetables and vegetable-based dishes" include raw and cooked vegetables, vegetable dishes and mixed salads. "Breakfast cereals" include porridge and muesli. "Potatoes, including products and dishes" include potatoes, potato products, dishes and salads. "Fish, including shellfish, products and dishes" includes shellfish and fish-based dishes. "Cooking fats and oils" include hard margarines, animal fats, vegetable fats and ghee. "Fruit" includes fresh, cooked and canned fruit. "Potato and cereal-based savoury snacks" include crisps and other potato-based snacks and bread/pastry type snacks. "Meat, including products and dishes" include unprocessed white and red meats (and products and dishes), processed meat products and offal, including products and dishes. "Nuts and seeds" include nuts, seeds and nut butters. "Dairy" includes milk, cheese, yoghurts and dairy-based desserts. "Other" includes sugar, herbs and spices and dietetic products. "Soups" include homemade, canned and reconstituted dried soups. "Sauces" include sweet and savoury sauces, gravies, salad dressings, pickles, chutneys and stuffing. "Eggs" include eggs and sweet and savoury egg dishes. "Beverages" include alcoholic and nonalcoholic drinks, such as tea, coffee, water, soft drinks, fruit juice and squashes. "Legumes" include beans, pulses and lentils, dried, cooked and canned, and legume-based salads.

3.4. Associations between Dietary Vitamin E Intakes and Indices of SMM

Significant positive associations were found between sex-specific quintiles of dietary vitamin E and FFM and FFM_{BMI} (p-trend < 0.001 in both men and women), after adjustments for covariates (Table 3), with significant interquintile differences (Q5 versus Q1) in FFM of +1.0% ($p < 0.001$) in both men, and women, and in FFM_{BMI} of +1.7% ($p < 0.001$) in men and +1.9% ($p < 0.001$) in women. The addition of the amount of vitamin E derived from supplements to the fully adjusted models did not alter the associations.

Table 3. Associations between quintiles of dietary vitamin E and skeletal muscle mass in men and women aged 42–82 years.

Men (*n* = 6266)	**Dietary α-Tocopherol Equivalents Intake (mg/day)**		**FFM**		**FFM_{BMI}**	
Quintile	Mean ± SD	Median	Unadjusted	Adjusted	Unadjusted	Adjusted
1 (*n* = 1254)	6.45 ± 1.19	6.72	60.56 ± 0.17	61.23 ± 0.16	2.25 ± 0.01	2.30 ± 0.01
2 (*n* = 1253)	9.00 ± 0.54	9.03	61.29 ± 0.16 **	61.45 ± 0.14	2.31 ± 0.01 ***	2.32 ± 0.01 *
3 (*n* = 1253)	10.93 ± 0.62	10.88	61.64 ± 0.17 ***	61.67 ± 0.14 *	2.32 ± 0.01 ***	2.32 ± 0.01 *
4 (*n* = 1253)	13.45 ± 0.90	13.36	62.20 ± 0.16 ***	61.86 ± 0.15 **	2.36 ± 0.01 ***	2.34 ± 0.01 **
5 (*n* = 1253)	19.67 ± 4.54	18.30	62.40 ± 0.16 ***	61.86 ± 0.15 **	2.38 ± 0.01 ***	2.34 ± 0.01 **
Q5–Q1 diff [1]			1.84	0.63	0.13	0.04
% diff [2]			3.04	1.03	5.78	1.74
p trend			<0.001	<0.001	<0.001	<0.001
Women (*n* = 7913)						
Quintile	Mean ± SD	Median	Unadjusted	Adjusted	Unadjusted	Adjusted
1 (*n* = 1583)	5.36 ± 0.99	5.58	39.95 ± 0.12	40.45 ± 0.12	1.52 ± 0.01	1.57 ± 0.01
2 (*n* = 1583)	7.41 ± 0.44	7.42	40.30 ± 0.11 *	40.47 ± 0.11	1.56 ± 0.01 ***	1.57 ± 0.01
3 (*n* = 1582)	8.94 ± 0.45	8.96	40.64 ± 0.11 ***	40.65 ± 0.11	1.59 ± 0.01 ***	1.59 ± 0.01
4 (*n* = 1583)	10.74 ± 0.64	10.70	41.04 ± 0.11 ***	40.80 ± 0.11 *	1.62 ± 0.01 ***	1.60 ± 0.01 **
5 (*n* = 1582)	15.14 ± 3.55	14.13	41.31 ± 0.11 ***	40.87 ± 0.12 *	1.64 ± 0.01 ***	1.60 ± 0.01 **
Q5–Q1 diff [1]			1.36	0.42	0.12	0.03
% diff [2]			3.4	1.04	7.89	1.91
p trend			<0.001	<0.001	<0.001	<0.001

Values are presented as means ± SEM. The *p*-trend was calculated using ANCOVA. [1] Q5–Q1 calculates the absolute difference between the means of quintile (Q) 5 and Q1. [2] % difference calculates the percentage difference between the means of Q5 and Q1. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus quintile 1, according to ANCOVA. FFM = fat-free mass. FFM and FFM_{BMI}—adjusted model includes age, total energy, protein intake as a percentage of total energy, smoking status, physical activity, corticosteroid use, menopausal status, HRT use, statins use, social class and fat mass (FFM only).

3.5. Associations between Plasma Vitamin E Concentrations and Indices of SMM

In general, similar linear trends were found for both men and women, with those across quintiles of α- and γ-tocopherol tending to be in the same direction, and the trend for the ratio of α-tocopherol: γ-tocopherol was in the opposite direction (Table 4). Linear trends in both men and women were most apparent across quintiles of plasma γ-tocopherol. In adjusted plasma model analyses, there was a significant positive trend across both α- and γ-tocopherol quintiles for FFM in men ($p < 0.001$) and women ($p < 0.01$). However, for FFM, a significant negative trend was found across quintiles of the ratio of α-tocopherol:γ-tocopherol in both men and women ($p < 0.001$). Whereas, across quintiles of the ratio of α-tocopherol: γ-tocopherol, significant positive trends were found for FFM in both men ($p < 0.001$) and women ($p < 0.01$), and significant negative trends for FFM_{BMI} in both men and women ($p < 0.001$). In the adjusted model for FFM, significant differences were found between quintile 1 and quintiles 4 and 5 of plasma γ-tocopherol in women ($p < 0.01$ and <0.05, respectively), whereas in men, significant differences were found between quintile 1 and quintiles 2 ($p < 0.01$), 4 ($p < 0.05$) and 5 ($p < 0.01$) of the ratio of α-tocopherol:γ-tocopherol. In the adjusted model for FFM_{BMI}, significant differences were found between Q1 and Q3 of plasma α-tocopherol in women ($p < 0.05$). Regarding plasma γ-tocopherol, significant differences were found between Q1 and Q5 of plasma in men ($p < 0.01$) and between Q1 and Q4 ($p < 0.05$) and 5 ($p < 0.01$) in women. In women, significant differences were found between Q1 and Q4 ($p < 0.01$) and Q5 ($p < 0.05$) of the ratio of α-tocopherol:γ-tocopherol.

3.6. Associations between Dietary Vitamin E Intakes and Bone Density Status

Mean calcaneal BUA values, stratified by sex and quintiles of dietary vitamin E, are shown in Table 5, for unadjusted data and the fully adjusted model. Significant positive associations across quintiles of dietary vitamin E intake were evident in both men and women, after adjustments for covariates (*p*-trend < 0.001 in both men and women). In the fully adjusted model, a significant difference was identified in men, for Q3 versus

Q1 (+1.8%; $p < 0.05$). Further adjustment for the amount of vitamin E derived from supplements did not modify the associations.

Table 4. Multivariate adjusted SMM indices for EPIC-Norfolk participants, stratified by sex and quintiles of plasma α-tocopherol, plasma γ-tocopherol and α-tocopherol:γ-tocopherol ratio, adjusted for blood cholesterol measurement.

Men (n = 2232)	**Plasma α-Tocopherol, Adjusted for Cholesterol (μmol/mmol)**		**FFM**		**FFM_{BMI}**	
Quintile	Mean ± SD	Median	Unadjusted	Adjusted	Unadjusted	Adjusted
1 (n = 447)	3.17 ± 0.36	3.25	61.03 ± 0.27	61.13 ± 0.24	2.31 ± 0.01	2.31 ± 0.01
2 (n = 446)	3.80 ± 0.13	3.80	61.13 ± 0.25	61.14 ± 0.24	2.31 ± 0.01	2.31 ± 0.01
3 (n = 447)	4.23 ± 0.13	4.23	61.76 ± 0.28	61.72 ± 0.24	2.31 ± 0.01	2.32 ± 0.01
4 (n = 446)	4.71 ± 0.15	4.70	61.24 ± 0.28	61.31 ± 0.24	2.31 ± 0.01	2.31 ± 0.01
5 (n = 446)	5.86 ± 1.14	5.47	61.36 ± 0.29	61.23 ± 0.24	2.28 ± 0.01 *	2.28 ± 0.01
Q5-Q1 diff [1]			0.33	0.10	−0.03	−0.03
% diff [2]			0.54	0.16	−1.30	−1.30
p trend			0.413	<0.001	0.039	<0.001
Women (n = 2051)	**Plasma α-Tocopherol, Adjusted for Cholesterol (μmol/mmol)**		**FFM**		**FFM_{BMI}**	
Quintile	Mean ± SD	Median	Unadjusted	Adjusted	Unadjusted	Adjusted
1 (n = 411)	3.22 ± 0.39	3.32	40.41 ± 0.24	40.48 ± 0.23	1.58 ± 0.01	1.58 ± 0.01
2 (n = 410)	3.86 ± 0.13	3.87	40.19 ± 0.23	40.22 ± 0.23	1.56 ± 0.01	1.56 ± 0.01
3 (n = 410)	4.31 ± 0.13	4.30	40.34 ± 0.24	40.33 ± 0.23	1.54 ± 0.01 *	1.54 ± 0.01 *
4 (n = 410)	4.79 ± 0.16	4.79	40.75 ± 0.22	40.70 ± 0.23	1.57 ± 0.01	1.57 ± 0.01
5 (n = 410)	5.96 ± 1.03	5.61	40.58 ± 0.23	40.53 ± 0.23	1.55 ± 0.01	1.55 ± 0.01
Q5-Q1 diff [1]			0.17	0.05	−0.03	−0.03
% diff [2]			0.42	0.12	−1.90	−1.90
p trend			0.251	0.006	0.239	<0.001
Men (n = 2232)	**Plasma γ-Tocopherol, Adjusted for Cholesterol (μmol/mmol)**		**FFM**		**FFM_{BMI}**	
Quintile	Mean ± SD	Median	Unadjusted	Adjusted	Unadjusted	Adjusted
1 (n = 447)	0.15 ± 0.04	0.16	60.78 ± 0.28	60.94 ± 0.24	2.32 ± 0.01	2.32 ± 0.01
2 (n = 446)	0.22 ± 0.01	0.22	60.96 ± 0.26	61.29 ± 0.24	2.33 ± 0.01	2.32 ± 0.01
3 (n = 447)	0.28 ± 0.02	0.28	61.27 ± 0.28	61.24 ± 0.24	2.30 ± 0.01	2.30 ± 0.01
4 (n = 446)	0.34 ± 0.02	0.34	61.58 ± 0.27 *	61.53 ± 0.24	2.30 ± 0.01	2.31 ± 0.01
5 (n = 446)	0.51 ± 0.14	0.47	61.94 ± 0.27 **	61.52 ± 0.24	2.27 ± 0.01 **	2.27 ± 0.01 **
Q5-Q1 diff [1]			1.16	0.58	−0.05	−0.05
% diff [2]			1.91	0.95	−2.16	−2.16
p trend			0.0007	$p < 0.0001$	0.0009	$p < 0.0001$
Women (n = 2051)	**Plasma γ-Tocopherol, Adjusted for Cholesterol (μmol/mmol)**		**FFM**		**FFM_{BMI}**	
Quintile	Mean ± SD	Median	Unadjusted	Adjusted	Unadjusted	Adjusted
1 (n = 411)	0.14 ± 0.04	0.14	39.94 ± 0.22	39.93 ± 0.23	1.58 ± 0.01	1.58 ± 0.01
2 (n = 410)	0.21 ± 0.02	0.21	40.40 ± 0.24	40.39 ± 0.23	1.58 ± 0.01	1.58 ± 0.01
3 (n = 410)	0.26 ± 0.02	0.26	40.50 ± 0.23	40.52 ± 0.23	1.57 ± 0.01	1.57 ± 0.01
4 (n = 410)	0.33 ± 0.02	0.33	40.81 ± 0.23 **	40.82 ± 0.23 **	1.54 ± 0.01 *	1.54 ± 0.01 *
5 (n = 410)	0.48 ± 0.12	0.44	40.62 ± 0.23 *	40.61 ± 0.23 *	1.54 ± 0.01 **	1.54 ± 0.01 **
Q5-Q1 diff [1]			0.68	0.68	−0.04	−0.04
% diff [2]			1.70	1.70	−2.53	−2.53
p trend			0.0254	0.0017	0.0013	$p < 0.0001$
Men (n = 2232)	**Plasma α:γ-Tocopherol Ratio, Adjusted for Cholesterol**		**FFM**		**FFM_{BMI}**	
Quintile	Mean ± SD	Median	Unadjusted	Adjusted	Unadjusted	Adjusted
1 (n = 447)	8.73 ± 1.48	8.99	62.22 ± 0.27	61.93 ± 0.24	2.29 ± 0.01	2.29 ± 0.01
2 (n = 446)	12.13 ± 0.82	12.15	61.04 ± 0.26 **	60.97 ± 0.24 **	2.29 ± 0.01	2.29 ± 0.01
3 (n = 447)	15.04 ± 0.90	14.96	61.37 ± 0.28 *	61.39 ± 0.24	2.32 ± 0.01	2.32 ± 0.01
4 (n = 446)	19.01 ± 1.46	18.87	61.11 ± 0.27 **	61.24 ± 0.24 *	2.31 ± 0.01	2.31 ± 0.01
5 (n = 446)	34.97 ± 30.04	26.65	60.78 ± 0.28 ***	61.00 ± 0.24 **	2.31 ± 0.01	2.31 ± 0.01

Table 4. *Cont.*

Q5-Q1 diff [1]			−1.44	−0.93	0.02	0.02
% diff [2]			−2.31	−1.50	0.87	0.87
p trend			0.002	$p < 0.001$	0.109	$p < 0.001$
Women (*n* = 2051)	**Plasma α:γ-Tocopherol Ratio, Adjusted for Cholesterol**		**FFM**		**FFM_{BMI}**	
Quintile	Mean ± SD	Median	Unadjusted	Adjusted	Unadjusted	Adjusted
1 (*n* = 411)	9.11 ± 1.60	9.44	40.51 ± 0.23	40.53 ± 0.23	1.54 ± 0.01	1.54 ± 0.01
2 (*n* = 410)	12.74 ± 0.90	12.78	40.87 ± 0.23	40.88 ± 0.23	1.55 ± 0.01	1.55 ± 0.01
3 (*n* = 410)	15.92 ± 1.04	15.83	40.37 ± 0.23	40.39 ± 0.23	1.55 ± 0.01	1.55 ± 0.01
4 (*n* = 410)	20.47 ± 1.74	20.31	40.30 ± 0.23	40.27 ± 0.23	1.59 ± 0.01 **	1.59 ± 0.01 **
5 (*n* = 410)	44.84 ± 50.09	31.67	40.22 ± 0.22	40.20 ± 0.23	1.58 ± 0.01 *	1.58 ± 0.01 *
Q5-Q1 diff [1]			−0.29	−0.33	0.04	0.04
% diff [2]			−0.72	−0.81	2.60	2.60
p trend			0.133	0.003	0.003	$p < 0.001$

Values are presented as means ± SEM. The *p*-trend was calculated using ANCOVA. [1] Q5-Q1 calculates the absolute difference between the means of quintile (Q) 5 and Q1. [2] % difference calculates the percentage difference between the means of Q5 and Q1. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus quintile 1. FFM = fat-free mass. For the FFM model, ratios were adjusted for age, smoking status, physical activity, corticosteroid and statin use, social class, fat mass, and menopausal status and HRT use in women. For the FFM_{BMI} model, ratios were adjusted for age, smoking status, physical activity, corticosteroid and statin use, social class, and menopausal status and HRT use in women.

Table 5. Associations between quintiles of dietary vitamin E and calcaneal BUA in men and women aged 42–82 years.

Men (*n* = 6443)	**Dietary α-Tocopherol Equivalents Intake (mg/day)**		**BUA**	
Quintile	Mean ± SD	Median	Unadjusted	Adjusted
1 (*n* = 1289)	6.45 ± 1.19	6.71	88.73 ± 0.50	89.03 ± 0.52
2 (*n* = 1289)	8.99 ± 0.53	9.01	90.06 ± 0.48	90.07 ± 0.49
3 (*n* = 1288)	10.93 ± 0.63	10.89	90.64 ± 0.48 **	90.64 ± 0.48 *
4 (*n* = 1289)	13.46 ± 0.90	13.38	90.51 ± 0.49 *	90.39 ± 0.49
5 (*n* = 1288)	19.71 ± 4.63	18.30	90.34 ± 0.49 *	90.15 ± 0.52
Q5-Q1 diff [1]			1.61	1.12
% diff [2]			1.81	1.26
p trend			0.044	<0.001
Women (*n* = 8251)				
Quintile	Mean ± SD	Median	Unadjusted	Adjusted
1 (*n* = 1651)	5.33 ± 0.98	5.54	70.85 ± 0.41	71.84 ± 0.39
2 (*n* = 1650)	7.38 ± 0.44	7.38	71.66 ± 0.40	72.16 ± 0.35
3 (*n* = 1650)	8.91 ± 0.46	8.93	71.13 ± 0.40	71.53 ± 0.35
4 (*n* = 1650)	10.72 ± 0.64	10.68	73.23 ± 0.41 ***	72.45 ± 0.35
5 (*n* = 1650)	15.13 ± 3.56	14.12	73.57 ± 0.41 ***	72.47 ± 0.38
Q5–Q1 diff [1]			2.72	0.63
% diff [2]			3.84	0.88
p trend			<0.001	<0.001

Values are presented as means ± SEM. The *p*-trend was calculated using ANCOVA. [1] Q5–Q1 calculates the absolute difference between the means of quintile (Q) 5 and Q1. [2] % difference calculates the percentage difference between the means of Q5 and Q1. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus quintile 1, according to ANCOVA. BUA = broadband ultrasound attenuation. BUA—adjusted model includes age, BMI, total energy, dietary calcium intake, dietary vitamin D intake, smoking status, physical activity, corticosteroid use, menopausal status, HRT use, calcium and vitamin D supplement use and family history of osteoporosis.

3.7. Associations between Plasma Vitamin E Concentrations and Bone Density Status

Analysis of mean calcaneal bone density measures, stratified by sex and quintiles of plasma vitamin E concentrations, is shown in Table 6, for both the unadjusted and fully adjusted models. In both men and women, mean BUA measures tended to significantly increase across quintiles of plasma α- and γ-tocopherol and decrease across quintiles of the ratio of α-tocopherol:γ-tocopherol ($p < 0.001$). No significant differences were found

between quintile 1 and any of the other quintiles in the adjusted models, in either men or women.

3.8. Associations between Dietary Vitamin E Intakes and Fracture Risk

In the fully adjusted dietary model analyses, significant positive associations were evident between quintiles of vitamin E intake and risk for total fractures and hip fractures in both men and women ($p < 0.001$), but negative associations were found for wrist fractures in women ($p < 0.01$) (Table 7). In men, both total fracture risk and wrist fracture risk were significantly lower in Q2 versus Q1 (0.79; 95% CI 0.64, 0.98; $p < 0.05$ and 0.51; 95% CI 0.29, 0.90; $p < 0.05$, respectively). In women, hip fracture risk was significantly lower in Q2 versus Q1 (0.81; 95% CI 0.66, 0.99; $p < 0.05$). The addition of supplement-derived vitamin E intake to the fully adjusted models did not alter the associations.

3.9. Associations between Plasma Vitamin E Concentrations and Fracture Risk

In the fully adjusted plasma vitamin E analyses, significant linear trends were found for risk of total and hip fractures and quintiles of plasma α-, γ-tocopherol and the ratio of α-tocopherol:γ-tocopherol in men ($p < 0.05$) (Table 8). The risk for both total and hip fracture decreased across quintiles of plasma α-tocopherol and the ratio of α-tocopherol:γ-tocopherol, but increased across quintiles of γ-tocopherol for hip fracture risk and tended to decrease for total fracture risk. In women, in the fully adjusted plasma vitamin E analyses, significant linear trends were found for risk of total and hip fractures and quintiles of plasma α-, γ-tocopherol and the ratio of α-tocopherol:γ-tocopherol ($p < 0.05$). The risk for total and hip fractures tended to decrease across quintiles of plasma α-tocopherol and the ratio of α-tocopherol:γ-tocopherol but increase across the quintiles of γ-tocopherol. In men, spine fracture risk was significantly higher in Q3 versus Q1 (2.00; 95% CI 1.00, 4.00; $p < 0.05$) in the fully adjusted ratio of α-tocopherol:γ-tocopherol model. Total fracture risk in women was significantly higher in Q4 versus Q1 (1.29; 95% CI 1.01, 1.65; $p < 0.05$) in the fully adjusted plasma γ-tocopherol model. However, hip fracture risk in women was significantly lower in Q3 versus Q1 (0.61; 95% CI 0.41, 0.89; $p < 0.05$) in the fully adjusted ratio of α-tocopherol:γ-tocopherol model. Wrist fracture risk in women was significantly lower in Q5 versus Q1 (0.56; 95% CI 0.32, 0.98; $p < 0.05$) in the fully adjusted plasma α-tocopherol model.

The associations of dietary intake and plasma concentrations of vitamin E with SMM, BUA and fracture risk are summarised in Table 9.

Table 6. Multivariate adjusted calcaneal BUA for EPIC-Norfolk participants, stratified by sex and quintiles of plasma α-tocopherol, plasma γ-tocopherol and α-tocopherol:γ-tocopherol ratio, adjusted for by blood cholesterol measurement.

Men (*n* = 2308)	**Plasma α-Tocopherol, Adjusted for Cholesterol (μmol/mmol)**		**BUA**	
Quintile	Mean ± SD	Median	Unadjusted	Adjusted
1 (*n* = 462)	3.17 ± 0.36	3.26	88.97 ± 0.82	89.11 ± 0.82
2 (*n* = 462)	3.80 ± 0.13	3.80	89.01 ± 0.81	88.87 ± 0.82
3 (*n* = 461)	4.24 ± 0.13	4.24	89.22 ± 0.83	89.01 ± 0.82
4 (*n* = 462)	4.72 ± 0.15	4.70	89.84 ± 0.83	89.84 ± 0.82
5 (*n* = 461)	5.90 ± 1.20	5.50	90.19 ± 0.84	90.38 ± 0.83
Q5–Q1 diff [1]			1.22	1.27
% diff [2]			1.37	1.42
p trend			0.209	<0.001
Women (*n* = 2149)	**Plasma α-Tocopherol, Adjusted for Cholesterol (μmol/mmol)**		**BUA**	
Quintile	Mean ± SD	Median	Unadjusted	Adjusted
1 (*n* = 430)	3.22 ± 0.41	3.33	68.74 ± 0.81	69.29 ± 0.66
2 (*n* = 430)	3.86 ± 0.13	3.87	69.22 ± 0.79	69.47 ± 0.66
3 (*n* = 430)	4.31 ± 0.13	4.30	69.38 ± 0.72	69.25 ± 0.66
4 (*n* = 430)	4.80 ± 0.16	4.80	70.86 ± 0.78	70.72 ± 0.66
5 (*n* = 429)	5.96 ± 1.02	5.63	70.95 ± 0.78 *	70.44 ± 0.67

Table 6. *Cont.*

Q5–Q1 diff [1]			2.21	1.15
% diff [2]			3.22	1.66
p trend			0.015	<0.001
Men (*n* = 2308)	**Plasma γ-Tocopherol, Adjusted for Cholesterol (μmol/mmol)**		**BUA**	
Quintile	Mean ± SD	Median	Unadjusted	Adjusted
1 (*n* = 462)	0.15 ± 0.04	0.16	88.93 ± 0.79	88.68 ± 0.83
2 (*n* = 462)	0.22 ± 0.02	0.23	89.16 ± 0.86	89.09 ± 0.82
3 (*n* = 461)	0.28 ± 0.02	0.28	89.10 ± 0.84	88.94 ± 0.82
4 (*n* = 462)	0.35 ± 0.02	0.34	89.50 ± 0.82	89.80 ± 0.82
5 (*n* = 461)	0.51 ± 0.14	0.47	90.54 ± 0.82	90.69 ± 0.82
Q5–Q1 diff [1]			1.61	2.01
% diff [2]			1.81	2.27
p trend			0.141	$p < 0.001$
Women (*n* = 2149)	**Plasma γ-Tocopherol, Adjusted for Cholesterol (μmol/mmol)**		**BUA**	
Quintile	Mean ± SD	Median	Unadjusted	Adjusted
1 (*n* = 430)	0.14 ± 0.04	0.14	69.45 ± 0.79	68.46 ± 0.67
2 (*n* = 430)	0.21 ± 0.02	0.21	69.22 ± 0.78	68.86 ± 0.66
3 (*n* = 430)	0.27 ± 0.02	0.26	68.93 ± 0.78	69.03 ± 0.66
4 (*n* = 430)	0.33 ± 0.02	0.33	70.34 ± 0.76	70.69 ± 0.66
5 (*n* = 429)	0.49 ± 0.13	0.44	71.21 ± 0.78	72.12 ± 0.66
Q5–Q1 diff [1]			1.76	3.66
% diff [2]			2.53	5.35
p trend			0.046	$p < 0.001$
Men (*n* = 2308)	**Plasma α:γ-Tocopherol Ratio, Adjusted for Cholesterol**		**BUA**	
Quintile	Mean ± SD	Median	Unadjusted	Adjusted
1 (*n* = 462)	8.73 ± 1.46	9.00	89.44 ± 0.82	89.67 ± 0.83
2 (*n* = 462)	12.10 ± 0.82	10.70	89.56 ± 0.81	89.67 ± 0.82
3 (*n* = 461)	14.97 ± 0.88	13.51	89.78 ± 0.85	89.76 ± 0.82
4 (*n* = 462)	18.91 ± 1.45	16.59	89.51 ± 0.82	89.31 ± 0.82
5 (*n* = 461)	35.15 ± 30.64	21.69	88.95 ± 0.82	88.79 ± 0.83
Q5–Q1 diff [1]			−0.49	−0.88
% diff [2]			−0.55	−0.98
p trend			0.602	$p < 0.001$
Women (*n* = 2149)	**Plasma α:γ-Tocopherol Ratio, Adjusted for Cholesterol**		**BUA**	
Quintile	Mean ± SD	Median	Unadjusted	Adjusted
1 (*n* = 430)	9.06 ± 1.62	9.42	70.31 ± 0.79	71.02 ± 0.66
2 (*n* = 430)	12.67 ± 0.90	12.75	70.03 ± 0.74	70.97 ± 0.66
3 (*n* = 430)	15.81 ± 1.02	15.75	69.32 ± 0.78	69.31 ± 0.66
4 (*n* = 430)	20.35 ± 1.76	20.13	69.68 ± 0.80	69.10 ± 0.66
5 (*n* = 429)	44.22 ± 57.93	31.23	69.81 ± 0.79	68.74 ± 0.68
Q5–Q1 diff [1]			−0.50	−2.28
% diff [2]			−0.71	−3.21
p trend			0.731	$p < 0.001$

Values are presented as means ± SEM. The *p*-trend was calculated using ANCOVA. BUA = broadband ultrasound attenuation. [1] Q5–Q1 calculates the absolute difference between the means of quintile (Q) 5 and Q1. [2] % difference calculates the percentage difference between the means of Q5 and Q1. * $p < 0.05$ versus quintile 1. Ratios were adjusted for age, BMI, smoking status, physical activity, family history of osteoporosis, corticosteroid use, dietary calcium intake, dietary vitamin D intake, calcium and vitamin D supplement use, and menopausal status and HRT use in women.

Table 7. Risk of total, hip, spine and wrist fractures in EPIC-Norfolk participants, stratified by sex and quintiles of dietary vitamin E intake (n = 25,223).

	Men (n = 11,427)					**Total Fractures**			**Hip Fracture**			**Spine Fracture**			**Wrist Fracture**		
		N	Mean	SD	Median	Incidence	HR	95% CI	Incidence	HR	95% CI	Incidence	HR	95% CI	Incidence	HR	95% CI
Dietary	Q1	2286	5.95	1.25	6.23	188/2286	1.00	Ref.	80/2286	1.00	Ref.	49/2286	1.00	Ref.	33/2286	1.00	Ref.
α-tocopherol	Q2	2285	8.65	0.59	8.67	161/2285	0.79 *	0.64 0.98	74/2285	0.89	0.64 1.23	47/2285	0.94	0.62 1.41	20/2285	0.51 *	0.29 0.90
equivalents	Q3	2286	10.65	0.61	10.62	176/2286	0.94	0.76 1.17	62/2286	0.86	0.61 1.22	49/2286	1.10	0.73 1.67	32/2286	0.80	0.48 1.34
intake	Q4	2285	13.22	0.92	13.11	158/2285	0.86	0.68 1.08	72/2285	1.08	0.76 1.53	33/2285	0.77	0.48 1.24	38/2285	0.88	0.53 1.47
(mg/day)	Q5	2285	19.71	4.92	18.22	194/2285	1.06	0.84 1.34	68/2285	1.05	0.73 1.52	45/2285	1.09	0.68 1.75	32/2285	0.68	0.38 1.19
						877/11,427			356/11,427			223/11,427			155/11,427		
p trend							$p < 0.001$			$p < 0.001$			0.21			0.11	
	Women (n = 13,796)					**Total Fractures**			**Hip Fracture**			**Spine Fracture**			**Wrist Fracture**		
		N	Mean	SD	Median	Incidence	HR	95% CI	Incidence	HR	95% CI	Incidence	HR	95% CI	Incidence	HR	95% CI
Dietary	Q1	2760	5.04	1.03	5.25	479/2760	1.00	Ref.	228/2760	1.00	Ref.	89/2760	1.00	Ref.	108/2760	1.00	Ref.
α-tocopherol	Q2	2759	7.14	0.45	7.15	438/2759	0.93	0.81 1.06	177/2759	0.81 *	0.66 0.99	80/2759	0.94	0.68 1.28	104/2759	1.05	0.80 1.39
equivalents	Q3	2759	8.70	0.46	8.68	427/2759	0.93	0.81 1.07	212/2759	1.04	0.85 1.27	63/2759	0.77	0.55 1.09	102/2759	1.08	0.81 1.44
intake	Q4	2759	10.55	0.66	10.50	377/2759	0.88	0.76 1.02	175/2759	0.93	0.75 1.16	67/2759	0.90	0.63 1.28	104/2759	1.20	0.89 1.63
(mg/day)	Q5	2759	15.01	3.48	14.01	371/2759	0.90	0.77 1.06	179/2759	1.02	0.81 1.29	58/2759	0.82	0.55 1.21	86/2759	1.07	0.77 1.50
						2092/13,796			971/13,796			357/13,796			504/13,796		
p trend							$p < 0.001$			$p < 0.001$			0.09			$p < 0.01$	

* $p < 0.05$ versus quintile 1. Ratios were adjusted for age, BMI, smoking status, physical activity, family history of osteoporosis, calcium intake, vitamin D intake, energy intake, calcium- and vitamin D-containing supplement use, corticosteroid use, and menopausal status and HRT use in women.

Table 8. Risk of total, hip, spine and wrist fractures in EPIC-Norfolk participants, stratified by sex and quintiles of plasma α-tocopherol, plasma γ-tocopherol and the ratio of α:γ, adjusted for cholesterol.

Men (*n* = 3727)				**Total Fractures**			**Hip Fractures**			**Spine Fractures**			**Wrist Fractures**		
	Mean	SD	Median	Incidence	HR	95% CI	Incidence	HR	95% CI	Incidence	HR	95% CI	Incidence	HR	95% CI
Plasma α-tocopherol, adjusted for cholesterol (μmol/mmol)	3.13	0.39	3.23	67/746	1.00	Ref.	31/746	1.00	Ref.	20/746	1.00	Ref.	4/746	1.00	Ref.
	3.79	0.13	3.79	52/745	0.79	0.54 1.13	20/745	0.62	0.35 1.11	16/745	0.84	0.43 1.62	10/745	2.41	0.75 7.72
	4.22	0.13	4.22	61/746	0.90	0.63 1.27	25/746	0.82	0.48 1.39	18/746	0.87	0.46 1.65	10/746	2.29	0.71 7.35
	4.70	0.16	4.69	65/745	0.88	0.63 1.24	27/745	0.78	0.47 1.32	17/745	0.78	0.41 1.50	8/745	1.76	0.52 5.87
	5.88	1.12	5.51	63/745	0.86	0.61 1.23	26/745	0.79	0.46 1.35	18/745	0.81	0.42 1.56	8/745	1.79	0.53 6.05
p trend					0.028			0.007			0.460			0.787	
Plasma γ-tocopherol, adjusted for cholesterol (μmol/mmol)	0.15	0.04	0.16	60/746	1.00	Ref.	23/746	1.00	Ref.	20/746	1.00	Ref.	7/746	1.00	Ref.
	0.23	0.02	0.23	52/745	0.85	0.58 1.24	24/745	1.03	0.58 1.85	13/745	0.63	0.31 1.28	9/745	1.25	0.46 3.39
	0.28	0.02	0.28	61/746	0.97	0.68 1.40	27/746	1.09	0.62 1.94	21/746	1.00	0.54 1.85	6/746	0.83	0.28 2.53
	0.35	0.02	0.35	75/745	1.21	0.85 1.71	30/745	1.22	0.69 2.14	21/745	0.98	0.53 1.83	9/745	1.28	0.47 3.49
	0.52	0.13	0.48	60/745	0.94	0.65 1.37	25/745	1.04	0.57 1.86	14/745	0.60	0.29 1.22	9/745	1.31	0.47 3.63
p trend					0.029			0.008			0.425			0.776	
Plasma α-tocopherol:γ-tocopherol ratio, adjusted for cholesterol	8.56	1.43	8.87	60/746	1.00	Ref.	28/746	1.00	Ref.	13/746	1.00	Ref.	7/746	1.00	Ref.
	11.90	0.77	11.93	64/745	1.00	0.70 1.42	31/745	0.94	0.56 1.57	19/745	1.53	0.74 3.17	5/745	0.67	0.21 2.12
	14.71	0.87	14.66	76/746	1.18	0.84 1.66	28/746	0.85	0.50 1.45	25/746	2.00*	1.00 4.00	11/746	1.42	0.55 3.68
	18.53	1.39	18.40	50/745	0.86	0.59 1.26	15/745	0.55	0.29 1.04	19/745	1.65	0.79 3.43	8/745	1.13	0.40 3.16
	34.08	27.74	25.88	58/745	0.96	0.66 1.40	27/745	0.96	0.55 1.68	13/745	1.09	0.49 2.44	9/745	1.24	0.44 3.45
p trend					0.027			0.007			0.490			0.765	
Women (*n* = 3564)				**Total Fractures**			**Hip Fractures**			**Spine Fracture**			**Wrist Fracture**		
	Mean	SD	Median	Incidence	HR	95% CI	Incidence	HR	95% CI	Incidence	HR	95% CI	Incidence	HR	95% CI
Plasma α-tocopherol, adjusted for cholesterol (μmol/mmol)	3.20	0.42	3.32	138/713	1.00	Ref.	66/713	1.00	Ref.	24/713	1.00	Ref.	37/713	1.00	Ref.
	3.86	0.13	3.86	124/713	0.87	0.68 1.11	66/713	0.99	0.70 1.39	25/713	1.05	0.60 1.84	26/713	0.67	0.40 1.12
	4.30	0.12	4.29	125/713	0.98	0.77 1.25	62/713	1.08	0.76 1.52	23/713	1.04	0.58 1.84	34/713	0.98	0.61 1.56
	4.78	0.17	4.77	132/713	1.03	0.81 1.31	56/713	0.93	0.65 1.33	27/713	1.20	0.69 2.10	36/713	1.04	0.65 1.65
	5.97	1.04	5.61	124/712	0.94	0.73 1.20	63/712	1.04	0.73 1.49	25/712	1.03	0.57 1.85	19/712	0.56 *	0.32 0.98
p trend					0.010			0.013			0.665			0.075	
Plasma γ-tocopherol, adjusted for cholesterol (μmol/mmol)	0.14	0.04	0.15	125/713	1.00	Ref.	62/713	1.00	Ref.	26/713	1.00	Ref.	32/713	1.00	Ref.
	0.21	0.02	0.21	132/713	1.11	0.87 1.42	60/713	1.01	0.71 1.45	23/713	0.91	0.52 1.62	31/713	1.04	0.63 1.71
	0.27	0.02	0.27	125/713	1.09	0.84 1.40	57/713	0.97	0.67 1.41	29/713	1.13	0.65 1.96	33/713	1.17	0.71 1.92
	0.33	0.02	0.33	143/713	1.29 *	1.01 1.65	76/713	1.36	0.96 1.93	28/713	1.20	0.69 2.09	30/713	1.04	0.62 1.75
	0.50	0.13	0.46	118/712	1.07	0.82 1.39	58/712	1.05	0.72 1.52	18/712	0.75	0.40 1.40	26/712	1.00	0.58 1.70

Table 8. *Cont.*

p trend					0.009				0.011				0.486				0.108		
Plasma α-tocopherol:γ-tocopherol ratio, adjusted for cholesterol	8.91	1.66	9.30	131/713	1.00	Ref.		69/713	1.00	Ref.		23/713	1.00	Ref.		29/713	1.00	Ref.	
	12.53	0.89	12.58	130/713	0.96	0.76	1.23	70/713	1.01	0.72	1.41	23/713	1.02	0.57	1.83	23/713	0.73	0.42	1.28
	15.64	0.99	15.56	123/713	0.90	0.70	1.15	43/713	0.61 *	0.41	0.89	29/713	1.30	0.75	2.26	42/713	1.40	0.87	2.26
	20.06	1.70	19.87	138/713	0.98	0.77	1.25	71/713	0.97	0.69	1.36	25/713	1.02	0.57	1.82	31/713	0.93	0.56	1.56
	42.51	50.07	30.12	121/712	0.84	0.65	1.10	60/712	0.83	0.58	1.19	24/712	0.97	0.53	1.79	27/712	0.84	0.48	1.44
p trend					0.007				0.011				0.507				0.099		

* $p < 0.05$ versus quintile 1. Ratios were adjusted for age, BMI, smoking status, physical activity, family history of osteoporosis, calcium intake, vitamin D intake, calcium- and vitamin D-containing supplement use, corticosteroid use and menopausal status and HRT use in women.

Table 9. Summary of the associations of dietary intake and plasma concentrations of vitamin E with SMM, BUA and fracture risk.

Measure of Interest		Dietary α-Tocopherol Equivalents Intake (mg/day)	% Difference [1]	Serum Cholesterol-Adjusted α-Tocopherol (μmol/mmol)	% Difference [1]	Serum Cholesterol-Adjusted γ-Tocopherol (μmol/mmol)	% Difference [1]	Serum Cholesterol-Adjusted α-Tocopherol:γ-Tocopherol Ratio	% Difference [1]
FFM	Men	positive ***	1.03	positive ***	0.16	positive ***	0.95	negative ***	−1.50
	Women	positive ***	1.04	positive **	0.12	positive **	1.70	negative **	−0.81
FFM_{BMI}	Men	positive ***	1.74	negative ***	−1.30	negative ***	−2.16	positive ***	0.87
	Women	positive ***	1.91	negative ***	−1.90	negative ***	−2.53	positive ***	2.60
BUA	Men	positive ***	1.26	positive ***	1.42	positive ***	2.27	negative ***	−0.98
	Women	positive ***	0.88	positive ***	1.66	positive ***	5.35	negative ***	−3.21
Total fracture risk [2]	Men	positive ***		positive *		positive *		positive *	
	Women	positive ***		positive *		negative **		positive **	
Hip fracture risk [2]	Men	positive ***		positive **		negative **		positive **	
	Women	positive ***		positive *		negative *		positive *	
Spine fracture risk [2]	Men	N.S.		N.S.		N.S.		N.S.	
	Women	N.S.		N.S.		N.S.		N.S.	
Wrist fracture risk [2]	Men	N.S.		N.S.		N.S.		N.S.	
	Women	negative **		N.S.		N.S.		N.S.	

FFM = fat-free mass; BUA = broadband ultrasound attenuation. [1] (Q5 mean − Q1 mean/Q1 mean) × 100. [2] Positive associations with fracture risk indicate lower risk with higher tocopherol, while negative associations indicate higher risk. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

4. Discussion

To our knowledge, this is the first study using data from a large population cohort of middle- and older-aged men and women in the UK to assess the associations between both dietary vitamin E intake and plasma vitamin E concentrations and indices of SMM, bone density status and fracture risk. Our results show significant positive associations between both dietary vitamin E intake and plasma concentrations of both serum cholesterol-adjusted α- and γ-tocopherol and FFM and BUA, and generally significant positive associations for fracture risk. The associations found with vitamin E for bone density status and fracture risk are independent of vitamin D and calcium intake, which are both known to be relevant for bone health. The results from this study indicate protection for musculoskeletal health with higher intakes and blood concentrations of vitamin E.

In the EPIC-Norfolk study, the mean daily dietary intakes of vitamin E in 11,535 men and 13,972 women, assessed using a 7dDD and expressed in milligrams of α-tocopherol equivalents, were 11.62 (SD 5.24) and 9.27 (SD 3.78) mg [48] which is very similar to the mean daily intakes in the cohorts in these analyses. These dietary intakes are in agreement with those from other European countries [62,63]. Although in relation to the AIs defined by EFSA [59], only 31% of men and 26% of women met the AIs.

Most studies conducted in the US reported higher average plasma γ-tocopherol levels as compared to studies conducted in Europe [32]. This is likely explained by the fact that γ-tocopherol is the major form (approximately 70%) of vitamin E in the US diet [31]. Therefore, this study focuses on European comparisons. The mean plasma α- and γ-tocopherol concentrations in our study cohorts are similar to those found in a healthy Irish adult population [63] and in the UK National Diet and Nutrition Survey (NDNS) [64]. More than 99% of both men and women in our study cohorts had higher plasma tocopherol concentrations or tocopherol:cholesterol ratios than the minimum satisfactory values [58].

Our study found a number of weak but significant correlations between the dietary intake of α-tocopherol equivalents and plasma concentrations of α-tocopherol, which has also been observed in a small German study [65] (n = 92; r = 0.14) but no associations have been found in a number of other European studies [66–68]. However, Kardinaal et al. found a significant age- and sex-adjusted correlation (r = 0.24, $p < 0.05$) for α-tocopherol between intake and adipose tissue levels in a small study of healthy adults [66], whereas no correlation was found between the adipose tissue level of alpha-tocopherol and dietary intake by Andersen et al. [67].

Significant positive trends in FFM and FFM_{BMI} were evident across increasing quintiles of dietary vitamin E intake for both sexes, after adjusting for important covariates. Similar linear trends were generally apparent in both men and women for plasma vitamin E concentrations, with those across quintiles of α- and γ-tocopherol tending to be in the same direction (positive for FFM but negative for FFM_{BMI}), and the trend for the ratio of α-tocopherol:γ-tocopherol in the opposite direction (negative for FFM but positive for FFM_{BMI}). These seemingly contradictory findings are not surprising as the increase in the ratio across quintiles is due to decreasing plasma α- but increasing γ-tocopherol concentrations. Whereas across increasing quintiles of plasma α-tocopherol, γ-tocopherol also increased, and across increasing quintiles of γ-tocopherol, plasma concentrations of α-tocopherol tended to decrease slightly. To date, most studies that have investigated the potential role of vitamin E in muscle health have focused on muscle function and strength rather than muscle mass and found that higher dietary vitamin E intakes [22,26] and plasma vitamin E concentrations were associated with higher strength measures and physical performance tests or lower levels of frailty [21,23,24,69–71]. Findings from our study support the importance of vitamin E to skeletal muscle health.

Significant trends were apparent for BUA across quintiles of dietary vitamin E intake and plasma concentrations in both men and women, with BUA tending to increase across quintiles of dietary vitamin E intake and plasma α- and γ-tocopherol but decrease across quintiles of the ratio of α-tocopherol:γ-tocopherol ($p < 0.001$). Significant positive associations were evident for dietary vitamin E intake and risk for total and hip fractures

in both men and women ($p < 0.001$), but a significant negative association was found for wrist fracture risk in women ($p < 0.01$), where the greatest number of fractures was found in Q1, and the lowest in Q5; it is possible that this association may be artefactual. In plasma vitamin E analyses, significant linear trends were found for total fracture risk in men ($p < 0.05$) with the risk of total fractures generally decreasing across the quintiles in all 3 models. Significant linear associations were also found for hip fracture risk in men ($p < 0.01$), with the risk of hip fracture decreasing across quintiles of plasma α-tocopherol and the ratio of α-tocopherol:γ-tocopherol but increasing across quintiles of γ-tocopherol. In women, significant linear trends were found for risk of total and hip fractures and plasma vitamin E ($p < 0.05$), with the risk of fractures generally decreasing across quintiles of plasma α-tocopherol and the ratio of α-tocopherol:γ-tocopherol but increasing across quintiles of γ-tocopherol.

In the Aberdeen Prospective Osteoporosis Screening Study (APOSS) cohort, no biologically meaningful changes in BMD or bone resorption and formation markers with dietary intakes or serum concentrations of tocopherols or the α/γ ratio were found in perimenopausal and postmenopausal women [72], although dietary vitamin E intake was negatively associated with femoral neck BMD in early postmenopausal women in Scotland [73]. A recent study of nutrient intake and BMD in postmenopausal women found that a high intake of vitamin E had a negative effect on BMD [74]. Low serum concentrations of α- tocopherol have been associated with an increased risk of hip fracture in elderly men and women [75] and an increased osteoporosis risk in postmenopausal women [27]. Both low intakes and serum concentrations of α-tocopherol were associated with an increased rate of fracture in elderly Swedish men and women [28]. There are a number of plausible explanations for the heterogeneity of the conclusions of the aforementioned epidemiological studies—the use of different covariates in the multivariable models, inconsistent measurement validity of biomarkers and the application of various exclusion criteria regarding the study sample—although most concur with our findings. Whether or not these study findings have any biological significance is unclear.

A recent review on the beneficial and detrimental effects of oxidative stress on human health concluded that α- and γ-tocopherol forms of vitamin E exert a differential set of biological effects, which cannot always be regarded as positive to human health [76]. Recent data have also suggested that plasma α-tocopherol concentrations are more dependent on mechanisms that control circulating lipids rather than those related to its absorption and initial incorporation into plasma [77]; α-tocopherol was found to remain in circulation longer in participants with higher serum lipids, but its absorption was not dependent on the plasma lipid status.

In contrast to a high affinity to α-tocopherol (100%), α-tocopherol transfer protein (α-TTP) has a much lower affinity towards other vitamin E forms; 50%, 10–30%, and 1% affinity to β-tocopherol, γ-tocopherol, and δ-tocopherol, respectively [78], and plays an important role in the maintenance of high concentrations of α-tocopherol in plasma and some tissues [79,80]. A reduction in plasma γ-tocopherol during enhanced intake of α-tocopherol, such as through supplemental intake, can be explained by the more rapid metabolism of γ-tocopherol occurring when α-tocopherol intake is increased [81]. Chylomicron-associated tissue uptake of vitamin E may contribute to the accumulation of non-α-tocopherol forms of vitamin E such as γ-tocopherol in human skin, adipose tissue, and muscle, where unexpectedly high concentrations of γ-tocopherol were observed, in contrast to its low levels in the plasma [82]. Many unique properties have been attributed to γ-tocopherol and its metabolites [83], which exhibit sometimes enhanced or different activities of α-tocopherol such as natriuretic, anti-inflammatory, antitumoural activities, as summarised in a recent review [84]. The ratio of α-tocopherol:γ-tocopherol is suggested as a correction method as it would respond to even a small increase in α-tocopherol from supplementation that may not be clearly evident in plasma α-tocopherol concentrations [85]. Findings from the analyses in this study have shown that adjustment for the amount of vitamin E from supplements did not affect the associations.

The interactions between these two tocopherols are complex within the body and it must also be remembered that the bioavailability of vitamin E is influenced by a number of factors, including other nutrients, genetics, absorption, transport and metabolism [86]. With regard to other nutrients and food intake, data from the NDNS found that α-tocopherol correlated directly with "healthy" nutrient choices (intrinsic sugars, dietary fibre, and vitamins) and inversely with "unhealthy" choices (extrinsic sugars and monounsaturated fats—i.e., avoidance of polyunsaturated fat), whilst γ-tocopherol and the γ-tocopherol:α-tocopherol ratio related inversely with "healthy" choices, with the authors concluding that the γ-tocopherol:α-tocopherol ratio may reveal poor dietary choices, which may subsequently lead to health issues in later life [87].

A number of possible mechanisms have been suggested illustrating how vitamin E may slow down the aging of skeletal muscle: (1) by improving antioxidant capacity, thereby reducing oxidative stress and inflammation; (2) improving membrane repair and increasing survival of damaged skeletal muscle by reducing oxidized phospholipid formation; (3) improving mitochondrial efficiency; (4) decreasing glycogen usage in skeletal muscle, while increasing fat metabolism; (5) enhancing muscle regeneration capacity; (6) stabilize insulin structure and improve insulin sensitivity of skeletal muscle [35]. It is thought that reduction in oxidative stress may also be a plausible mechanism whereby vitamin E protects bone, although the reported opposing inflammatory functions of α- and γ-tocopherol may result in an increase in bone resorption without affecting bone formation [36]. However, further research is needed to investigate the potential effects of other tocopherols and tocotrienols on sarcopenic and osteoporotic risk factors.

The strengths of our study include a large population size of middle-aged and elderly men and women, from whom we had measures of dietary and supplemental intake, obtained from 7dDDs, in addition to plasma concentrations of vitamin E (α- and γ-tocopherol), in order to study the potential associations of vitamin E with indices of SMM, BUA and fracture risk (over 18.5 years of follow-up). Limitations of our study include the observational and cross-sectional study design regarding SMM and BUA measurements, precluding us from inferring causation, and the use of self-reported measures for dietary intake and physical activity. However, the prospective nature of our study of fracture risk and long follow-up for end points of 18.5 years are advantages. In addition, the 7dDDs developed for use in the EPIC-Norfolk study have previously been validated and are expected to produce a more precise measure of dietary intake than 24 h diet recalls or food frequency questionnaires [48]. Plasma vitamin E concentrations were only available for a small subset of the cohort, which may have reduced the power of our analyses. Nevertheless, the availability of plasma concentrations, which are not subject to nonrandom biases that can affect questionnaire-based measurements, is a strength of our study, although these concentrations may be affected by various physiological effects. SMMs were calculated from weight, height and bioelectrical impedance measurements, and not from potentially more accurate and precise methods, such as DEXA, computer tomography or magnetic resonance imaging; however, this method has comparable acceptability in population studies [88]. The dietary and lifestyle data, including the consumption of corticosteroids, HRT and dietary supplements, used in the longitudinal analyses were collected at 1HE and we were unable to account for any changes in exposures which may have occurred over time and potentially affected the associations.

5. Conclusions

Our research has found significant positive associations between greater intakes of dietary vitamin E and SMM indices, bone density status and total and hip fracture risk in both middle-aged and elderly men and women, with the scale of effects ranging from 0.88% to 1.91% ($p < 0.001$). Associations found with circulating plasma α- and γ-tocopherol generally agreed with the dietary data. These findings suggest that dietary vitamin E intake may play a role in musculoskeletal health and provides evidence of the benefits of higher vitamin E intakes, similar to the AIs of α-tocopherol for adults of 13 mg/day for

men and 11 mg/d for women, as recommended by EFSA. These intakes can be achieved by eating a varied and balanced diet, including the consumption of foods rich in vitamin E, such as oily seeds and their derivatives, nuts and cereals rich in vitamin E, including fortified breakfast cereals. Where it is not possible to obtain adequate intakes through the diet, vitamin E supplements should be consumed, especially in those at sarcopenic or osteoporotic risk. Further investigation is required to understand the relationships with plasma concentrations and musculoskeletal health.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Norwich District Authority Ethics Committee (98CN01).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The authors will make the dataset available under a Data Transfer Agreement to any bona fide researcher who wishes to obtain the dataset in order to undertake a replication analysis. Although the dataset is anonymized, the breadth of the data included and the multiplicity of variables that are included in this analysis file as primary variables or confounding factors, means that provision of the dataset to other researchers without a Data Transfer Agreement would constitute a risk. Requests for data sharing/access should be submitted to the EPIC Management Committee (epic-norfolk@mrc-epid.cam.ac.uk).

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Chapter 6. The Dietary Inflammatory Index and its associations with biomarkers of nutrients with antioxidant potential, a biomarker of inflammation and multiple long-term conditions

6.1 Key points

What is already known on this subject?

- The low-grade inflammation that occurs with ageing has been related to a number of chronic diseases that contribute to multiple long-term conditions (MLTCs), the prevalence of which is increasing.
- Research indicates that diets rich in antioxidants, such as β-carotene, vitamins A, C and E, and magnesium may play an important role in modulating inflammation.

After my investigations into associations of vitamin E, I extended my research from a single nutrient to diet as a whole, by studying the relationship between the Dietary Inflammatory Index (DII®) and MLTCs. The DII® is a literature-based dietary score, developed to measure the potential impact of diet on an individual's inflammatory status.

What this study adds.

- Micronutrient biomarker concentrations were significantly lower as the diet became more pro-inflammatory and concentrations of hs-CRP, a well-known inflammatory biomarker, significantly higher in men, indicating criterion validity of the DII® score.
- Lower concentrations of vitamin C and higher concentrations of hs-CRP were associated with higher odds of MLTCs.
- Subgroups of the population, including manual workers, current smokers and those who had no qualifications or were physically inactive were more likely to consume a more pro-inflammatory diet.

- A lower DII® score (anti-inflammatory diet) was associated with higher odds of having MLTCs.

6.2 Published journal article

Mulligan, A.A.; Lentjes, M.A.H.; Skinner, J.; Welch, A.A. The Dietary Inflammatory Index and Its Associations with Biomarkers of Nutrients with Antioxidant Potential, a Biomarker of Inflammation and Multiple Long-Term Conditions. *Antioxidants* 2024, *13*, 962. https://doi.org/10.3390/antiox130809626.3

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Article

The Dietary Inflammatory Index and Its Associations with Biomarkers of Nutrients with Antioxidant Potential, a Biomarker of Inflammation and Multiple Long-Term Conditions

Angela A. Mulligan [1,*], Marleen A. H. Lentjes [1,2], Jane Skinner [1] and Ailsa A. Welch [1,*]

[1] Centre for Population Health Research, Faculty of Health, University of East Anglia, Norwich NR4 7TJ, UK; maria.lentjes@oru.se (M.A.H.L.); jane.skinner@uea.ac.uk (J.S.)
[2] School of Medical Sciences, Faculty of Medicine and Health, Örebro University, 70182 Örebro, Sweden
* Correspondence: a.mulligan@uea.ac.uk (A.A.M.); a.welch@uea.ac.uk (A.A.W.)

Abstract: We aimed to validate the Dietary Inflammatory Index (DII®) and assess the cross-sectional associations between the DII® and multiple long-term conditions (MLTCs) and biomarker concentrations and MLTCs using data from the European Prospective Investigation into Cancer (EPIC-Norfolk) study (11,113 men and 13,408 women). The development of MLTCs is associated with low-grade chronic inflammation, and ten self-reported conditions were selected for our MLTC score. Data from a validated FFQ were used to calculate energy-adjusted DII® scores. High-sensitivity C-reactive protein (hs-CRP) and circulating vitamins A, C, E, β-carotene and magnesium were available. Micronutrient biomarker concentrations were significantly lower as the diet became more pro-inflammatory (p-trend < 0.001), and hs-CRP concentrations were significantly higher in men (p-trend = 0.006). A lower DII® (anti-inflammatory) score was associated with 12–40% higher odds of MLTCs. Lower concentrations of vitamin C and higher concentrations of hs-CRP were associated with higher odds of MLTCs. The majority of the associations in our study between MLTCs, nutritional biomarkers, hs-CRP and the DII® were as expected, indicating that the DII® score has criterion validity. Despite this, a more anti-inflammatory diet was associated with higher odds of MLTCs, which was unexpected. Future studies are required to better understand the associations between MLTCs and the DII®.

Keywords: multiple long-term conditions; MLTCs; multi-morbidity; MM; dietary inflammatory index; biomarker; validation; antioxidant

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1. Introduction

The Dietary Inflammatory Index (DII®) is a literature-based dietary score that was developed to measure the potential impact of diet on the inflammatory status of an individual [1]. The biological damage resulting from reactive oxygen species (ROS) is known as oxidative stress, which is induced by inflammation, and it results in a lowering of the antioxidant capacity of cells [2,3]. Diets rich in antioxidants, such as vitamins A, C and E, β-carotene, selenium, flavonoids and phytoestrogens, which are included in the DII® score, may potentially play an important role in modulating inflammation [4]. Antioxidants offer protection against a number of chronic conditions [5], including cancer [6], depression [7], cardiovascular disease [8], stroke [9] hypertension [10,11], type 2 diabetes [12–14] and obesity [14].

The National Institute for Health and Care Excellence (NICE) defines multiple long-term conditions (MLTCs), or multi-morbidity (MM), as the presence of two or more long-term health conditions in an individual [15]. These conditions can include defined physical or mental health conditions, such as type 2 diabetes or schizophrenia; ongoing conditions, such as learning disability; symptom complexes, such as frailty or chronic pain; sensory impairment, such as sight or hearing loss; and alcohol or substance misuse [15]. However, there is currently no international consensus on how to define and measure MLTCs, but a

number of recent reviews have attempted to progress towards reaching a more standardised approach [16–18].

The prevalence of MLTCs in ageing populations is increasing, leading to huge healthcare and personal costs. 'Inflamm-aging' is used to describe chronic low-grade inflammation that is characteristic of increasing age [19], which has been related to a number of chronic diseases, including cancer [20], cardiovascular disease [21], type 2 diabetes [22] and depression [23], that contribute to MLTCs. Research indicates that diets rich in antioxidants, such as β-carotene, vitamins A, C and E and magnesium, may play an important role in modulating inflammation. High-sensitivity C-reactive protein (hs-CRP) is a well-known inflammatory biomarker, and previous studies have reported that elevated concentrations of hs-CRP are associated with a higher risk of cancer and the incidence of other chronic diseases [20,24]. Lifestyle factors, such as diet, smoking and physical activity, can affect an individual's state of systemic inflammation [25–27], which has been shown to promote the development of diseases such as cancer [20], cardiovascular disease [21], type 2 diabetes [22], musculoskeletal conditions [28] and depression [23].

Using data for England, in 2015, 54.0% of people aged 65 and over suffered from MLTCs; by 2035, this is predicted to have risen to 67.8% [29]. The authors estimate that there will be 17.0% of people aged 65 and over living with four or more conditions in 2035, compared with 9.8% in 2015; by disease, most people aged 65 and over will be affected by arthritis (62.6%), hypertension (55.9%), respiratory disease (24.4%), cancer (23.7%) and type 2 diabetes (21.6%) [29]. In 2019, the UK spent GBP 50.5 billion on related long-term chronic conditions [30], making chronic diseases one of the major socio-economic challenges of our time. There are numerous adverse consequences of MLTCs; people will die prematurely [31] and have more hospital admissions, which will be of a longer duration [32]. Having MLTCs has an enormous effect on an individual's quality of life, tending to impact more on physical than mental health [33].

Given the relevance of inflammation to the development of MLTCs and the potential for a more antioxidant and anti-inflammatory diet to influence the onset and progression of MLTCs, research is lacking on the associations between the DII® and prevalence or onset of MLTCs. Furthermore, whilst the DII® has been validated or associated with circulating CRP concentrations [34], few studies have investigated the associations between clinical nutritional biomarkers of nutrient intake concurrently with the presence of MLTCs [35,36]. Additionally, as it is difficult to accurately measure dietary intake, we therefore chose to validate the DII® score against available concentrations of nutritional biomarkers, which are also DII® parameters, to establish criterion validity, as there is currently a paucity of data in this area.

Considering these findings, further exploration of the potential associations between the consumption of an inflammatory diet and MLTCs, supported by nutritional data and circulating CRP, are required. Therefore, using cross-sectional data, this study firstly aims to validate the DII® score against available nutritional biomarkers and hs-CRP. A secondary aim is to assess the associations between the DII® score and MLTCs, and a third aim is to investigate the associations between biomarker concentrations and MLTCs. We will additionally assess the associations between hs-CRP and nutritional biomarkers.

2. Materials and Methods

2.1. EPIC-Norfolk Study Design

The European Prospective Investigation into Cancer (EPIC) Norfolk study is part of the Europe-wide EPIC study, which has more than half a million participants from ten countries [37]. The EPIC-Norfolk cohort study was primarily set up to investigate diet and the risk of developing cancer, but its research interests widened to study additional lifestyle exposures and the causes of other chronic conditions and mortality [38].

2.2. Study Population

Men and women, aged between 39 and 79 years, were recruited from 35 general practitioners' surgeries located in the Norfolk region of East Anglia from 1993 to 1997. As the vast majority of the UK population is registered with a general practitioner's surgery through the National Health Service, the general practitioner's age sex registers are an ideal population-sampling frame. The Norfolk District Health Authority Ethics Committee granted approval for the study (98CN01), and all participants provided written, informed consent, adhering to the Declaration of Helsinki.

2.3. Assessment of Dietary Intake and Supplement Use

Dietary intake at the baseline examination was assessed using a semi-quantitative food frequency questionnaire (FFQ) consisting of a food list of 130 lines with an additional question on milk intake at the back of the questionnaire. This FFQ is designed to capture the average daily intakes of foods and drinks during the previous year. The EPIC-Norfolk FFQ has been extensively validated in this study population [39–41]. The FFQ data were calculated for nutrient contribution using the FETA (FFQ EPIC Tool for Analysis) tool [42] based on our earlier in-house system, CAFÉ (Compositional Analyses from Frequency Estimates) [43]. Outliers in energy intake were identified by using the ratio of energy intake (EI) to the basal metabolic rate (BMR), where the BMR was calculated using sex-specific Henry equations [44]. Participants in the top and bottom 0.5% of the EI:BMR ratio were excluded, as were those with FFQs containing 10 or more missing answers.

Intakes of foods and drinks from the FFQ were combined into crude food groups (expressed in grams). The food groups consisted of alcoholic beverages; grains and cereal-based products; eggs; fats and oils; fish and fish products; meat, including products and dishes; milk and dairy products; non-alcoholic beverages; nuts and seeds; potatoes; soups and sauces; sugars, preserves and snacks; and fruits, vegetables and legumes.

Participants who answered 'Yes' to the following question in the FFQ were classified as supplement users: 'Have you taken any vitamins, minerals, fish oils, fibre or other food supplements during the past year?'.

2.4. The Dietary Inflammatory Index (DII®)

This is a literature-derived, population-based DII®, whose purpose is to compare diverse populations based on the inflammatory potential of their diets [1]. Qualifying articles (N = 1943) were scored according to whether each dietary parameter increased (+1), decreased (−1) or had no (0) effect on six inflammatory biomarkers: IL-1β, IL-4, IL-6, IL-10, TNF-α and C-reactive protein. Articles were weighted by study characteristics, and using these weighted values, the pro- and anti-inflammatory fractions for each food parameter were calculated.

2.5. Creation of the DII®

Figure 1 illustrates the multi-step process required to create the DII® score. The DII® score was calculated using 37 dietary parameters. All of the pro-inflammatory parameters were included in the score: energy, carbohydrate, protein, total fat, saturated fat, trans fat, cholesterol, iron and vitamin B12. The anti-inflammatory parameters included alcohol, monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids, (PUFAs), n-3 fatty acids, n-6 fatty acids, fibre, pyridoxine (B6), folic acid, riboflavin (B2), thiamine (B1), niacin, vitamins A, C, D and E, β-carotene, magnesium, selenium, zinc, flavan-3-ols, flavonols, flavones, anthocyanidins, isoflavones, pepper, onion, garlic and green/black tea. The residual method was used to obtain the energy-adjusted intakes for all nutrients. For the DII® score calculation, dietary intakes were adjusted to a 2000 kcal/day diet to assess diet quality independently of diet quantity and to, in part, reduce measurement error, as energy intake is related to both under- and over-reporting of dietary intakes [45]. The most negative DII® score implies the maximum anti-inflammatory diet, while the most positive score implies the maximum pro-inflammatory diet.

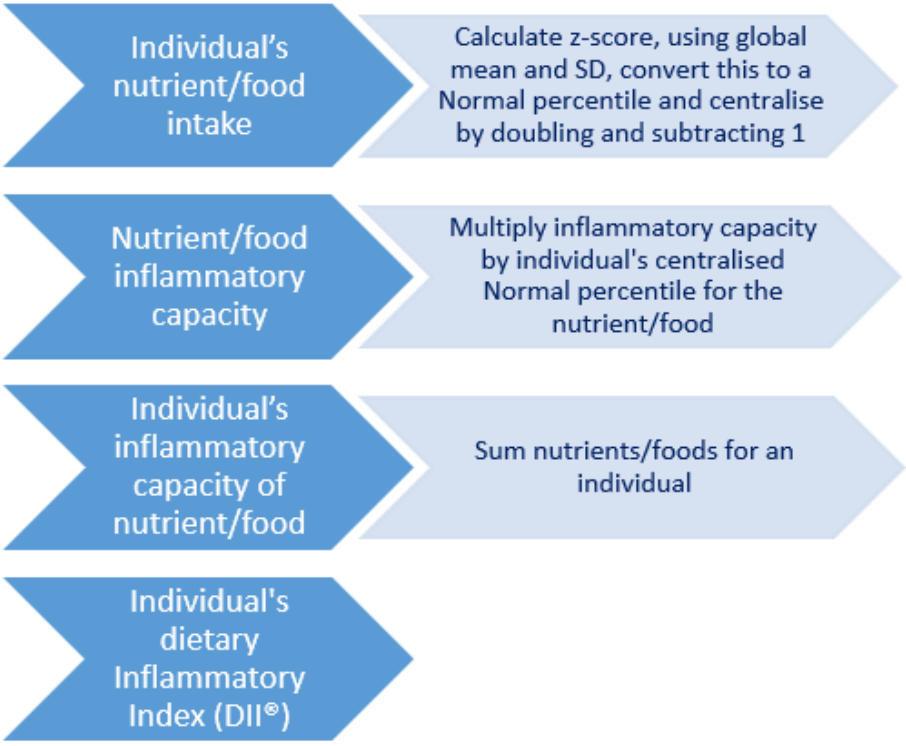

Figure 1. Steps in the creation of the DII® score by Shivappa et al. [1].

2.6. Blood Sample and Biomarker Analyses

A non-fasting blood sample was provided by 95% of participants at the baseline health examination. Blood was taken by venipuncture into plain and citrate monovettes. The blood was stored in a dark container overnight in a refrigerator at 4–7 °C and then spun at 2100× *g* for 15 min at 4 °C to obtain plasma and serum samples, which were stored at −196 °C.

Concentrations of vitamin A (retinol) and vitamin E, in the form of α-tocopherol, were available for a subset of the cohort (n = 6656) that consisted of previous nested case–control studies, where cases were defined by incident cardiovascular disease or cancer and four matched, disease-free controls [46]. Plasma concentrations were analysed at IARC, Lyon (France), using HPLC. Plasma vitamin E concentration was adjusted for cholesterol, as this is perceived to be a more reliable marker for vitamin E nutritional status [47,48]. The adjusted concentration is presented in μmol/mmol, calculated by dividing the plasma vitamin E concentration (μmol/L) by the total cholesterol concentration (mmol/L).

Plasma β-carotene concentration was available for 7495 participants selected from case–control studies nested within the EPIC-Norfolk study. Plasma samples were analysed for β-carotene concentrations by reversed-phase HPLC (HPLC-1100 system, Hewlett Packard) at IARC, Lyon (France), using a method based on that of Steghens et al. [49].

Concentrations of β-carotene and vitamins A and E were not used to investigate associations with MLTCs, as they came from nested case–control studies.

Approximately six months after the study had started, available funding enabled samples to be taken for vitamin C analysis using citrated plasma. Plasma for vitamin C was stabilised in a standardised volume of metaphosphoric acid, which was then stored at −70 °C. Plasma vitamin C concentration was determined using a fluorometric assay within one week of sampling [50].

Serum magnesium concentration was determined using blood samples that were prepared using a technique optimised for use in the EPIC study and stored in liquid nitrogen at −196 °C until analysed using an Olympus AU640 Chemistry Immuno Analyser (Quotient Bioresearch, Fordham, UK) to perform a xylidyl blue-based colorimetric assay (Beckman Coulter, Brea, CA, USA).

In 2008, previously frozen samples of serum collected were analysed for concentration of high-sensitivity CRP (hs-CRP) in 18,586 available samples using the AU640 Chemistry Immuno Analyser (Olympus Diagnostics, Watford, UK).

2.7. Calculation of the MLTC Score

Ten chronic conditions were selected to contribute to the MLTC score—myocardial infarction, stroke, type 2 diabetes, cancer, asthma, arthritis, depression, osteoporosis, hypertension and obesity—taking into account the most prevalent conditions included in the Quality and Outcomes Framework (QOF) of the UK General Practice [51]. The MLTC score was calculated by assigning one point for each condition, enabling a maximum score of ten.

Conditions were ascertained with the help of measurements taken (blood pressure, weight and height) or questionnaire data for the eight remaining conditions. At the baseline health examination, a trained nurse measured participants' weight (to the nearest 0.1 kg) using digital scales (Salter, Oldham, UK). Height was measured (to the nearest 0.1 cm) using a free-standing stadiometer. Participants wore light clothing and no shoes for both measurements. Body mass index (BMI) was calculated as the body mass (weight) divided by the square of the height and is expressed in kg/m^2. The body mass index (BMI) calculated using the measured height and weight at the baseline health examination was used to categorise the participants as underweight (<18.5 kg/m^2), normal weight ($\geq$18.5 to <25 kg/m^2), overweight ($\geq$25 to 30 kg/m^2) or obese ($\geq$30 kg/m^2).

A trained nurse took two measurements of systolic and diastolic blood pressures using an Accutorr sphygmomanometer with participants in a seated position after having rested for three minutes. The most appropriate cuff size was selected to consider the arm circumference, and the mean of the two blood pressure readings was used in the analyses.

A self-administered health and lifestyle questionnaire (HLQ) before the baseline examination provided data on the prevalence of a number of conditions. Participants were asked about their medical histories with the question "Has the doctor ever told you that you have any of the following?", followed by a list of conditions that included heart attack, stroke, type 2 diabetes, cancer, asthma, arthritis, depression, osteoporosis and hypertension. Where participants did not answer the question relating to any of the chronic conditions, it was assumed that they did not have the condition. The number of participants affected were as follows: heart attack (n = 31), stroke (n = 22), type 2 diabetes (n = 29), cancer (n = 22), asthma (n = 28), arthritis (n = 58), depression (n = 44) and osteoporosis (n = 50).

Participants were classified as having hypertension if they fulfilled any of the following criteria: measured systolic blood pressure $\geq$ 140 mmHg, measured diastolic blood pressure $\geq$ 90 mmHg, stated that the doctor had diagnosed them as having high blood pressure (hypertension) requiring treatment with drugs or reported taking anti-hypertensive medication [52].

We summed the number of chronic conditions per individual and created a binary variable, i.e., those with zero or one chronic condition and those with two or more conditions.

2.8. Measurement of Other Associated Variables

The HLQ, which was completed by participants just before the baseline examination, provided data to enable the categorisation of a number of variables. Social class at HLQ was defined using the Registrar General's occupation-based classification system. Non-manual occupations were represented by the following codes: I (professional), II (managerial and technical) and IIIa (non-manual skilled), whilst the codes for manual occupations were as follows: IIIb (manual skilled), IV (partly skilled) and V (unskilled) [53]. In this paper, these five classes were categorised into two groups, manual and non-manual, with a 'missing' third group for those who did not answer the question.

Educational status was based on the highest qualification achieved, which was categorised into four groups: degree or equivalent, A level or equivalent, O level or equivalent and less than O level or no qualifications. In our analyses, those with an educational status

of O level and above were combined into one category, and a 'missing' category was created for participants who did not answer the question.

Participants were categorised as either 'current smokers' if they currently smoked cigarettes, 'former smokers' if they were a smoker previously and 'never smokers' were those who had never smoked (derived from the HLQ). A 'missing' category was created for those who did not provide an answer to the question.

Usual physical activity was derived using data from questions in the HLQ, relating to occupational and recreational activity over the previous year. Using a simple index, participants were assigned to one of four groups: inactive, moderately inactive, moderately active and active [54–56].

2.9. Inclusion and Exclusion Criteria for Analysis

Figure 2 shows the numbers of participants available for analyses. In order to minimise data exclusions, missing data for a number of variables were treated in the following ways. A "missing category" was created for those with missing data on educational level, social class or smoking status (n = 16, 520 and 202, respectively). Data were available for analyses for 11,113 men and 13,408 women.

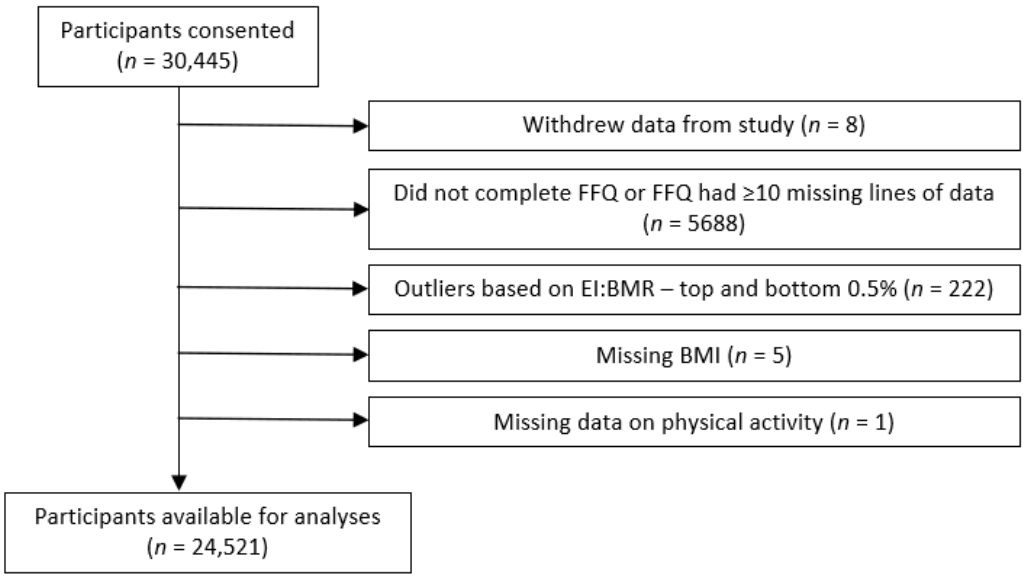

Figure 2. Study population included in analyses.

2.10. Statistical Analyses

All analyses were stratified by sex, as an independent *t*-test showed significant differences existed in the DII® score ($p < 0.001$) between men and women. $p < 0.05$ was considered to be statistically significant in the analyses. The analyses were performed with the Stata statistical software version 17.0 (Stata Corp., College Station, TX, USA). Our analysis strategy is best observed in Figure 3.

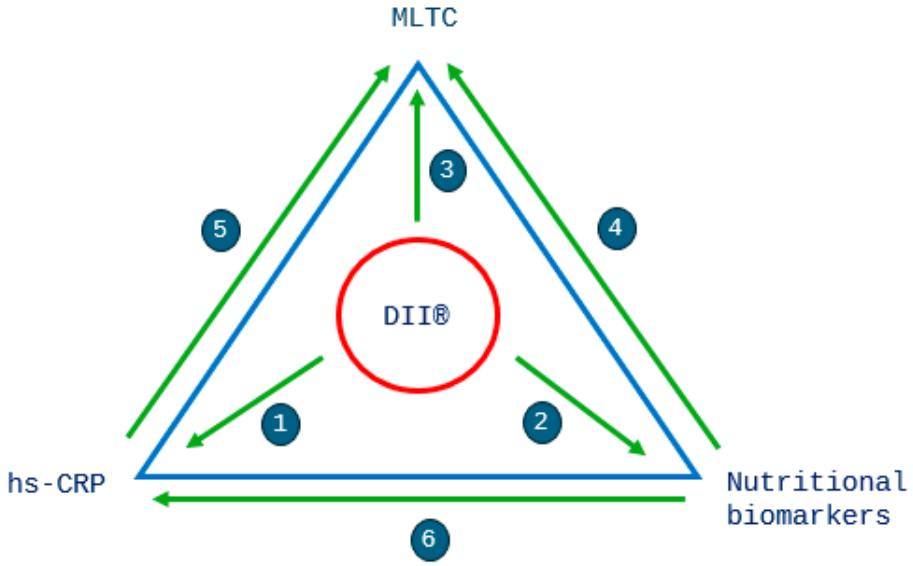

Figure 3. Overview of the research questions and analyses. The following associations were investigated: 1—DII® and hs-CRP; 2—DII® and nutritional biomarkers (vitamin C and Mg); 3—DII® and MLTCs; 4—nutritional biomarkers and MLTCs (vitamin C and Mg); 5—hs-CRP and MLTCs; 6—hs-CRP and nutritional biomarkers (β-carotene, vitamins A, C and E and Mg).

2.10.1. Descriptive Analyses

Descriptive statistics (means and SDs for continuous variables and frequencies and percentages for categorical variables) were analysed for all participants by sex-specific quintiles of the DII® score, adjusted for a 2000 kcal diet. Linear regression and the chi-squared test for trends were used to test for trends for selected continuous and categorical variables, respectively, across sex-specific quintiles of the DII® score. Where the percentage difference in biomarker concentrations between quintiles 1 and 5 is shown, this was calculated as $Q5 - Q1/Q1 \times 100$. Reported intakes of crude food groups are described to assess their contribution over the DII® spectrum. Food group data were not adjusted for energy intake.

2.10.2. Associative Analyses

Binary logistic regression was used to determine odds ratios (ORs) of having two or more MLTCs (as opposed to zero or one) for quintiles 1 to 4 of the DII® score (with quintile 5—most pro-inflammatory diet—as the reference category), using a series of cumulative adjustment models (Figure 3—research question 3). Model 1: unadjusted; model 2: adjusted for age; model 3: adjusted for age, smoking, physical activity, social class and educational level. ORs are presented with 95% confidence intervals (CIs). Trends in the results by DII® quintile were calculated by replacing the quintile number with the median values of the DII® within each quintile and modelling this as a continuous variable in the logistic regression [57].

To assess the association between the biomarkers and MLTCs, we used the same strategy as above (Figure 3—research questions 4 and 5). The highest concentrations (quintile 5) were used as the reference category. We assessed concentrations of hs-CRP and nutritional biomarkers across quintiles of the DII® score (Figure 3—research questions 1 and 2). Additionally, we investigated associations between hs-CRP and nutritional biomarkers (Figure 3—research question 6).

3. Results

Not having any of the ten conditions included in the MLTC score was reported by 3052 (27%) men, 4376 (39%) reported having one condition and 3685 (33%) reported having two or more conditions (see Supplementary Figure S1). Not having any of the conditions was reported by 3437 (26%) women, while 4711 (35%) reported having one condition and

5260 (39%) reported having two or more conditions. The mean (SD) of chronic conditions in men was 1.18 (1.01), and in women, it was 1.32 (1.10). In this study, 2.1% of men and 3.4% of women were classified as having four or more of the ten conditions included in our MLTC score. More than 50% of men and more than 40% of women had hypertension (Supplementary Figure S2), with the second most common condition being arthritis in both men and women. In both men and women, more than 10% reported being obese, and more than 10% of women reported depression.

3.1. Characteristics of the Study Population

Selected characteristics of men (n = 11,113) and women (n = 13,408) by quintiles of the DII® score, adjusted for a 2000 kcal diet, are shown in Table 1. The median DII® scores were lower (i.e., less inflammatory) in women than in men.

Mean age, weight and BMI were significantly lower in men as the diet became more pro-inflammatory (p-trend < 0.001), whereas in women, only mean weight and BMI were significantly lower if the diet was more pro-inflammatory (p-trend < 0.001 and <0.05 respectively). Men and women whose diet was classified as the most anti-inflammatory (Q1) had the highest usage of supplements, with a significantly lower supplement consumption observed with the consumption of a more pro-inflammatory diet (p < 0.001). In both men and women, the percentage of manual workers, those who had no qualifications, current smokers and those who were physically inactive was significantly higher with a more pro-inflammatory diet (p < 0.001).

3.2. Food Group Consumption

Additionally, we studied the associations between quintiles of the DII® score and the percentage contribution of weights of food groups. In both men and women, the intake of fruit, vegetables and legumes was lower with a more pro-inflammatory diet (see Supplementary Figure S3a,b). Intakes of milk and dairy products, non-alcoholic beverages, and sugars, preserves and snacks were generally higher as the diet was more pro-inflammatory. However, the gradients of intakes across the quintiles were generally small, with the greatest proportional differences between Q1 and Q5 observed for fruits, vegetables and legumes (−52% in men and −50% in women) and sugars, preserves and snacks (+136% in men and +180% in women).

3.3. Validation of the DII® Score

We studied the associations between the DII® score and directly measured inflammation (CRP) and nutritional biomarkers, vitamin C and magnesium, which were available for most of the study population, and β-carotene and vitamins A and E, available from nested case–control studies, to validate the obtained DII® score. We chose antioxidant nutritional biomarkers previously associated with diet as well as disease risk.

Table 2 shows that, in men, the mean concentrations of β-carotene, vitamin A, cholesterol-adjusted vitamin E, magnesium and vitamin C were all generally lower as the diet became more pro-inflammatory (p-trend < 0.001). In women, the mean concentrations of β-carotene, vitamin A, cholesterol-adjusted vitamin E and vitamin C were lower as the diet became more pro-inflammatory (p-trend < 0.001), but not magnesium. In terms of inflammation, the hs-CRP concentrations were significantly higher in men with increasing DII® quintile (p-trend = 0.006) but not in women (p-trend = 0.125).

Table 1. Selected characteristics of men and women by quintiles of the DII® score.

	Quintile 1: Most Anti-Inflammatory	Quintile 2	Quintile 3	Quintile 4	Quintile 5: Most Pro-Inflammatory	*p* Trend
MEN	n = 2223	n = 2223	n = 2222	n = 2223	n = 2222	
DII® range	−6.76 to −1.31	−1.31 to −0.02	−0.02 to 1.03	1.03 to 2.15	2.15 to 7.60	
DII®(median)	−2.24	−0.62	0.50	1.55	2.95	
MLTCs (n, %)	839 (38)	768 (35)	710 (32)	713 (32)	655 (29)	
Age (years)	60.5 (9.0)	60.1 (9.3)	59.4 (9.2)	59.5 (9.4)	58.9 (9.5)	<0.001
Weight (kg)	80.9 (11.2)	80.7 (11.4)	80.7 (11.2)	80.0 (11.4)	79.0 (11.5)	<0.001
BMI (kg/m^2)	26.6 (3.3)	26.6 (3.3)	26.6 (3.2)	26.5 (3.3)	26.2 (3.2)	<0.001
Supplement user, %	50	42	38	32	29	<0.001
Social class, %						<0.001
Non-manual	65	61	58	55	48	
Manual	33	37	40	44	49	
Missing	2	2	2	1	2	
Education, %						<0.001
No qualifications	25	27	29	32	38	
O level and above	75	73	71	68	61	
Missing	0	0	0	0	0	
Smoking status, %						<0.001
Current	5	7	11	15	22	
Former	58	58	55	53	48	
Never	36	35	34	32	30	
Missing	1	0	1	1	1	
Physical activity, %						<0.001
Inactive	27	28	31	34	33	
Moderately inactive	26	26	26	25	20	
Moderately active	24	23	23	22	24	
Active	24	22	20	20	23	
BMI, %						0.012
Underweight	0	0	0	0	0	
Normal weight	31	32	31	32	36	
Overweight	56	54	55	54	52	
Obese	14	14	13	13	12	

Table 1. *Cont.*

	Quintile 1: Most Anti-Inflammatory	Quintile 2	Quintile 3	Quintile 4	Quintile 5: Most Pro-Inflammatory	*p*Trend
WOMEN	n = 2682	n = 2682	n = 2681	n = 2682	n = 2681	
DII® range	−6.62 to −2.17	−2.17 to −1.02	−1.02 to 0.01	0.01 to 1.18	1.18 to 6.71	
DII® (median)	−2.95	−1.55	−0.50	0.55	2.08	
MLTCs (n, %)	1081 (40)	1073 (40)	1018 (38)	1045 (39)	1043 (39)	
Age (years)	59.1 (9.1)	58.8 (9.2)	58.7 (9.2)	58.7 (9.3)	58.9 (9.6)	0.569
Weight (kg)	68.6 (12.0)	68.1 (11.6)	67.8 (11.1)	68.1 (12.1)	67.1 (11.8)	<0.001
BMI (kg/m^2)	26.3 (4.3)	26.2 (4.3)	26.1 (4.0)	26.3 (4.5)	26.0 (4.4)	0.001
Supplement user, %	64	58	53	49	42	<0.001
Social class, %						<0.001
Non-manual	65	64	60	58	55	
Manual	32	34	38	39	42	
Missing	2	2	2	3	3	
Education, %						<0.001
No qualifications	35	39	40	45	50	
O level and above	65	61	60	55	50	
Missing	0	0	0	0	0	
Smoking status, %						<0.001
Current	6	7	9	13	20	
Former	36	34	32	30	29	
Never	58	58	58	56	50	
Missing	1	1	1	1	1	
Physical activity, %						<0.001
Inactive	25	27	30	32	36	
Moderately inactive	31	33	34	34	30	
Moderately active	24	24	22	21	21	
Active	21	16	14	13	13	
BMI, %						0.005
Underweight	1	1	0	1	1	
Normal weight	42	43	43	43	46	
Overweight	41	40	41	38	37	
Obese	17	17	16	18	16	

Values are mean ± SD unless specified otherwise. *p*-value for trend for continuous variables was calculated using linear regression; chi-squared test for trend was used for categorical variables (missing category excluded from trend analyses).

Table 2. Biomarker concentrations for men and women by quintiles of the DII® score.

	Quintile 1: Most Anti-Inflammatory	Quintile 2	Quintile 3	Quintile 4	Quintile 5: Most Pro-Inflammatory	Q5–Q1 Diff	% Diff	*p* Trend
MEN	n = 2223	n = 2223	n = 2222	n = 2223	n = 2222			
hs-CRP (nmol/L)	27.4 (51.2) (n = 1603)	27.7 (50.4) (n = 1609)	26.5 (45.2) (n = 1590)	27.5 (54.0) (n = 1622)	33.1 (72.7) (n = 1610)	5.7	20.9	0.006
β-carotene (μmol/L)	0.42 (0.25) (n = 761)	0.39 (0.25) (n = 737)	0.35 (0.22) (n = 727)	0.33 (0.18) (n = 741)	0.30 (0.18) (n = 707)	−0.12	−28.6	<0.001
Vitamin A (μmol/L)	1.87 (0.44) (n = 761)	1.86 (0.45) (n = 737)	1.84 (0.43) (n = 727)	1.82 (0.46) (n = 741)	1.77 (0.43) (n = 707)	−0.1	−5.4	<0.001
Vitamin E, adjusted for cholesterol (μmol/mmol)	4.56 (1.18) (n = 755)	4.42 (1.13) (n = 723)	4.39 (0.98) (n = 714)	4.31 (1.00) (n = 734)	4.02 (0.92) (n = 697)	−0.55	−12.0	<0.001
Vitamin C (μmol/L)	54.3 (17.5) (n = 1993)	50.9 (17.7) (n = 1973)	47.5 (17.6) (n = 1967)	44.2 (18.6) (n = 1981)	38.7 (18.8) (n = 1952)	−15.6	−28.8	<0.001
Magnesium (mmol/L)	0.82 (0.12) (n = 1602)	0.81 (0.12) (n = 1608)	0.82 (0.12) (n = 1591)	0.81 (0.12) (n = 1617)	0.81 (0.12) (n = 1611)	−0.001	−0.15	<0.001
WOMEN	n = 2682	n = 2682	n = 2681	n = 2682	n = 2681			
hs-CRP (nmol/L)	27.5 (48.2) (n = 1960)	29.4 (65.2) (n = 1997)	27.7 (56.5) (n = 2003)	30.7 (66.2) (n = 1976)	31.7 (62.5) (n = 1925)	4.2	15.2	0.125
β-carotene (μmol/L)	0.59 (0.35) (n = 693)	0.49 (0.27) (n = 696)	0.48 (0.29) (n = 723)	0.44 (0.28) (n = 704)	0.40 (0.23) (n = 701)	−0.19	−32.4	<0.001
Vitamin A (μmol/L)	1.78 (0.44) (n = 693)	1.80 (0.48) (n = 696)	1.75 (0.42) (n = 723)	1.72 (0.45) (n = 704)	1.70 (0.42) (n = 701)	−0.08	−4.3	<0.001
Vitamin E, adjusted for cholesterol (μmol/mmol)	4.64 (1.11) (n = 690)	4.50 (1.09) (n = 687)	4.45 (1.05) (n = 711)	4.37 (1.02) (n = 699)	4.14 (0.97) (n = 689)	−0.51	−10.9	<0.001
Vitamin C (μmol/L)	65.4 (18.6) (n = 2360)	62.1 (18.3) (n = 2351)	59.8 (18.4) (n = 2363)	57.0 (19.4) (n = 2323)	49.6 (21.2) (n = 2305)	−15.9	−24.3	<0.001
Magnesium (mmol/L)	0.79 (0.13) (n = 1959)	0.80 (0.12) (n = 1990)	0.80 (0.12) (n = 1998)	0.80 (0.12) (n = 1966)	0.80 (0.12) (n = 1920)	0.002	0.2	0.610

Values are mean ± SD. %diff = (Q5 − Q1)/Q1 × 100. *p*-value for trend for continuous variables was calculated using linear regression.

3.4. Associations between the DII® Score and MLTCs

Table 3 presents the results for the associative analyses between the DII® and MLTCs. The percentages of people with a chronic condition did not vary greatly across quintiles of the DII® score, nor did the percentages of each of the individual ten conditions (see Supplementary Figures S2 and S3, respectively).

In men, all three models indicated higher ORs of having two or more chronic conditions when the diet was more anti-inflammatory (p-trend < 0.001). The most anti-inflammatory diet had 45% higher odds than the most pro-inflammatory diet (model 1). The addition of age (model 2) slightly attenuated the associations in the lowest quintiles. Adjusting for additional factors (model 3) had minimal effect on the ORs.

For women, the unadjusted model 1 and model 2 showed no statistically significant associations for any quintile; also, the trend was non-significant (p-trend = 0.21 and 0.24, respectively). The addition of other covariates (model 3) increased the ORs proportionately more for the more anti-inflammatory diets, resulting in a significant trend (p-trend = 0.02).

Table 3. Odds ratios of having MLTCs by quintiles of the DII® score in men and women.

	Q1 (Most Anti-Inflammatory)		Q2		Q3		Q4		Q5 (Most Pro-Inflammatory)	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	***p* Trend**
MEN (n = 11,113)										
Model 1	1.45	1.28–1.64	1.26	1.11–1.43	1.12	0.99–1.28	1.13	0.99–1.28	1.00	<0.001
Model 2	1.35	1.19–1.54	1.20	1.05–1.36	1.10	0.96–1.25	1.10	0.96–1.25	1.00	<0.001
Model 3	1.40	1.23–1.60	1.22	1.07–1.39	1.11	0.97–1.26	1.09	0.95–1.24	1.00	<0.001
WOMEN (n = 13,408)										
Model 1	1.06	0.95–1.18	1.05	0.94–1.17	0.96	0.86–1.07	1.00	0.90–1.12	1.00	0.209
Model 2	1.06	0.94–1.19	1.06	0.95–1.19	0.97	0.87–1.09	1.02	0.91–1.14	1.00	0.243
Model 3	1.12	1.00–1.26	1.10	0.98–1.24	1.00	0.89–1.12	1.03	0.92–1.16	1.00	0.024

The outcome is having two or more chronic conditions. Q5 (most pro-inflammatory diet) is the reference category for the exposure. Model 1—unadjusted; model 2—adjusted for age; model 3—adjusted for age, smoking status, physical activity, educational level and social class.

Trend testing was achieved by replacing the quintile number with the median value of the DII® score in the respective quintile.

3.5. Associations between Nutritional Biomarker Concentrations, Inflammation and MLTCs

Figures 4 and 5 illustrate the associations observed between concentrations of nutritional biomarkers and inflammation and odds ratios of having MLTCs in men and women, respectively (research questions 2 and 3). Similar significant trends were observed for models 1 and 2; we therefore present the results for model 3 only (adjusted for age, smoking, physical activity, social class and educational level). In both men and women, higher ORs were observed of having two or more chronic conditions when concentrations of vitamin C were lower (p-trend < 0.001). In terms of inflammation, lower ORs of having two or more chronic conditions were associated with lower concentrations of hs-CRP (p-trend < 0.001) in both men and women. No significant associations were observed for magnesium in either men or women.

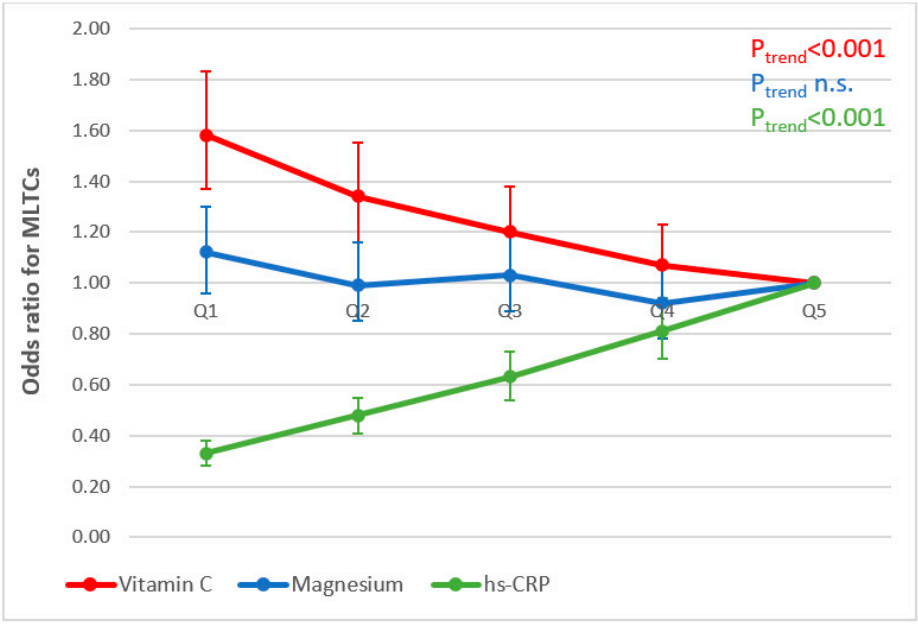

Figure 4. Associations between mean biomarker concentrations (quintiles, Q) and MLTCs in men (adjusted for age, smoking status, physical activity, educational level and social class).

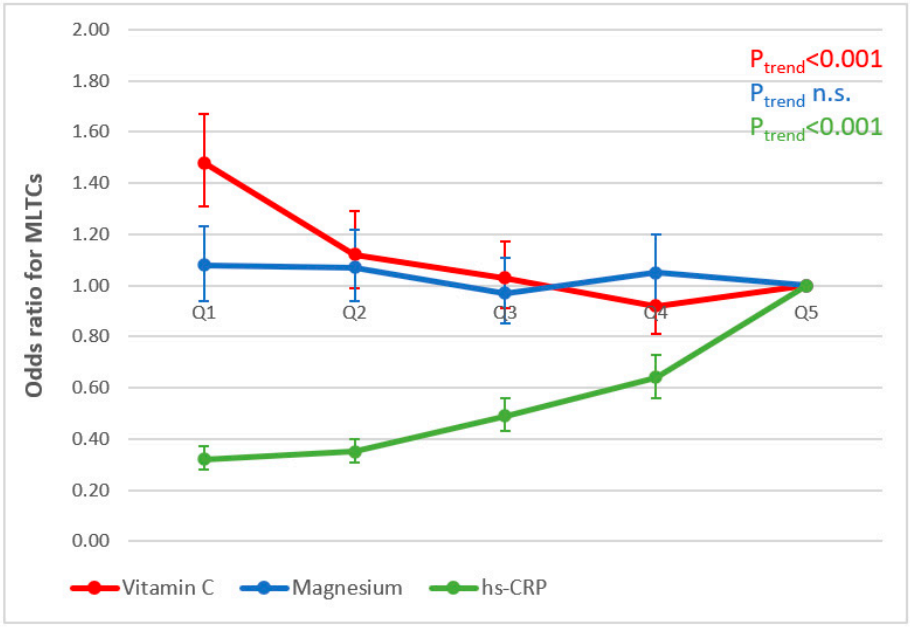

Figure 5. Associations between mean biomarker concentrations (quintiles, Q) and MLTCs in women (adjusted for age, smoking status, physical activity, educational level and social class).

3.6. Associations between hs-CRP and Nutritional Biomarkers (Research Question 6)

For every standard deviation higher in the biomarker concentration, the hs-CRP concentrations were observed to be lower, with exception of magnesium and vitamin E, where the hs-CRP concentrations were observed to be higher (see Table 4).

Table 4. Regression of hs-CRP on nutritional biomarkers *.

	MEN				WOMEN			
	N	**Mean (SD)**	**Exp (Coeff)**	**95% CI**	**N**	**Mean (SD)**	**Exp (Coeff)**	**95% CI**
β-carotene	2767	19.4 (12.1)	0.81	0.78–0.84	2642	25.5 (15.8)	0.75	0.72–0.78
Vitamin A	2767	52.6 (12.8)	0.90	0.87–0.94	2642	49.9 (12.4)	1.04	0.99–1.08
Vitamin C	7728	46.9 (18.6)	0.80	0.78–0.82	9499	58.7 (19.8)	0.81	0.79–0.83
Vitamin E	2725	4.35 (1.02)	1.07	1.02–1.12	2612	4.34 (1.06)	1.05	1.01–1.09
Magnesium	7678	0.81 (0.12)	1.17	1.14–1.20	9430	0.80 (0.12)	1.16	1.13–1.19

* hs-CRP was log-transformed. The nutritional biomarker concentrations were divided by their standard deviation. The value of 0.81 exp(coeff) for β-carotene in men, for example, can thus be interpreted as a 19% fall in the geometric mean of hs-CRP with a one-SD increase in β-carotene.

4. Discussion

We observed that a more pro-inflammatory diet was statistically significantly associated with higher hs-CRP, whilst circulating concentrations of β-carotene and vitamins A, C and E, anti-inflammatory and antioxidant vitamins, were lower. We also observed statistically significant higher ORs of having two or more chronic conditions when circulating concentrations of vitamin C were lower and lower ORs with lower concentrations of hs-CRP. Socio-economic and lifestyle factors, including social class, educational level, smoking status and physical activity, which are risk factors for chronic disease, were associated with the DII® score in the direction that was expected. However, a more anti-inflammatory diet was associated with higher odds of MLTCs, which was the opposite from what we had hypothesised. The findings from this study (summarised in Figure 6), using direct measures of status of nutritional antioxidants, β-carotene and vitamins A, C and E, and directly measured CRP, indicate that the DII® score has criterion validity for the inflammatory potential of diet in this population. However, the results relating to the DII® score and ORs for having MLTCs warrant further scrutiny and may in part be explained by the cross-sectional design of our research. It is plausible that participants suffering from a chronic condition before the start of the study may have increased their consumption of certain foods such as fruits and vegetables, reflecting a more anti-inflammatory diet, which may lead one to incorrectly conclude that a more anti-inflammatory diet is associated with disease.

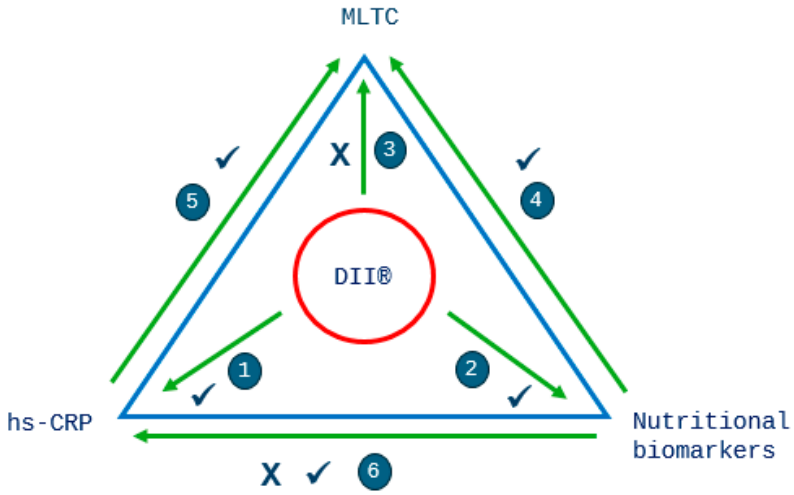

Figure 6. Summary of our findings in relation to the research questions, indicating expected and unexpected associations. Research questions: 1—DII® and hs-CRP (Table 2); 2—DII® and nutritional biomarkers (vitamin C and Mg) (Table 2); 3—DII® and MLTCs (Table 3); 4—nutritional biomarkers and MLTCs (vitamin C and Mg) (Figures 4 and 5); 5—hs-CRP and MLTCs (Figures 4 and 5); 6—hs-CRP and nutritional biomarkers (β-carotene, vitamins A, C and E and Mg) (Table 4). A ✓indicates an expected association and a X indicates an unexpected association.

We observed that a diet with greater inflammatory potential (a higher DII® score) was associated with higher hs-CRP concentrations. Results from cross-sectional studies on the association between the DII® score and CRP have been mixed, but a recent systematic review and meta-analysis shows that higher DII® scores are associated with a higher odds ratio of having raised plasma CRP levels [34]. Anti-inflammatory components of the DII® include unsaturated fatty acids, vitamins and minerals, a number of which additionally have antioxidant properties, consumed in foods such as fruits, vegetables, legumes and wholegrains. These foods are also important components of other healthy dietary patterns, such as the Mediterranean Diet, which has been shown to be associated with lower CRP concentrations in cross-sectional studies [58]. Circulating concentrations of vitamin C are widely recognised as a valid biomarker for the consumption of fruits and vegetables [59], and our findings that a more anti-inflammatory DII® score was associated with higher vitamin C concentrations and that lower concentrations of this antioxidant vitamin were related to higher MLTCs seem to support this. Data from the EPIC-Norfolk study have previously shown that higher concentrations of ascorbic acid (also when excluding supplement users) are associated with lower mortality [60]. Zhang et al. found that more frequent consumption of processed meat and poultry was associated with higher risks of MLTCs, whereas a higher intake frequency of total fish, fruits and cereal was associated with lower risks, in UK Biobank participants [61]. Less than 1% of total body magnesium is found in the blood, and under normal conditions, the body maintains tight homeostatic control of its concentration [62]. It is therefore unsurprising that we did not find any associations between magnesium concentrations and MLTCs.

We are unaware of previous studies that have investigated the DII® and MLTCs or direct measures of nutrient and inflammatory status (scoping review in preparation [63]), making our findings an important contribution to the literature on MLTCs, inflammation and diet. Ruel et al. found that a high consumption of fruit and vegetables and grain products other than rice and wheat could prevent the development of MLTCs in the Chinese population [64]. Protective associations have been found for higher fruit consumption and MLTCs in two cross-sectional studies in South Korea [65] and China [66]. Diets high in red meat and chicken were found to be among the main risk factors for MLTCs in middle-aged Australians [67]. Our observation that associations between the DII® score and MLTCs were non-significant or in the opposite direction from what we expected (when associations between biomarkers and MLTCs were observed in the hypothesised directions) may have several reasons. Firstly, although the DII® score parameters were classified into anti-inflammatory (e.g., vitamins A, C and E) or pro-inflammatory (e.g., saturated fats) [1], nutrients are seldom eaten in isolation. Moreover, the balance between the included nutrients and foods in the DII® score does not represent the balance in daily dietary habits. Secondly, dietary assessment methods, especially FFQs, are known for misreporting, thereby impacting on nutrient intake and potentially misrepresenting the proportions between food groups included in the DII® [68]. It is possible that there is a lack of capacity for the DII® score, measured using an FFQ in this population, to appropriately assess associations with MLTCs.

The major strengths of our study include its large population of community-living, middle-aged and elderly men and women and the availability of information on a large number of directly measured or self-reported chronic conditions that comprise the presence of MLTCs as well as factors associated with MLTCs, including age, smoking habit, physical activity, social class and education. The availability of concurrent direct measures of nutrition (β-carotene and vitamins A, C and E and magnesium) and inflammation are also a strength. Objectively measured height and weight at the same time-point, to enable the classification of obesity, are also an advantage. The capacity to establish criterion validity for relationships between the DII®, biochemistry and socio-economic factors are also a major strength.

The main limitations of our research include the self-reported measures for a number of variables, including dietary intake, physical activity and disease history (from which we

obtained the MLTC score). The self-reporting of chronic conditions lacked information on date of diagnosis, and time at risk for MLTCs could therefore not be assessed. Moreover, reverse causality may have played a role in our findings. Although dietary and anthropometric assessments, blood sampling and questions on medical history were collected concurrently, the absence of the date of onset of chronic conditions may have resulted in reverse causality. Participants may have changed their diet because they were unwell prior to entry to the study [69]. For example, participants suffering from a chronic condition some time before the study started may have increased their consumption of foods such as fruits, vegetables and fish, reflecting a more anti-inflammatory and antioxidant diet, which may lead to the incorrect conclusion that a more anti-inflammatory diet is associated with disease [69]. It is well established that participants who enrol in cohort studies are less likely to be disabled or seriously unwell, and this may impact the generalisability of our results [70]. Nevertheless, the data from the baseline examination show that this cohort was comparable to the UK national population for a number of characteristics, including age, sex and anthropometric measurements, but the cohort did have a lower percentage of current smokers [71].

In our study, the conditions contributing to the MLTC score were myocardial infarction, stroke, type 2 diabetes, cancer, asthma, depression, arthritis, osteoporosis, hypertension and obesity. We were unable to include certain conditions, such as chronic kidney disease, as this was not asked about in the HLQ. Since a large number of individuals had existing hypertension, arthritis or depression, this may have dominated our MLTC score. Seven of the conditions are a sub-set of the eleven that Diederichs et al. recommend should be included in MLTC indices [72]. Moreover, many of the most prevalent conditions listed in the Quality and Outcomes Framework (QOF) of the UK General Practice were included in the HLQ and thereby counted towards the MLTC score [51]. Dodds et al. recently compared the prevalence of MLTCs, defined using two-count and two-index approaches, using UK Biobank data and found a higher prevalence using the count than the index methods [73]. A recent study by MacRae et al. used English primary care data to investigate the impact of varying the conditions considered when measuring MLTCs [74] and recommend that researchers should consider using existing condition lists that are associated with the highest prevalence of MLTCs to enable comparisons across studies [75–77]. However, these researchers acknowledge that data availability may influence condition choice [74].

Although the DII® developed by Shivappa et al. [1] includes 45 food parameters, only 37 food parameters were included in our study. However, the missing food parameters likely make up a small proportion of the total nutrients consumed within our study population (e.g., eugenol, ginger, rosemary, saffron, turmeric), and despite these missing parameters, we did observe associations with diet-related biomarkers. Findings from a previous study have validated the association between the DII® score and circulating inflammatory marker concentrations, even when the number of available food parameters is limited [78]. Our data included three of the four flavonoid parameters, although previous research has shown that tea and fruits are the highest contributors to flavonoid intake in the UK, which are included in the score, either as a specific parameter or through a number of the vitamin components [79]. Isoflavone intake data were also included in the DII® score, even though intake in the EPIC-Norfolk population is low and therefore unlikely to have made an important contribution to the overall score [80].

Research has shown how inflammation may contribute to the development of a number of chronic conditions. Dysfunction of the endothelium, induced by inflammation, has been associated with CVD and hypertension [81] and has also been linked to the development of insulin resistance and type 2 diabetes [82,83]. Infections have been estimated to be responsible for approximately 15% of cancers worldwide via a number of mechanisms, including chronic inflammation [84]. There is also evidence that inflammasome-mediated pathways may be associated with depression, cognitive decline and dementia, including Alzheimer's disease [85]. A recent review found that age-related oxidative stress is potentially a contributing factor to the progression of a number of diseases, includ-

ing CVD, neurodegenerative diseases, cancer and arthritis [86]. The consumption of an anti-inflammatory diet rich in antioxidants would therefore seem beneficial.

Future research in this area should ensure that the presence of MLTCs is clearly defined, preferably using a more established and accepted consensus. Data on when a chronic condition was first diagnosed, in relation to the period of dietary data collection, must be available. More high-quality analyses are required to add to the limited evidence on this topic.

5. Conclusions

We found that higher inflammation, measured by direct measurements of hs-CRP, and lower concentrations of the antioxidant nutrients β-carotene and vitamins A, C and E were consistently significantly associated with higher odds of having MLTCs. Given this, our findings that a more anti-inflammatory diet was associated with higher odds of MLTCs were unexpected given the associations we found with biochemical and nutritional biomarkers. Possible explanations lie in the complexity of dietary habits and inter-relationships between nutrients not covered in the DII® score as well as methodological issues. However, based on the results from our analyses on biomarkers of diet and inflammation risk, the findings from our study show that the DII® score has criterion validity for the inflammatory potential of the diet in this population of middle-aged and old men and women. Future studies require better and concurrent capture of the individual conditions comprising MLTCs, as well as more discriminating methods for defining MLTCs, in addition to direct biomarkers of inflammation, in order to unravel how the anti-inflammatory potential of diet may help in preventing diseases of ageing.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox13080962/s1, Supplementary Figure S1. Percentages of men (A) and women (B) who reported having zero, one or two or more chronic conditions by quintiles of the DII® score; Supplementary Figure S2. Percentage of men (A) and women (B), who reported having any of the ten conditions, by quintiles of the DII® score; Supplementary Figure S3a. Percentage contribution of weights of food groups to total weight of food and drinks by quintiles of the DII® score in men; Supplementary Figure S3b. Percentage contribution of weights of food groups to total weight of food and drinks by quintiles of the DII® score in women.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Norwich District Authority Ethics Committee (98CN01).

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: The authors will make the dataset available under a Data Transfer Agreement to any bona fide researcher who wishes to obtain the dataset in order to undertake a replication analysis. Although the dataset is anonymised, the breadth of the data included and the multiplicity of variables that are included in this analysis file as primary variables or confounding factors means that provision of the dataset to other researchers without a Data Transfer Agreement would constitute a risk. Requests for data sharing/access should be submitted to the EPIC Management Committee (epic-norfolk@mrc-epid.cam.ac.uk).

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Conflicts of Interest: The authors declare no conflicts of interest.

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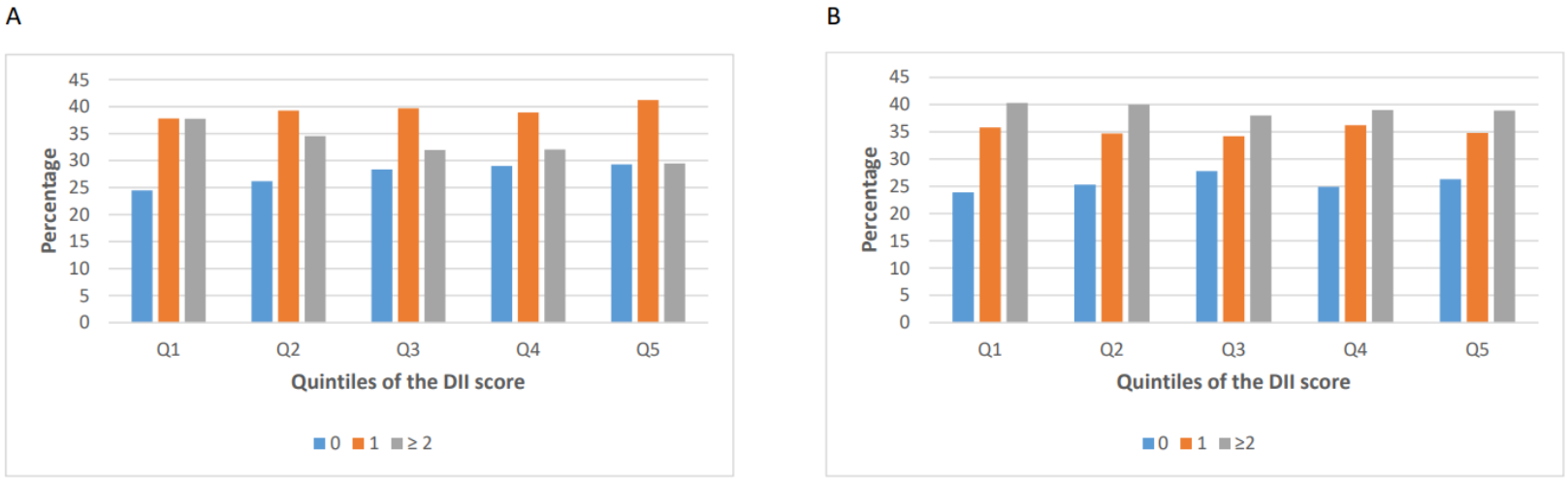
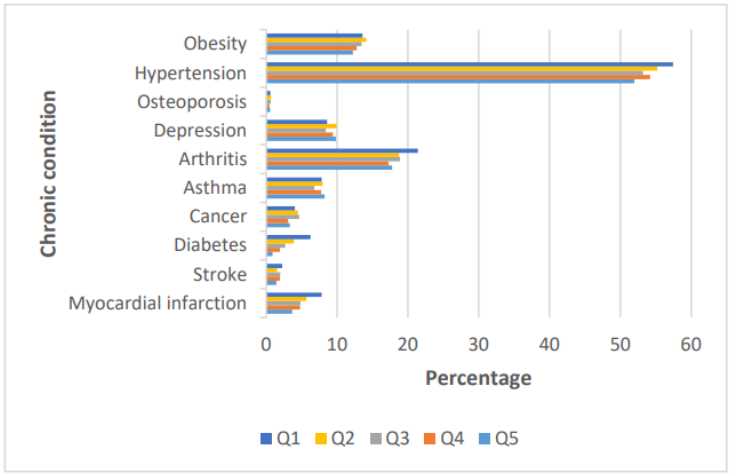
6.3 Supplementary material

(https://www.mdpi.com/article/10.3390/antiox13080962/s1)



Supplementary Figure S1. Percentages of men (A) and women (B) who reported having zero, one, or two or more chronic conditions by quintiles of the DII® score (a higher quintile category indicates a more pro-inflammatory diet).

A



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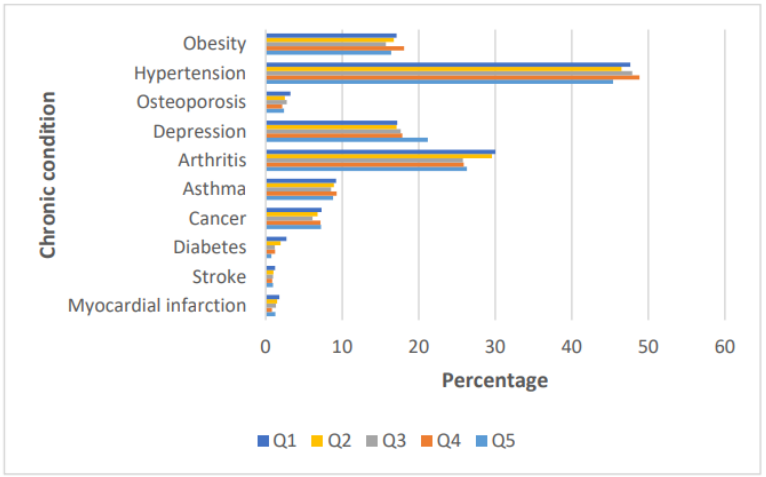
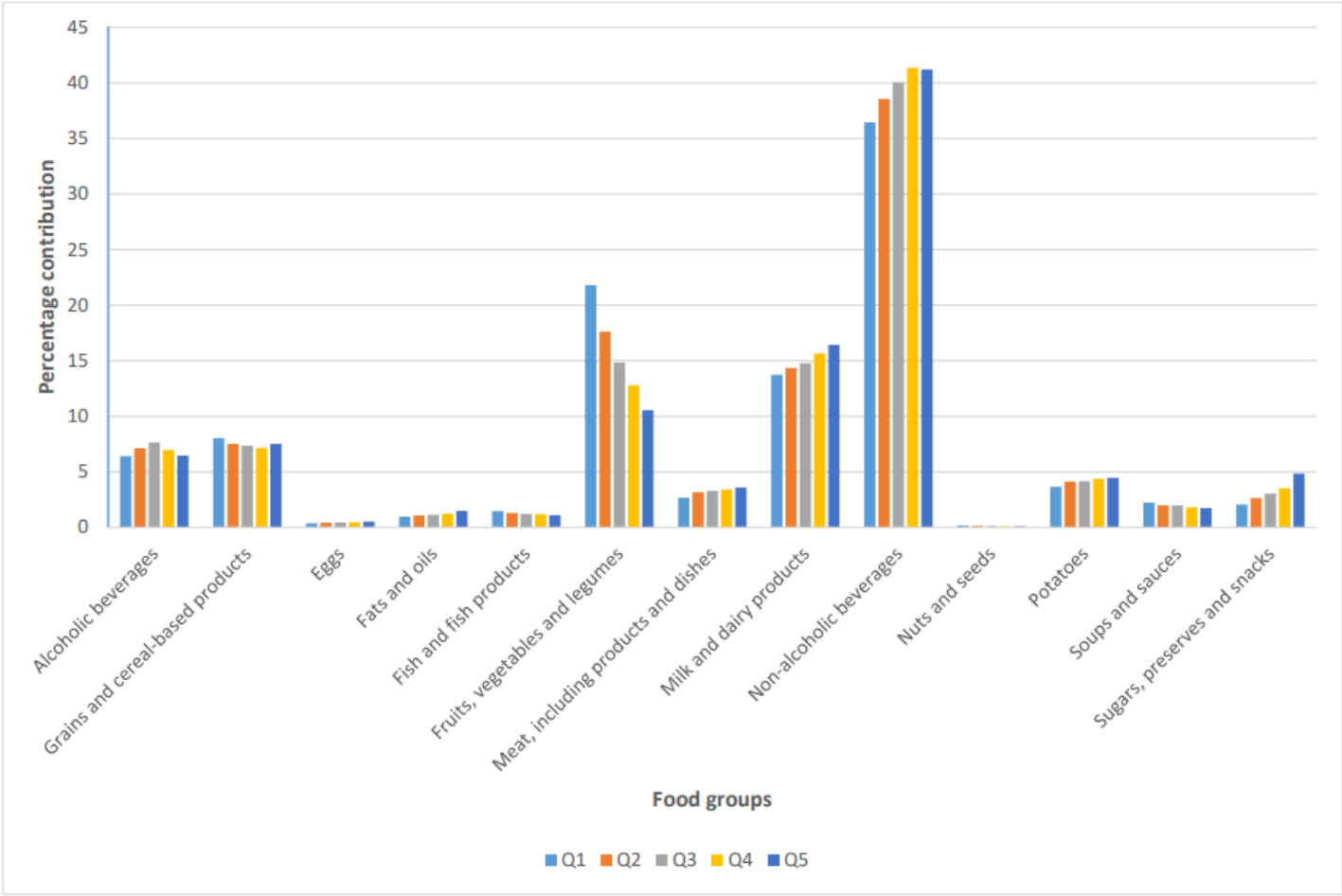
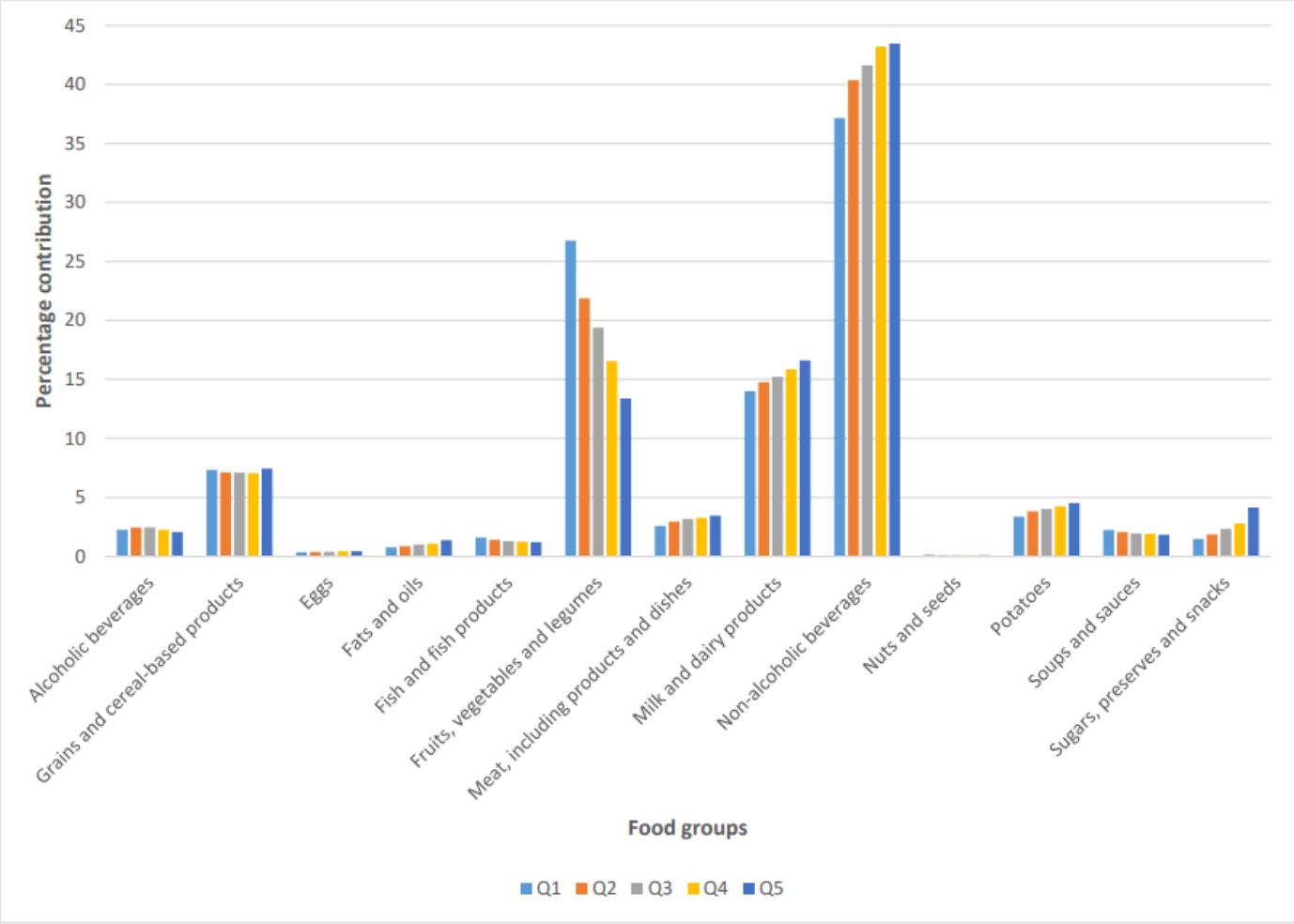


Supplementary Figure S2. Percentage of men (A) and women (B), who reported having any of the ten conditions, by quintiles of the DII® score (a higher quintile category indicates a more pro-inflammatory diet).



Supplementary Figure S3a. Percentage contribution of weights of food groups to total reported weight of food and drinks by quintiles of the DII® score in men (a higher quintile category indicates a more pro-inflammatory diet).



Supplementary Figure S3b. Percentage contribution of weights of food groups to total reported weight of food and drinks by quintiles of the DII® score in women (a higher quintile category indicates a more pro-inflammatory diet).

Chapter 7. Discussion

7.1 Chapter overview

The aim of this thesis was to investigate the impact of diet and changes in body composition on mortality risk, chronic conditions and inflammation, to ascertain how these lifestyle factors could benefit the ageing process. The rationale for this research stems from the growing percentage of older people in the UK (5), many of whom have one or more chronic condition, such as obesity, hypertension and depression (13), potentially linked to age-related inflammation (107). Furthermore, currently in England, heart disease, cancer and respiratory diseases are among the top five causes of mortality (26). The research papers included in this thesis used data from the EPIC-Norfolk cohort study of middle- and older-aged free-living men and women to study the impact of nutrition and changes in body composition on mortality risk, diseases of ageing and inflammation.

In this chapter, a summary of the findings from the four papers included in this thesis is presented. The strengths and limitations of this research are then discussed, followed by the significance and contributions of my research, in light of recent findings from the literature. I will also incorporate findings from studies that have been published in the last few years, relating to the overarching theme of my papers. Implications for public health and future research are then highlighted, followed by the overall conclusion.

7.2 Summary of findings

My research makes a valuable contribution to the field of healthy ageing, taking Hu's dietary-related components (3) and ABCD into consideration, by examining the impact of changes in weight and WC on mortality, and of diet on chronic conditions and inflammation, to ascertain how aspects of diet and anthropometry could contribute towards healthy ageing. The key findings from each of my papers are summarised in the following paragraphs, followed by a brief summary of my thesis in its entirety.

7.2.1 Analysis of the effects of weight change on long-term mortality (Chapter 3)

I assessed the associations between measured weight change over approximately four years and all-cause mortality, as well as mortality from CVD,

cancer and respiratory causes, in 12,580 middle-aged and older men and women. Findings showed that weight loss over this period, which was predominantly unintentional, was significantly associated with higher mortality over the next 15 years of follow-up. Both men and women who lost more than 2.5 kg had statistically significant higher hazards of all-cause mortality, and men who had a weight loss greater than 2.5 kg had statistically significant higher hazards of CVD mortality than those who maintained their weight, after adjustment for covariates. Weight loss over approximately 4 years was also associated with higher mortality during the next 15 years of follow-up in subgroups of the population, after stratification for age, smoking, BMI, physical activity and the exclusion of participants who said they had lost weight due to illness and deaths within the first 5 years of follow-up, as well as in dieters who reported losing more than 5 kg. Results for weight gain were inconclusive.

7.2.2 Analysis of the effects of changes in waist circumference and long-term mortality, and in relation to concurrent changes in weight (Chapter 4)

The study on waist circumference (WC) and changes therein builds on the investigations into weight change and mortality. Results from this study showed that a gain in WC of more than 5 cm over approximately 4 years was significantly associated with higher total mortality in both men and women and higher CVD mortality in men over the next 16 years of follow-up, after adjustment for covariates. Similar associations were also evident, particularly in men, after stratification for age, smoking, BMI, physical activity, educational level, social class and after the exclusion of deaths within the first 5 years of follow-up. Associations for a loss in WC and mortality were inconsistent. In analyses of concurrent changes in WC and weight, the greatest risk in men was observed with weight loss and a gain in WC for both all-cause and CVD mortality, whereas in women, the greatest risk for both all-cause and CVD mortality was found in those with weight loss and maintenance of WC. These differences observed between men and women may, in part, be explained by men being more likely to be apple-shaped, storing their fat around the midsection, whereas women tend to be pear-shaped, storing their fat in the gluteal-femoral depots.

7.2.3 Analysis of dietary intake and plasma concentrations of vitamin E with musculoskeletal health, and fracture risk during 18.5 years of follow-up (Chapter 5)

I continued my investigations into body composition, investigating the associations of dietary intake and circulating concentrations of vitamin E, with measures of SMM and bone density status. Dietary intake and circulating concentrations of vitamin E were also examined in relation to fracture risk during 18.5 years of follow-up. Lower SMM is a risk factor for sarcopenia, and it is also associated with osteoporosis and fracture risk. I showed that a higher dietary intake of α-tocopherol equivalents was significantly correlated with circulating concentrations of cholesterol-adjusted α-tocopherol in both men and women, in each of the three cohorts of SMM, BUA and fracture risk. Significant positive associations were observed between dietary vitamin E intake and circulating concentrations of both serum cholesterol-adjusted α- and γ-tocopherol and FFM and BUA, and mainly significant positive associations for fracture risk; a higher vitamin E intake was associated with a lower significant risk for total and hip fractures in both men and women ($p < 0.001$), but a higher significant risk was found for wrist fractures in women ($p < 0.01$). In both men and women, the risk for both total and hip fractures tended to display a downward trend for incremental quintiles of circulating α-tocopherol ($p < 0.05$). Chapter 5 in this thesis focuses on studying the associations of an antioxidant vitamin with musculoskeletal health, in an ageing population, where rates of loss of bone density and SMM are high. Additionally, weight loss in older age may indicate loss of muscle mass, as well as fat. As skeletal muscle is the organ with the highest consumption of oxygen in the body, vitamin E may potentially offer some protection regarding musculoskeletal health in middle-aged and older adults. Concentrations of vitamin E could conceivably decrease with weight loss, through a reduction in dietary intake.

7.2.4 Analysis of the Dietary Inflammatory Index with biomarkers and multiple long-term conditions (Chapter 6)

The use of a dietary score, in the form of the DII® score combining 37 dietary components, takes the analysis a step up from a single nutrient exposure, vitamin E, with musculoskeletal health, to a wide range and inter-relationships of diet with MLTCs. The prevalence of MLTCs, which is already high, is projected to rise with the

ageing world population. As MLTCs are associated with a poor quality of life, physical disability, depression, and premature death, in addition to high treatment costs, addressing the management of them is an important and growing area of research. 'Inflamm-aging' is used to describe chronic low-grade inflammation that is a common attribute of increasing age (59) and a high concentration of hs-CRP, an inflammatory biomarker, is associated with a number of chronic conditions.

Ten chronic conditions were selected to contribute to my MLTCs score, including conditions that were investigated in earlier chapters in this thesis, namely obesity, CVD and cancer. The results showed that a more pro-inflammatory diet was statistically significantly associated with higher hs-CRP, whilst circulating concentrations of anti-inflammatory and antioxidant vitamins, β-carotene and vitamins A, C and E, were lower. Significantly higher odds ratios (ORs) of having MLTCs were observed when circulating concentrations of vitamin C were lower, but lower ORs with lower concentrations of hs-CRP. Risk factors for chronic diseases, including social class, educational level, smoking status and physical activity, were associated with the DII® score in the expected direction, with the percentage of manual workers, those who had no qualifications, current smokers and those who were physically inactive found to be significantly higher with a more pro-inflammatory diet. However, a more anti-inflammatory diet was associated with higher odds of MLTCs, which was the opposite from what I had hypothesised. This may be partly due to the cross-sectional design of my research, with participants, who were suffering from a chronic condition before the start of the study, potentially having increased their consumption of certain foods such as fruits and vegetables, reflecting a more anti-inflammatory diet, which may lead one to incorrectly conclude that a more anti-inflammatory diet is associated with disease. Although our results regarding the association between the DII® score and MLTCs was in the opposite direction to what was hypothesised, I observed associations as expected between the DII® score and biomarkers and between biomarkers and MLTCs.

7.2.5 Summary of thesis findings

My research into the impact of diet and body composition on mortality risk, chronic conditions and inflammation, using anthropometric, biochemical, clinical and dietary data, has highlighted a number of behaviours in this population of middle- and older-aged men and women, which could benefit the ageing process. As weight loss of more than 2.5 kg, and a gain in waist circumference (WC) of more than 5 cm, were associated with higher mortality risk, achieving a healthy weight in early adulthood and maintaining it throughout life would seem advantageous. Since my investigations suggest that dietary vitamin E, an antioxidant and anti-inflammatory nutrient, has a protective role in musculoskeletal health and that a more pro-inflammatory diet is associated with lower micronutrient biomarker concentrations and higher concentrations of hs-CRP, consuming a diet that is sufficient in anti-inflammatory nutrients and optimal with regard to energy, to maintain body weight, would appear beneficial. However, as my analyses unexpectedly showed that a more pro-inflammatory diet was associated with lower odds of having MLTCs, further research is required to add to the limited analyses on this topic.

7.3 Strengths and limitations of my research

The EPIC-Norfolk study is a large, prospective, population-based cohort study, of more than 25,000 men and women, aged 39–79 in 1993–1997, recruited from general practitioners' surgeries. Data from the 1HE were representative of the UK national population for several characteristics, including age, sex, blood pressure and anthropometric measurements but the prevalence of smoking was lower in the EPIC-Norfolk cohort (108). Where I have used data from the 2HE for my investigations (Chapters 3, 4 and 5), the findings may have been affected by selection bias as a comparison of the characteristics of participants who attended both the 1HE and the 2HE, compared to those who attended the 1HE showed that the prevalence of self-reported CVD and cancer was lower in those who attended both HEs, as was the percentage of current smokers and physically inactive participants, illustrating that healthier individuals were more likely to return for a follow-up assessment.

A strength of my research is the availability of follow-up data on disease incidence and mortality through the use of health records linkage with the appropriate local and national organisations. This enabled me to assess the

associations of dietary vitamin E intake and incident fractures, as well as associations of changes in weight and WC and mortality. Three of the four studies used prospective data, which is a major strength, as they provide evidence to suggest causality: to assess (1) associations between weight change (Chapter 3) and (2) changes in WC and mortality (Chapter 4) and (3) associations between vitamin E and fracture risk (Chapter 5). Advantages of having measurements of weight and WC in the same participants at both HEs include increased statistical power, the ability to control for individual differences, and an increased confidence in the accuracy of the findings. The large population of free-living men and women and long follow-up time are also strengths of this research, enabling sensitivity analyses to rule out the potential effects on the results of including participants who die shortly after the start of the follow-up. Furthermore, weight, height and WC were objectively measured by trained nurses at both HEs, rather than self-reported by the study participants, providing confidence in the accuracy of these measurements. Additionally, information on a number of variables that are known to be statistically related to the dependent variables in my chapters had been collected, such as physical activity, social class and educational status.

Limitations of this research include the cross-sectional study design of two of the papers, regarding SMM and BUA measurements and their associations with dietary intakes and circulating concentrations of vitamin E (Chapter 5), and associations of the DII® and biomarker concentrations with MLTCs (Chapter 6), preventing me from inferring causation. However, consistency of findings from previous research, dose-response relationships, the use of biomarker data, the existence of plausible mechanisms and having adjusted for confounding factors support the existence of causal relationships. Another limitation is the use of self-reported measures in this thesis, for dietary intake, physical activity and disease history, affecting the reliability and validity of the data. 7dDD data were used to investigate associations between vitamin E intake and musculoskeletal health. However, the 7dDDs developed for use in the EPIC-Norfolk study have previously been validated and are expected to produce a more valid measure of dietary intake than 24hDRs or FFQs, though mis-reporting occurs (83). FFQ data were used to study associations of the DII® with MLTCs, which

also have issues regarding mis-reporting, and may therefore potentially misrepresent the proportions between food groups included in the DII® (109) and be unable to appropriately assess associations with MLTCs.

The dietary and lifestyle data used in the longitudinal analyses were collected at 1HE and I was unable to account for all changes in behaviours, including physical activity, which may have resulted in non-differential misclassification of participants, potentially attenuating the observed associations. Although, as alluded to earlier, study findings are likely to have been affected by selection bias, as healthier individuals were more likely to attend a follow-up health examination.

7.3.1. Proposed investigations to further explore unexpected or unclear findings from my research

My research found that weight loss was associated with higher mortality risk, but I am uncertain whether the weight loss itself was the cause, or if it was an early indicator of the presence of life-limiting diseases and / or age-related frailty, which were actually the cause of the weight loss. As some EPIC-Norfolk participants will have attended four or five health examinations, completing additional HLQs, and follow-up mortality data are available, analysing weight changes and mortality risk using subsequent health check data will help clarify my observations.

I also found that an increase in WC was associated with a higher mortality risk. However, my analyses do not definitively clarify whether the associations of weight loss and a gain in WC with higher mortality risk are evident in the same population or whether they are associated with distinct population groups. However, in analyses of concurrent changes in WC and weight, the highest mortality risk in men occurred with weight loss and waist circumference gain (WCG) for all-cause and CVD mortality, whereas in women, the highest risk for both all-cause and CVD mortality was observed in those with weight loss and maintenance of WC. For future research assessments of physical functioning measured in EPIC-Norfolk, carried out at the 3HE (2004 – 2011), the 4HE (2012 – 2016) and the 5HE (2016 – 2018), could be used to add to my research using longitudinal analyses. Additionally, at the 4HE, a total body iDEXA scan enabled the investigation of total body fat distribution (overall and regional fat and muscle mass), as well as providing data for total BMD. The availability

of these supplementary data, and further objective measurements of weight and WC, will enable future analyses of changes in both weight and WC, and sarcopenic obesity (SO), which is a growing health concern for older people, in relation to mortality risk.

My analyses, using cross-sectional data, showed that associations between the DII® score and MLTCs were non-significant or in the opposite direction from what was expected (when associations between biomarkers and MLTCs were observed in the hypothesised directions), potentially due to reverse causation. These unexpected findings could be further explored with the inclusion of data from subsequent HEs. The use of FFQ data from additional timepoints coupled with information on when participants were first diagnosed with a chronic condition, will enable investigations into associations between an anti-inflammatory diet and the odds of having a single chronic condition or MLTCs, in participants who are free from chronic diseases at the 1HE. Using simple counts of conditions when creating a MLTC score has limitations, if a large number of individuals have the same conditions, e.g. hypertension or arthritis, as they may dominate the MLTC score. Analyses from a carefully designed study using longitudinal data, minimising reverse causation, will help clarify my initial findings.

Another potential explanation for my unexpected finding that an anti-inflammatory diet was associated with higher odds of having MLTCs may in part be due to the nutrients and foods included in the score and their classification. Alcohol is classified as an anti-inflammatory nutrient in the DII® score, although alcohol can be both pro-inflammatory and anti-inflammatory, depending on the amount consumed and the length of time. The DII® score does not include sugar, refined carbohydrates or highly processed foods which are pro-inflammatory foods. Creating an alternative DII® score, adjusting for the aforementioned components, and re-analysing my cross-sectional data will show whether misclassifications have contributed to my unexpected observations.

7.4 Significance and contributions of my research and additional research in these areas

Here I will reflect on the significance of my papers, considering the latest publications and how these topics have evolved over recent years. I will also include

findings from studies that have carried out research encompassing the overarching theme of my thesis.

7.4.1 Recent findings relating to changes in weight and anthropometric variables and mortality

Research into the associations between weight change and mortality is ongoing. This topic continues to be of great significance as the global prevalence of overweight and obesity continues to increase (110), and the number of older people is growing (111). It has been recognised that comprehending the associations between weight change and mortality in older adults is paramount, because of the changes in body composition that come with ageing, such as increases in fat mass and WC and decreases in SMM, which is associated with sarcopenia (112). A recent systematic review and meta-analysis (search up to 11th June 2020) on the association of weight change and all-cause mortality in adults, aged 65 years and older, concluded that weight changes, either loss (hazard ratio (HR): 1.59; 95% confidence interval (CI): 1.45–1.74; $p < 0.001$), gain (HR: 1.10; 95%CI: 1.02, 1.17; $p = 0.01$) or fluctuation (HR: 1.66; 95%CI: 1.28, 2.15; $p < 0.001$), in community-dwelling older men and women were associated with a higher all-cause mortality risk compared to maintaining a stable weight (113). The review assessed the risk of bias, with 25 of the 35 studies rated as good, 2 as fair and 8 as poor.

Since the publication of this systematic review and meta-analysis, conflicting results on the associations between weight change and mortality in middle-aged and older adults continue to be reported. However, the most consistent observation from these recent studies is that weight change in middle-aged and older participants is associated with a higher all-cause mortality risk, with generally stronger associations observed for weight loss than for weight gain. Associations between weight loss and mortality risk from all causes have been reported in European (114,115), American (116,117), Australian (117) and Asian (118–123) populations. Recent studies have also reported significant associations for weight loss and a higher mortality risk from CVD (114,115,117,118,121,123–126), ischaemic heart disease (IHD) (123–125), stroke (123,124), respiratory diseases (118,123), cancer (114,117,121,123) and other causes (117,123). Although I did not find any significant associations between weight

gain and all-cause mortality in the EPIC-Norfolk cohort population, a number of these studies have also shown that weight gain is associated with a higher risk of all-cause mortality (114,118,119,121–123) and mortality from CVD (114,118,123,125), IHD (123,125) and cancer (114,119,123). A few studies from Asia have observed that weight fluctuation or cycling is associated with a higher all-cause mortality risk (127–129) and a higher risk for mortality from IHD (127), digestive systems (129) and other causes (128,129). However, Mehran *et al.* observed that the greatest variability in weight, assessed using BMI, was protective against mortality from CVD (128). Kwon *et al.* found that participants who continued to lose weight after two years had an even higher all-cause mortality risk, whereas those who regained weight after two years had a lower mortality risk (123).

Although these studies are not without their limitations, the existing evidence suggests that weight change in middle-aged and older participants may not be beneficial, and that maintaining weight during these later stages of life might be the most prudent strategy. Comparisons between studies are difficult due to certain limitations and differences in methodology, including

- small sample sizes, especially in stratified analyses
- self-reported versus measured weights
- time interval over which the change in weight occurred
- categorisation of weight change and weight stability
- inclusion of participants with certain conditions
- whether weight loss was intentional or unintentional
- adjustment for confounding factors and changes in these variables over time.

Additionally, whether findings relating to weight loss are from community-dwelling or institutionalised individuals is also an important consideration.

I assessed associations between weight change and mortality in participants who said that they had lost weight due to dieting and observed that those who said that they had lost more than 5 kg due to dieting during the 5 years prior to the 2HE (N=1044), 29% of them gained weight between the health examinations. It is possible

that during this time, they did lose weight but then regained it, and possibly more, but I was unable to verify this. Findings from my research showed that a higher mortality risk was found in dieters who lost weight, although this was not significant, as well as in those gained between 5 and 10 kgs (HR:1.92; 95% CI: 1.14–3.23), although the low number of participants in the various weight change categories must be highlighted (130). Having objectively measured weights, rather than self-reported data at both health examinations was essential to study this association. Assessing associations between objectively measured weight change and mortality in participants who said they had lost weight due to dieting demonstrates originality of research in this area and highlights the methodological difficulties due to reporting/information bias.

Three of the aforementioned studies on weight change and mortality risk additionally investigated the associations between changes in WC with mortality risk and observed that a decrease in WC was significantly associated with a higher risk for all-cause mortality (117,119,122), CVD mortality (119) and cancer mortality (117). These studies were carried out in healthy middle-aged and/or older adults, and adjustment for potential confounders and sensitivity analyses provide confidence in their findings, although two of the studies had small numbers of deaths, possibly due to the relatively short follow-up period (117,119). A plausible explanation for the observed associations between a decrease in WC and a higher mortality risk is that a loss in WC may indicate the presence of an undiagnosed condition, which is a risk factor for mortality. No associations were found in two of the studies between mortality risk and weight gain, but Yuan *et al.* observed a higher mortality risk for a gain in WC (122). Findings from a recent study in an older-aged Chinese population found that a gain in WC is associated with a higher CVD mortality risk, except in those with a high baseline WC, whereas a decrease in WC was only associated with a lower CVD mortality risk in those with a moderate-high baseline WC (131); findings for associations between changes in WC and all-cause mortality varied by baseline WC but significant associations were found for both losses (men and women) and gains (men only) in WC. I investigated associations of changes in WC by baseline WC and all-cause mortality risk and observed that only in women with a very high baseline

WC, was a significantly higher all-cause mortality risk with a gain in WC of > 5 cm observed but that a WC gain of > 5 cm was significantly associated with a higher all-cause mortality risk in men, irrespective of baseline WC. I did not find any significant associations between losses in WC and mortality risk in the multivariate-adjusted models, in either men or women, indicating the importance of preventing an increase in central adiposity in older age.

My work reaffirms that weight loss in later life is associated with a higher mortality risk and that monitoring changes in both weight and body composition, such as WC, are essential for assessing morbidity and mortality risks, including whether weight changes are intentional. However, as weight loss and accompanying subsequent changes in body composition may be a prodromal sign of the presence of diseases which may reduce longevity, it is possible that it is undiagnosed, pre-existing disease which is associated with a higher mortality risk. Whether weight loss in older age is a confounder or a mediator with regard to subsequent higher mortality risk, regular monitoring to identify unintentional changes are necessary to diagnose potential health conditions that may require treatment.

7.4.2 Changes in weight and anthropometric variables and chronic conditions, including fracture risk

A recent study used evidence from a UK primary care database to investigate the associations of intentional weight loss and 10 obesity-related conditions in 0.5 million adults aged > 18 years, finding that weight loss showed the greatest benefits for hypertension, type 2 diabetes mellitus, dyslipidaemia, chronic kidney disease and sleep apnoea (132). Results for heart failure, atrial fibrillation and unstable angina/myocardial infarction were inconclusive. The certainty of intentional weight loss was a strength in this study, although the authors did not provide data by sex or age categories, to ascertain if associations differed.

Although gaining weight results in an increase in BMD, this increase in BMD is associated with a higher fracture risk, due to bone fragility and an increased risk of falls, according to a recent review (133). Ensrud *et al.* observed that women aged ≥ 65 years who lost weight had a higher risk of bone loss and hip fracture, irrespective of their current BMI or whether or not they were trying to lose weight (134). This

study provides further evidence of the importance of monitoring weight change, particularly weight loss, in older age. Zahedi *et al.* recently carried out a systematic review (23 papers) and dose-response meta-analysis (21 papers) of prospective cohort studies to assess the associations of abdominal obesity with bone fractures in adults aged ≥ 18 years, observing that abdominal obesity was significantly associated with a higher risk of hip and vertebral fractures (135) and that a 10 cm increase in WC was associated with a 3% higher risk of vertebral fracture. The paper did not present data for younger and older participants separately, but the findings also suggest that monitoring changes in abdominal indices is important for bone health.

Other recent studies have focussed on associations of changes in WC and morbidity. An increase in WC was associated with increased disability in 8,000 Indonesians, aged ≥ 50 years whereas a decrease in WC in non-obese women was associated with more disability (136), where disability refers to difficulties in daily physiological, personal and social functioning. This study also showed that with an increase in WC, disability increased considerably with age and in those who self-reported having a chronic disease; the main shortcoming of this research was the absence of data on BMI.

Ma *et al.* observed that WC gain over approximately eight years was significantly and positively associated with a higher risk of chronic obstructive pulmonary disease (COPD) in more than 8,000 Chinese adults, aged 18 years and older; both men and women with abdominal obesity at follow-up had a higher risk of having COPD irrespective of whether or not they were classified as having abdominal obesity at baseline (137). The risk of COPD risk was significantly lower among men with a WC loss of more than 2.5%. This was a well-designed study with the exclusion of participants at baseline who had an existing lung disease, asthma or asthma-like symptoms, diabetes and cancer and adjustment for related covariates, including forced expiratory volume in one second and forced vital capacity at baseline (137).

Another study in 2,900 Chinese adults, aged 65 years and older, observed that those who gained WC and those who had a high WC at both baseline and follow-up had a higher risk of developing MLTCs (2 or more out of 18 chronic conditions) (138). This study also found that changes in WC, waist-to-height ratio (WHtR), and a

relatively new anthropometric index, the weight-adjusted-waist index (WWI), calculated as WC (cm) divided by the square root of weight (kg) (cm/√kg), in this older-aged population were associated with a higher risk of MLTCs. Stratified analyses by a number of covariates, including age, sex, education level and physical activity showed that these factors did not significantly modify any of the associations between WC or WHtR and MLTC risk.

The above studies reinforce the importance of monitoring changes in weight and body composition in middle- and older-age.

7.4.3 Recent findings relating to vitamin E and musculoskeletal health

There have been a few publications on associations between both dietary and circulating concentrations of α-tocopherol and musculoskeletal health since my research, suggesting that higher dietary intakes of vitamin E confer protection. However, Niu *et al.* found that concentrations of α-tocopherol were negatively associated with femoral neck strength in 878 middle- and older-aged Americans (139). However, the authors only adjusted their data for age, sex and BMI and not for other potentially confounding variables such as disease history, menopausal status or the use of supplement or relevant medication, which may have impacted their results.

Inconsistent findings from analyses of associations between vitamin E and BMD using NHANES data have recently been published in two papers, although one used the sum of α- and γ-tocopherol concentrations, whereas the other used α-tocopherol concentrations in their analyses. Peng *et al.* observed that higher serum vitamin E (sum of α- and γ-tocopherols) concentrations were associated with lower BMD and positively correlated with femoral BMD in 378 middle- and older-aged men and women; additionally, the authors noted that vitamin E was better at maintaining normal BMD in people with normal BMI (< 25kg/m^2) than in those with a BMI ≥ 25kg/m^2 (140). No associations between circulating concentrations of α-tocopherol and BMD were observed by Zhang *et al.* in 2757 NHANES participants, aged ≥ 20 years, but findings from weighted quantile sum regression and principal component analysis methods showed a negative association between co-exposure to fat-soluble vitamins and BMDs, which was mainly driven by vitamin E, suggesting that vitamin E

may have a damaging effect on bone health (141). Some of the differences in findings from the above two studies can potentially be explained by the age of the participants, the type(s) of vitamin E investigated, analyses used, and variables used for adjustment. Taking these factors into consideration, I find the results from Peng *et al*. to be more robust. In my paper, mean BUA measures tended to be significantly higher across quintiles of both cholesterol-adjusted plasma α- and γ-tocopherols.

Results from a Mendelian randomization investigation using data from the UK Biobank study suggest that higher circulating α-tocopherol levels were associated with greater BMD (142). A strength of a Mendelian randomization study is a reduction in potential confounding and reverse causation bias, thereby resulting in more reliable data on the causal inference in the association between circulating α-tocopherol and BMD.

Out of the four aforementioned studies (139–142), only Michaëlsson and Larsson (142), using data from the UK Biobank study, adjusted the circulating concentration of α-tocopherol for blood lipids. I did adjust concentrations of α-tocopherol for total cholesterol in my analyses when investigating its associations with musculoskeletal health, as this is seen as a more reliable marker for vitamin E nutritional status as tocopherols are transported via the circulation through lipoproteins (79). Comparing findings from studies where the blood concentration of α-tocopherol has not been adjusted for cholesterol is not ideal, nor is the comparison of associations where α-tocopherol has and has not been adjusted for cholesterol or concentrations for α- and γ-tocopherols have been combined.

Findings from a study of 153 Koreans, aged 50 – 80 years, observed a significant association between femur BMD, measured using DEXA, and vitamin E intake, although the dietary intake was from a single 24hDR; additional analyses suggested that sufficient intakes of the antioxidant vitamins C (100 mg/day) and E (12 mg α-tocopherol equivalents/day) are important for maintaining BMD and lean mass (143). Data from this Korean study, with a small study size and the estimation of α-tocopherol intake from one 24hDR raises questions regarding the reliability of their findings. Otsuka *et al.* observed that higher dietary intakes of α-tocopherol were associated with a lower prevalence of sarcopenia in a community-dwelling older

Japanese population (n = 1345, aged ≥ 60 years) (144). In this study, a self-administered brief diet history questionnaire was used to estimate the dietary intake frequency of 56 foods and beverage items during the previous month to calculate the daily intake of energy and selected nutrients using a specific computer algorithm. The estimated intake of α-tocopherol may not be very accurate if participants were unable to quantify the portion sizes of their foods and beverages.

In my analyses in Chapter 5, I have shown that adjustment for the amount of vitamin E from supplements did not affect the associations. However, Vallibhakara and colleagues, in a double-blinded, randomized, placebo-controlled trial study, observed that vitamin E supplementation in postmenopausal, osteopenic women may have a preventive effect on bone loss (145).

Given the existing evidence, including my research into the associations of dietary intakes and circulating concentrations of α-tocopherol with musculoskeletal health, α-tocopherol seems to confer some protection, potentially through its antioxidant and anti-inflammatory properties.

7.4.4 Vitamin E and healthy ageing

A recent review by Traber highlights the important role of vitamin E in healthy ageing, suggesting its ability to confer protection against chronic diseases, potentially through its antioxidant and anti-inflammatory properties (146). Ciarcia *et al.* recently reviewed associations between vitamin E concentrations, dietary intake and/or supplementation, and the main non-communicable diseases, including diabetes mellitus, asthma, CVD and four common cancers (breast, lung, colorectal and prostate) (147). They conclude that the conflicting evidence in the literature regarding the potential beneficial effects of vitamin E against chronic conditions may be due to a number of factors, including the high heterogeneity of many diseases, the presence of different isoforms of vitamin E with their differing biological actions on the body, as well as differences in inter-individual metabolic activity. Additionally, antioxidant systems in humans are multiple, complex and often integrated, and therefore the incorporation of vitamin E alone may not be adequate to restore the physiological redox balance. It is important to also consider the importance of the role played by the intestine and gut microbiota in the processes of absorption, transformation, and

subsequent transport of the different isoforms of vitamin E. In my paper investigating associations of vitamin E with musculoskeletal health, I used both dietary intakes (α-tocopherol equivalents) and available data on circulating concentrations (α- and γ-tocopherols) of vitamin E, which I adjusted for total cholesterol, in an attempt to deal with some of the known issues in this area of research.

Middle- and older-aged women without MLTCs participating in the United Kingdom Women's Cohort Study had significantly higher intakes of vitamin E, expressed in μg/MJ, than those with either one or two or more conditions (148), suggesting that vitamin E may offer some protection against the development of chronic conditions. Although in multivariate analyses, borderline significant associations were found across quintiles of vitamin E intake and MLTC risk (*p*-linearity = 0.058), with higher intakes associated with a lower risk of MLTCs, this research in women shows that the anti-inflammatory and antioxidant properties of vitamin E may be beneficial in delaying the onset of chronic conditions and that further studies to assess potential associations of MLTCs and both intakes and circulating concentrations of vitamin E in older adults are warranted, and to investigate if associations are dependent on BMI.

In a study of older Koreans, higher circulating concentrations of α and the sum of β- and γ-tocopherols were associated with a lower risk of cognitive impairment, although the findings were only significant for the sum of β- and γ-tocopherols (149). However, this was a small study of 230 participants and the authors did not adjust the serum vitamin E concentrations for blood lipids. Mangialasche *et al*. found that higher levels of tocopherol and tocotrienol forms were associated with a lower risk of cognitive impairment in older Finnish adults (150). These researchers measured all eight forms of vitamin E, adjusting their concentrations for cholesterol and a number of potential confounders; the main limitation of this study was the small sample size of 140 participants. Significantly higher risks of new-onset hypertension were found among the general Chinese population in the lowest (< 18.75 mg/day) and highest (≥ 40.53 mg/day) quintiles of energy-adjusted vitamin E intake (sum of α-, β, γ- and δ-tocopherols) compared with those in the second–fourth quintiles, with the highest risk observed in the lowest quintile (151). This was a large study of more than 12,000

adults, with stratified analyses showing a stronger association in older (≥ 60 years) versus younger participants in the lowest quintile of vitamin E intake, compared with those in the second–fourth quintiles.

Additionally, findings from a large, prospective cohort study of more than 29,000 Finnish male smokers, aged 50 – 69 years at baseline, observed that higher serum concentrations of α-tocopherol were significantly associated with moderately lower all-cause mortality, as well as deaths from CVD, cancer and other causes, independent of several potentially confounding variables (152). Zhang *et al.* recently reviewed 27 articles with 28 health outcomes, regarding vitamin E intake, and observed that higher intakes of vitamin E intake were associated with a lower risk of certain cancers, CVD, Parkinson's disease, depression, age-related cataracts and fracture (153). However, most of the data came from observational studies, and the quality of the evidence was generally defined as low or very low, with only stroke, age-related cataracts and obesity identified as having a moderate level of quality. The vitamin E intake from the studies included both dietary and supplemental intakes and the form of the dietary intake was unclear.

Findings from the above research indicate that vitamin E seems to be considered a beneficial micronutrient with regard to healthy ageing, possibly through its antioxidant and anti-inflammatory properties.

7.4.5 Recent findings relating to the consumption of an inflammatory diet and MLTCs

One of the main limitations of existing research into associations between the consumption of an inflammatory diet and MLTCs is the absence of an international consensus on how to define and measure MLTCs and the number and types of conditions included in different studies results in comparability issues of the findings (154). Also, the use of cross-sectional data, which does not allow the inference of causality as it is unclear whether or not the exposure, i.e. the consumption of an inflammatory diet, preceded the disease, and a lack of data on when the chronic condition was diagnosed, in relation to the collection of data on dietary intake (106). If a study participant who was diagnosed with a chronic condition three years ago, is asked to complete an FFQ about their diet over the previous year, or multiple 24hDRs, it is possible that their dietary intake data does not reflect their habitual diet

prior to the diagnosis of their chronic condition. This may result in researchers concluding that the consumption of a pro-inflammatory diet is not associated with the prevalence of a chronic condition or MLTCs or even that an anti-inflammatory diet is associated with MLTCs. In Chapter 6, I was able to use biomarker data to show that lower concentrations of vitamin C and higher concentrations of hs-CRP were associated with higher odds of having MLTCs, although I found that a more anti-inflammatory diet was unexpectedly associated with higher odds of having MLTCs (see Figure 6 in this paper for a summary of the associations). Having biomarker data for both nutrients and inflammation are a strength of research in this area.

However, a recent paper, using UK Biobank data from more than 100,000 chronic disease-free men and women has shown than a low-inflammatory diet is associated with a lower risk and slower aggregation of MLTCs, especially in those aged >60 years (155). To my knowledge, this is the first longitudinal study to investigate associations of a low-inflammatory diet and incidence of MLTCs over a period of approximately 10 years, in middle- and older-aged community-dwelling men and women. This study has a number of strengths, including the use of two separate scores, the inflammatory diet index (IDI) and the empirical dietary inflammatory pattern (EDIP), which are based on food intakes; the presence of 59 chronic diseases, which was determined annually, through the use of medical records; and a number of sensitivity analyses. The detailed analyses within this study provide evidence of the potential benefits of consuming a low-inflammatory diet, although the scores were created using dietary data from a single 24hDR.

7.4.6 Inflammatory diets, body fat distribution, SMM and sarcopenia

Zelicha and colleagues (156) assessed the effect of the Mediterranean diet, enriched with additional polyphenols, and lower in both red and processed meat on visceral adiposity in the 18-month Dietary Intervention Randomized Controlled Trial-Polyphenols, Unprocessed (DIRECT-PLUS) trial, finding that loss of visceral adipose tissue, measured by magnetic resonance imaging, was significantly higher than in the group who followed a traditional Mediterranean diet or those who received healthy dietary guidelines. A similar loss in weight and WC was observed in participants on both the Mediterranean-based diets, but the diet enriched with additional polyphenols, and lower in both red and processed meat resulted in more than double

the loss of visceral adipose tissue than the traditional Mediterranean diet. Polyphenols are anti-inflammatory components of the DII®, providing further evidence of the possible benefits of an anti-inflammatory diet on visceral adiposity. However, future research is required to determine the mechanisms by which polyphenol-rich foods might cause a reduction in visceral adipose tissue.

A recent study investigated the associations of body shape phenotypes and measures of body fat distribution with inflammatory biomarkers in EPIC and the UK Biobank, observing that a number of anthropometric indexes, including WC and BMI, were positively associated with CRP in both men and women (157). The authors carried out a number of sub-group and sensitivity analyses, which produced similar results. I have shown that an increase in WC, with little weight gain, is significantly associated with a higher mortality risk and this research makes a significant contribution to our knowledge of associations between body fat distribution and inflammation, providing additional evidence in support of preventing a gain in WC in middle and older age, to potentially delay the onset of MLTCs.

As previously noted, developing classifications of MLTCs is problematic, as it is difficult to decide upon what conditions to include, which may be determined by data availability. My recent paper on the DII® and its associations with MLTCs did not include musculoskeletal health, although osteoporosis and obesity were included as chronic conditions, which are associated with FFM and bone density status. I have therefore included the findings from some recent publications which have used the DII® to assess associations with components of sarcopenia, to see if an anti-inflammatory diet might confer protection.

Findings from a small, cross-sectional study of 300 Iranians aged ≥ 55 years found that participants in the top tertile of the DII® were 2.18 times (95% CI: 1.01 – 4.74) more likely to have sarcopenia than those in the bottom tertile, although no significant associations were observed between the DII® and low muscle mass, abnormal handgrip strength or abnormal gait speed (158). This study population was also used to assess associations between three nutrient-based dietary patterns and sarcopenia (159). The authors found that participants in the top tertile of the anti-

inflammatory pattern had lower odds of sarcopenia (OR 0.25; 95% CI 0.10 – 0.63) than those in the bottom tertile. Liu *et al.* (160) investigated cross-sectional associations between sarcopenia and SMM and three different micronutrient intake patterns, including (1) B vitamins and minerals; (2) vitamins A, D, B12 and calcium; and (3) antioxidant vitamins A, C, E, and K, observing that intakes in the top tertile of the three micronutrient patterns were significantly associated with a lower risk of sarcopenia and greater SMM. However, the dietary data used in the study came from a single 24hDR and is therefore unlikely to reflect habitual diet and the analyses were not adjusted for energy or macronutrients.

Wizgier *et al.* observed that in men aged ≥ 75 years, a low intake of each of four antioxidant micronutrients, vitamins A, C and E and zinc, and a high Energy-adjusted Dietary Inflammatory Index (E-DII™) were significantly associated with incident activities of daily living disability over a follow-up period of three years, although incident poor grip strength was only associated with low vitamin C intake and high E-DII scores (161). Limitations of the study by Wizgier *et al.* included the use of a diet history, which only assessed the previous 3 months, and the inclusion of only 24 out of the 47 parameters in the DII® (161). A more pro-inflammatory diet was significantly associated with low muscle mass, low muscle strength and the combination of both low muscle mass and strength in older adults, (n = 1863, aged > 50 years), participating in the NHANES study, suggesting that a reduction in the consumption of a more pro-inflammatory diet might reduce the risk of sarcopenia (162). Limitations of this study include the use of a single 24hDR to estimate dietary intake, which is unlikely to reflect habitual dietary intake, in addition to the known shortcomings of cross-sectional studies, namely the inability to infer causality and the possibility of residual confounding (162). Interestingly, of the prevalence of chronic diseases in this study, (diabetes, hypertension, coronary artery disease, congestive heart failure, angina, heart attack, stroke, cancer, and renal failure), significant differences were only observed for hypertension, stroke and cancer across tertiles of the E-DII, with the lowest prevalence observed in the highest/most pro-inflammatory tertile. This agrees with my research findings, which may in part be due to an absence of information on the date of diagnosis of the self-reported chronic conditions, and

therefore an inability to assess the time at risk for MLTCs, which may have resulted in reverse causality.

These papers suggest that the consumption of antioxidant nutrients and an anti-inflammatory diet may potentially reduce the risk of sarcopenia and activities of daily living disability in later life.

7.5 Implications for public health

In a 1735 issue of the *Pennsylvania Gazette*, Benjamin Franklin wrote that "an ounce of prevention is worth a pound of cure", referring to the importance of fire safety and the need for Philadelphians to be better prepared with regard to fire prevention. Recently, UK governmental attention has begun to focus more on prevention than cure in our ageing population, and the need to improve the quality of life, rather than longevity (8) has been highlighted. Additionally, having a more holistic approach to treating patients with MLTCs (163), rather than treating individual diseases separately, needs to be the strategy going forward.

If weight loss is deemed important for the overall health of individuals or indeed population groups, intervention methods that can better ensure successful weight loss need to be developed, including nutrition education and the promotion of physical activity, before weight fluctuations begin to occur. However, monitoring weight loss in older adults who are frail or pre-frail is of the utmost importance. Physicians should be aware of the significance of weight loss, especially among older men (164). Underweight and older people living in the community should ideally be monitored for unintentional weight loss, to identify the need for early investigations and interventions. Significant unintentional or unexplained weight loss can be caused by diabetes, cancer or mental health conditions, such as depression.

As evidence suggests that changes in both weight and WC have been significantly associated with a higher morbidity and mortality risk, regular monitoring of weight and WC in adults would identify loss, gain or cycling issues which may be a cause for concern, prompting them to seek advice from their GP regarding diagnosis and treatment. Early detection of such changes may potentially result in preventing or at least delaying the onset of certain chronic conditions, thereby extending both

longevity and quality of life. A recent Consensus Statement from the International Atherosclerosis Society (IAS) and International Chair on Cardiometabolic Risk (ICCR) Working Group on Visceral Obesity recommends that WC is routinely measured in clinical practice due to the extensive evidence that it provides both independent and additional information to BMI for predicting morbidity and mortality (165). However, the feasibility of implementing this in GP practices is unknown. A recent draft guideline from NICE recommends that adults with a BMI < 35 kg/m² measure their own WHtR and seek further clinical measurements and advice from a healthcare practitioner if the measurement shows that the waist size is more than half of the height, as this indicates an increased health risk (166). The relatively new anthropometric index, WWI, was used in a study of older Koreans, where it was found to be positively associated with fat mass but negatively associated with muscle mass (167). Additionally, WWI was associated with a high probability of combined low muscle mass and high fat mass. These findings, and results from other studies on its associations with morbidity (168–173) and mortality (126) suggest that routinely monitoring both weight and WC to calculate this index may be beneficial, particularly in middle-aged and older populations.

Although conflicting evidence exists, the general consensus seems to be that vitamin E is important for healthy ageing, potentially through its antioxidant and anti-inflammatory properties. Good sources of vitamin E include plant oils, such as rapeseed, sunflower, soya, corn and olive oil, nuts, seeds, wheatgerm, but it is also found in meats, dairy products, green leafy vegetables and fortified cereals. Although no UK Reference Nutrient Intake (RNI) value has been established for vitamin E, safe intakes of α-tocopherol equivalents have been set at more than 4 mg for men and more than 3 mg for women (174). Adhering to the UK dietary guidelines and consuming a healthy balanced diet should result in achieving at least these intakes of vitamin E. In Chapter 5, the mean intake of α-tocopherol equivalents (mg/day) in 11,427 men in the fracture dietary analysis group was 11.63 (SD=5.24), with 1.7% having an intake <4mg (n = 194); the mean intake in 13,796 women was 9.29 (SD=3.78), with 1.0% having an intake <3 mg (n = 132) (74). The European Food Safety Agency (EFSA) Panel on Dietetic Products, Nutrition and Allergies set Adequate

Intakes (AIs) of α-tocopherol for adults at 13 mg/day for men and 11 mg/d for women (175), which are slightly higher than the mean intakes achieved by men and women in my cohort analyses. So, although no RNI currently exists for vitamin E in the UK, intakes of α-tocopherol equivalents in my analyses are similar to the AIs set by EFSA, suggesting that intakes in the UK are generally sufficient.

The majority of evidence to date indicates that an anti-inflammatory diet confers protection against the onset and progression of a number of chronic conditions. Anti-inflammatory parameters of the DII® include alcohol, monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids, (PUFAs), n-3 fatty acids, n-6 fatty acids, fibre, pyridoxine (B6), folic acid, riboflavin (B2), thiamine (B1), niacin, vitamins A, C, D and E, β-carotene, magnesium, selenium, zinc, flavan-3-ols, flavonols, flavonones, flavones, anthocyanidins, isoflavones, pepper, onion, garlic, green/black tea, caffeine, eugenol, ginger, saffron, turmeric, thyme/oregano and rosemary, whereas the pro-inflammatory parameters consist of energy, carbohydrate, protein, total fat, saturated fat, trans fat, cholesterol, iron and vitamin B12 (66). A number of the anti-inflammatory components are found in foods, such as fruits, vegetables, legumes, wholegrains and fish, that are also important components of other healthy dietary patterns, such as the Mediterranean Diet, which has been shown to be associated with lower CRP concentrations in cross-sectional studies (176). Advocating the consumption of a healthy plant-based diet would seem beneficial, both for our own health and that of the planet (177).

A NICE guideline has recently been published (2025), since submission of this thesis, on the prevention and management of overweight, obesity and central adiposity in children, young people and adults (178). It includes new and updated recommendations, such as encouraging people to improve their dietary intake even if this does not result in them losing weight, and to eat a nutritionally balanced diet in the long term, which agrees with research discussed in my thesis on the importance of consuming a healthy diet for longevity. I have discussed the Mediterranean diet and its anti-inflammatory properties, and adherence to the UK's

Eatwell Guide, which will provide a healthy, balanced diet, including micronutrients and anti-inflammatory components, such as vitamin E (179).

The new NICE guideline also discusses the added benefit of measuring WC to calculate WHtR and of the importance of providing support with maintaining weight loss. Although I am unsure whether the weight loss itself was the cause of the higher mortality risk in my analyses, or if it was an early indicator of the presence of life-limiting diseases and / or age-related frailty, which were actually the cause of the weight loss, this document advises to interpret BMI with caution in people aged ≥ 65 years, considering MLTCs, conditions that may affect functional capacity and the possible protective effect of having a slightly higher BMI when older.

7.6 Implications for future research

7.6.1 Changes in weight and anthropometric variables and mortality and morbidity

Additional research needs to be carried out in older people, both to identify how to improve their quality of life as well the health of subsequent generations.

I investigated only associations between changes in WC and mortality but there are other anthropometric measurements, that are generally straightforward to measure, that may also be beneficial to monitor in older adults, for both morbidity and mortality risks. Ashwell *et al.* suggest that WHtR is able to identify more people at 'early health risk' and mortality risk than either BMI alone or in combination with WC (180–183). Park *et al.* showed that a new anthropometric index, the WWI, was a strong predictor of cardiometabolic morbidity and mortality in more than 460,000 participants of the Korean National Health Insurance Cohort study (184). In future research, I could potentially use EPIC-Norfolk data to investigate associations of both WHtR and WWI with mortality risk and compare the results with my findings for WC, to see which measurement is more sensitive.

I did not investigate associations between weight fluctuations and mortality, but I believe that the EPIC-Norfolk cohort, where participants have attended up to five HEs, provides a useful data source to study the effects of weight cycling on mortality. However, as those who attended all five HEs are likely to be the healthier participants, this caveat will limit the generalisability of the findings to the UK population.

More recently, measurements of muscle mass and fat mass at both time-points have been highlighted as important areas for improvement into research in this area. As measured data on percentage body fat, weight, height and WC are available from the EPIC-Norfolk 2HE (1998 - 2000) and 3HE (2004 – 2011), in the future, I could investigate concurrent changes in weight and body composition between these HEs. However, it is possible that participants who attended both of these HEs are healthier than those who did not attend, and therefore not representative of the general UK population.

Intervention studies with a long follow-up are required to elucidate the relationships between weight change and morbidity and mortality, especially in overweight and obese adults. Clinical trials should be carried out to examine whether maintaining current body weight should be recommended to reduce mortality risk in middle- and older-aged participants. Further research is required to clarify whether the observed associations between weight change and mortality vary depending upon baseline weight, and whether the weight change was intentional or unintentional. Identification of the underlying causes of weight loss is essential to capture in future studies. Additional investigations will be needed to determine more precisely the association between weight loss and the onset of fatal diseases and whether clinical or laboratory investigations can identify individuals for whom early intervention may be effective. Accounting for key anthropometric variables, such as height, hip circumference, fat mass and FFM is important when assessing relationships between changes in weight and/or WC and mortality to minimise observations of distorted causal associations (185).

Future cohort studies need to examine the factors for unintentional weight loss and the detrimental health outcomes in the old and thin. Appropriate programmes to support with maintaining body weight after weight loss should be developed to avoid weight cycling, especially in those who are not obese.

7.6.2 Vitamin E and musculoskeletal health and its role in healthy ageing

The NHS states that the amount of vitamin E (α-tocopherol equivalents) needed is at least 4mg a day for men and at least 3mg a day for women (186), although recommendations are higher in other countries (187). However, it should be noted

that these recommended or safe intakes or AIs are set for people who are well. As the majority of older people suffer from one or more chronic condition, assessing their needs of micronutrients, such as vitamin E, may be necessary. Additionally, clinical trials are required to investigate the effect on SMM, BMD and fracture risk of dietary intakes, corresponding to a range of intakes of vitamin E (α-tocopherol equivalents), from 3-4mg/day up to 15mg/day. Ascertaining blood concentrations of vitamin E is essential in these trials, in addition to blood lipids, including total cholesterol. It must be kept in mind that vitamin E consists of two families, tocopherols and tocotrienols, each of which can be further divided into four analogues (α-, β-, γ- and δ-). In the UK, synthetic forms of α-, γ- and δ-tocopherols and a natural, tocopherol-rich extract, predominantly in the form of α-tocopherol, are approved antioxidants. They are found in more processed foods, including high-fat products such as margarines, vegetable oils, meat pies, dessert sauces, and ready-made desserts, to prevent rancidity. Ideally, examining all of the vitamin E analogues could result in a more comprehensive understanding of the role of vitamin E in musculoskeletal health and perhaps even in other age-related chronic conditions.

A new project, Zero Hidden Hunger EU (https://www.zerohiddenhunger.eu/), running from 2024 – 2027, aims to provide estimates of the prevalence of micronutrient deficiencies, focussing on population subgroups considered to be at high risk, such as older adults. The project aims to provide high-quality evidence to create solutions to ensure adequate intakes of dietary vitamins and minerals from sustainable sources. Such an initiative will result in an important data source on both the prevalence of vitamin E deficiency in the EU and suggestions on how older adults can improve their dietary intake of this antioxidant with anti-inflammatory potential.

7.6.3 Consumption of an inflammatory diet and MLTCs

Although a number of studies have shown that the consumption of an inflammatory diet is associated with a number of long-term chronic conditions, to date, there has been little research into the possible associations of the consumption of an inflammatory diet and the presence of MLTCs. This is an important area, deserving of future research, due to the ageing population worldwide and a projected increase in the prevalence of MLTCs. However, in order to investigate

these potential associations, a number of factors relating to data quality must be addressed, including

(1) an agreed minimum number/appropriate list of long-term chronic conditions that should be included in the MLTCs score

(2) the collection of reliable data on the date of diagnosis of these individual conditions

(3) the assessment of habitual dietary intake, both before and after diagnosis of the conditions.

Improvements in data quality will result in more robust evidence to help ascertain if the consumption of a pro-inflammatory diet is associated with the onset of MLTCs. Data must also be collected on factors associated with MLTCs, as well as concentrations of inflammatory biomarkers. The study population should ideally be nationally representative and include those who are perhaps less inclined to participate in research, such as people from minority ethnicities, as well as those who are socioeconomically deprived. In order to assess associations between the consumption of a pro-inflammatory diet and the incidence of MLTCs, participants should be recruited before the onset of chronic conditions and relevant data captured during follow-up, including changes in factors associated with MLTCs, where possible, such as physical activity and smoking status.

7.6.3.1 Complexity of dietary patterns

There currently exists a plethora of dietary scores, indexes and patterns, most of which are used to investigate associations of morbidity and mortality. However, more recently, these scores have also been used to assess their effects on sustainability, greenhouse gas emissions and carbon footprint. Some of these scores are based solely on nutrients (Dietary Antioxidant Quality Score (DAQS)), others are based on foods (Nordic diet, NOVA classification), but the majority of scores are based on a combination of foods and nutrients (DII®, Mediterranean diet score (MDS), Dietary Approach to Stop Hypertension (DASH) diet, Healthy Eating Index (HEI), Diet Quality Index-International (DQI-I)).

A number of similarities exist across many of the scores, with an emphasis on plant-based foods and a limitation on intakes of processed foods, sweets and red

meat. Lassale *et al*. investigated the associations of 10 diet quality scores on the risk of all-cause, CVD and cancer mortality in more than 450,000 healthy EPIC participants, finding that all 10 scores were associated with mortality, but the authors highlighted the strong predictive power of other factors, especially age and sex, as well as study population, smoking status, BMI, physical activity and educational level (188). This study emphasises the importance of adjusting for variables associated with mortality when assessing associations of diet and mortality risk.

There are methodological issues relating to the type of dietary instrument used, which may be due to data availability, and whether nutrient data have been energy-adjusted, and if so, in what way. On top of this, there are often a number of versions of a score, each of which is created slightly differently from the other, further complicating study findings. Tong *et al*. investigated the associations of four different versions of the MDS (Mediterranean diet pyramid, literature-based MDS, median-based MDS and tertile-based MDS) with CVD incidence and all-cause and CVD mortality in the EPIC-Norfolk cohort study, using FFQ data, adjusted to 2000 kcal/day (189). Although generally similar associations were found for all four MDSs, with greater adherence to the Mediterranean diet being associated with lower CVD incidence and mortality, the strongest associations were found for the Mediterranean diet pyramid. This is an important study, illustrating how associations may be dependent on the version of the dietary score that is used. Similar work can be carried out on the various inflammatory scores that have been published to establish if they produce different findings.

Care must therefore be taken with regard to the creation of these dietary patterns, scores and indexes and the interpretation of findings based on them. The creators should provide clear steps in their publications on how they should be created and researchers who use them should clarify if they have adhered to these specifications. As I have shown in Table 3 and Table 4 in Chapter 2, differences in the number of parameters included in the DII® score affected how participants were assigned to quintiles, which may have important consequences for public health reasons and diet-disease associations.

7.6.4 Suggestions for future research into healthy ageing

Taking my findings into consideration, including some of the limitations and uncertainties from my research, I will now summarise what I think is the best course of action for future research into healthy ageing.

Prospective cohort studies are expensive, and it takes a long period of time before data are available for research. Therefore, new studies to enable more rigorous investigations into assessments of diet-disease associations and mortality should be designed with care, ensuring that all essential data on factors of interest, e.g. habitual diet, anthropometric measurements, biomarker data, clinical measurements, lifestyle characteristics and chronic conditions, and that changes in these factors are captured over time. New cohort studies should store blood in aliquots to enable new and novel research in the future, as this approach in the EPIC-Norfolk study has resulted in recent epidemiological research using metabolomics.

Existing big data sources, with the aid of machine learning and artificial intelligence, could be used to potentially identify new modifiable factors that have an effect on healthy ageing. There are a number of big data sources in the UK, including the UK Biobank, which is one of the data sources being studied in the InflAIM project (https://www.inflaim.com/), to find out why some people are susceptible to developing MLTCs. The availability of big data on the microbiome, metabolomics, accelerometery, in combination with high-quality dietary data, would enable more advanced research into healthy ageing.

7.7 Conclusions

My research has focused on a broad spectrum of nutritional assessment, covering all dimensions from the acronym ABCD: Anthropometry, Biological markers, Clinical symptoms and Dietary intake. Findings from my analyses on middle- and older-aged men and women indicate that weight loss of more than 2.5 kg, but a gain in waist circumference of more than 5 cm, was associated with higher mortality risk, in this study population. Higher dietary intakes and circulating concentrations of vitamin E were positively associated with measures of fat-free mass and bone density status, and generally significant positive associations for a lower risk of total and hip fractures, suggesting that dietary vitamin E has a protective role in musculoskeletal

health. A more pro-inflammatory diet was associated with lower micronutrient biomarker concentrations and higher concentrations of high-sensitivity C-reactive protein (hs-CRP), a well-known inflammatory biomarker. Higher concentrations of hs-CRP were associated with higher odds of having multiple long-term conditions (MLTCs). However, unexpectedly, a more pro-inflammatory diet was associated with lower odds of having MLTCs.

The work from my thesis has highlighted the need for agreement on definitions and criteria for future epidemiological research using longitudinal data, with regard to understanding changes in anthropometric variables and the creation of a MLTC score. Further studies of healthy ageing require objective measurements to improve the quality of research and should therefore include biomarker measurements, of both dietary intake and inflammation, as well as clinical assessments, including of physical functioning. These enhancements will result in more robust evidence of the impact of nutritional factors on inflammation, diseases of ageing and mortality risk and enable the comparison of data from different study populations.

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Appendices

Appendix 1: List of my publications cited in this dissertation (* submitted for PhD by Publication)

Appendix 2: Copies of statements from co-authors confirming my contribution to the papers submitted for PhD by Publication

Appendix 1: List of my publications

(** Chapter paper; * cited in this thesis)

Authors	Title	Publication	Volume	Number	Pages	Year
Amoutzopoulos, Birdem; Steer, Toni; Roberts, Caireen; Collins, David; Trigg, Kirsty; Barratt, Rachel; Abraham, Suzanna; Cole, Darren James; **Mulligan, Angela**; Foreman, Jackie.	Rationalisation of the UK Nutrient Databank for Incorporation in a Web-Based Dietary Recall for Implementation in the UK National Diet and Nutrition Survey Rolling Programme	Nutrients	14	21		2022
Andersson, Susan W; Skinner, J; Ellegård, L; Welch, AA; Bingham, S; **Mulligan, A**; Andersson, H; Khaw, KT.	Intake of dietary plant sterols is inversely related to serum cholesterol concentration in men and women in the EPIC Norfolk population: a cross-sectional study	European journal of clinical nutrition	58	10	1378-1385	2004
Bingham, SA; Vorster, H; Jerling, JC; Magee, E; **Mulligan, A**; Runswick, SA; Cummings, JH.	Effect of black tea drinking on blood lipids, blood pressure and aspects of bowel habit	British Journal of Nutrition	78	1	41-55	1997
* Bingham, Sheila A; Welch, Ailsa A; McTaggart, Alison; **Mulligan, Angela A**; Runswick, Shirley A; Luben, Robert; Oakes, Suzy; Khaw, Kay Tee; Wareham, Nicholas; Day, Nicholas E.	Nutritional methods in the European prospective investigation of cancer in Norfolk	Public health nutrition	4	3	847-858	2001
Boker, LK; Peeters, PHM; **Mulligan, AA**; Navarro, C; Slimani, N; EPIC Working Group on Dietary Patterns, Sub-Group on Soy Consumption.	Consumption of soy products among European consumers of a health-conscious diet	IARC scientific publications	156		109-12	2002
Grace, Philip B; Taylor, James I; Low, Yen-Ling; Luben, Robert N; **Mulligan, Angela A**; Botting,	Phytoestrogen concentrations in serum and spot urine as	Cancer Epidemiol	13	5	698-708	2004

Authors	Title	Publication	Volume	Number	Pages	Year
Nigel P; Dowsett, Mitch; Welch, Ailsa A; Khaw, Kay-Tee; Wareham, Nick.	biomarkers for dietary phytoestrogen intake and their relation to breast cancer risk in European prospective investigation of cancer and nutrition-norfolk	ogy Biomarkers & Prevention				
Hayhoe, Richard PG; Lentjes, Marleen AH; **Mulligan, Angela A**; Luben, Robert N; Khaw, Kay-Tee; Welch, Ailsa A.	Cachexia, Sarcopenia and Muscle Wasting, Maastricht, The Netherlands, 7–9 December 2018	Journal of Cachexia, Sarcopenia and Muscle	9		1121-1184	2018
Hayhoe, Richard PG; Lentjes, Marleen AH; **Mulligan, Angela A**; Luben, Robert N; Khaw, Kay-Tee; Welch, Ailsa A.	Carotenoid dietary intakes and plasma concentrations are associated with heel bone ultrasound attenuation and osteoporotic fracture risk in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort	British Journal of Nutrition	117	10	1439-1453	2017
Hayhoe, Richard PG; Lentjes, Marleen AH; **Mulligan, Angela A**; Luben, Robert N; Khaw, Kay-Tee; Welch, Ailsa A.	Cross-sectional associations of dietary and circulating magnesium with skeletal muscle mass in the EPIC-Norfolk cohort	Clinical Nutrition	38	1	317-323	2019
***** Jennings, Amy; **Mulligan, Angela A**; Khaw, Kay-Tee; Luben, Robert N; Welch, Ailsa A.	A Mediterranean Diet Is Positively Associated with Bone and Muscle Health in a Non-Mediterranean Region in 25,450 Men and Women from EPIC-Norfolk	Nutrients	12	4		2020

Authors	Title	Publication	Volume	Number	Pages	Year
Kaptoge, S; Welch, A; McTaggart, A; **Mulligan, A**; Dalzell, N; Day, NE; Bingham, S; Khaw, KT; Reeve, J.	Effects of dietary nutrients and food groups on bone loss from the proximal femur in men and women in the 7th and 8th decades of age	Osteoporosis International	418	5	418-28	2003
Keinan, Boker L; Peeters, PHM; **Mulligan, AA**; Navarro, C; Slimani, N; EPIC Working Group on Dietary Patterns.	Consumption of soy products in 10 European countries	IARC scientific publications	156		105-108	2002
Keinan-Boker, L; Peeters, PHM; **Mulligan, AA**; Navarro, C; Slimani, N; Mattisson, Iréne; Lundin, E; McTaggart, A; Allen, NE; Overvad, K.	Soy product consumption in 10 European countries: the European Prospective Investigation into Cancer and Nutrition (EPIC) study	Public Health Nutrition	5	6b	1217-1226	2002
Kelly, Rebecca K; Pollard, Zoe; Young, Heather; Piernas, Carmen; Lentjes, Marleen; **Mulligan, Angela**; Huybrechts, Inge; Carter, Jennifer L; Key, Timothy J; Perez-Cornago, Aurora.	Evaluation of the New Individual Fatty Acid Dataset for UK Biobank: Analysis of Intakes and Sources in 207,997 Participants	Nutrients	14	17		2022
Key, Timothy J; Appleby, Paul N; Cairns, Benjamin J; Luben, Robert; Dahm, Christina C; Akbaraly, Tasnime; Brunner, Eric J; Burley, Victoria; Cade, Janet E; Greenwood, Darren C; Stephen, Alison M; Mishra, Gita; Kuh, Diana; Keogh, Ruth H; White, Ian R; Bhaniani, Amit; Borgulya, Gabor; **Mulligan, Angela A**; Khaw, Kay Tee.	Dietary fat and breast cancer: comparison of results from food diaries and food-frequency questionnaires in the UK Dietary Cohort Consortium	The American journal of clinical nutrition	94	4	1043-1052	2011
Kiely, Mairead; Faughnan, Marian; Wähälä, Kristiina; Brants, Henny; **Mulligan, Angela**.	Phyto-oestrogen levels in foods: the design and construction of the VENUS database	British Journal of Nutrition	89	S1	S19-S23	2003

Authors	Title	Publication	Volume	Number	Pages	Year
Klingberg, S; Andersson, H; **Mulligan, A**; Bhaniani, A; Welch, A; Bingham, S; Khaw, KT; Andersson, S; Ellegård, L.	Food sources of plant sterols in the EPIC Norfolk population	European journal of clinical nutrition	62	6	695-703	2008
Klipstein-Grobusch, K; Slimani, N; Krogh, V; Keil, U; Boeing, H; Overvad, K; Tjønneland, A; Clavel-Chapelon, F; Thiébaut, A; Linseisen, J; Schulze, MB; Lagiou, P; Papadimitrou, A; Saieva, C; Veglia, F; Bueno-De-Mesquita, HB; Peeters, PH; Kumle, M; Brustad, M; Martínez, García C; Barricarte, A; Berglund, G; Weinehall, L; **Mulligan, A**; Allen, N; Ferrari, P; Riboli E.	Trends in self-reported past alcoholic beverage consumption and ethanol intake from 1950 to 1995 observed in eight European countries participating in the European Investigation into Cancer and Nutrition (EPIC)	Public health nutrition	5	6b	1297-1310	2002
Kuhnle, Gunter GC; Dell'Aquila, Caterina; Aspinall, Sue M; Runswick, Shirley A; Joosen, Annemiek MCP; **Mulligan, Angela A**; Bingham, Sheila A.	Phytoestrogen content of fruits and vegetables commonly consumed in the UK based on LC–MS and 13C-labelled standards	Food Chemistry	116	2	542-554	2009
Kuhnle, Gunter GC; Dell'Aquila, Caterina; Aspinall, Sue M; Runswick, Shirley A; **Mulligan, Angela A**; Bingham, Sheila A.	Phytoestrogen content of beverages, nuts, seeds, and oils	Journal of agricultural and food chemistry	56	16	7311-7315	2008
Kuhnle, Gunter GC; Dell'Aquila, Caterina; Aspinall, Sue M; Runswick, Shirley A; **Mulligan, Angela A**; Bingham, Sheila A.	Phytoestrogen content of foods of animal origin: dairy products, eggs, meat, fish, and seafood	Journal of agricultural and food chemistry	56	21	10099-10104	2008
Kuhnle, Gunter GC; Dell'Aquila, Caterina; Aspinall, Sue M; Runswick, Shirley A; **Mulligan, Angela A**; Bingham, Sheila A.	Phytoestrogen content of cereals and cereal-based foods consumed in the UK	Nutrition and cancer	61	3	302-309	2009

Authors	Title	Publication	Volume	Number	Pages	Year
Kuhnle, Gunter GC; Tasevska, Natasha; Lentjes, Marleen AH; Griffin, Julian L; Sims, Matthew A; Richardson, Larissa; Aspinall, Sue M; **Mulligan, Angela A**; Luben, Robert N; Khaw, Kay-Tee.	Association between sucrose intake and risk of overweight and obesity in a prospective sub-cohort of the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk)	Public health nutrition	18	15	2815-2824	2015
Kuhnle, Gunter GC; Ward, Heather A; Vogiatzoglou, Anna; Luben, Robert N; **Mulligan, Angela**; Wareham, Nicholas J; Forouhi, Nita G; Khaw, Kay-Tee.	Association between dietary phyto-oestrogens and bone density in men and postmenopausal women	British journal of nutrition	106	7	1063-1069	2011
Lachman, Sangeeta; Peters, Ron JG; Lentjes, Marleen AH; **Mulligan, Angela A**; Luben, Robert N; Wareham, Nicholas J; Khaw, Kay-Tee; Boekholdt, S Matthijs.	Ideal cardiovascular health and risk of cardiovascular events in the EPIC-Norfolk prospective population study	European journal of preventive cardiology	23	9	986-994	2016
Lahmann, Petra H; Hughes, Maria Celia; Ibiebele, Torukiri I; **Mulligan, Angela A**; Kuhnle, Gunter GC; Webb, Penelope M.	Estimated intake of dietary phyto-oestrogens in Australian women and evaluation of correlates of phyto-oestrogen intake	Journal of nutritional science	1			2012
***** Lentjes, MAH; **Mulligan, Angela Assumpta**; Welch, Ailsa A; Bhaniani, Amit; Luben, RN; Khaw, K–T.	Contribution of cod liver oil-related nutrients (vitamins A, D, E and eicosapentaenoic acid and docosahexaenoic acid) to daily nutrient intake and their associations with plasma concentrations in the EPIC-Norfolk cohort	Journal of human nutrition and dietetics	28	6	568-582	2015

Authors	Title	Publication	Volume	Number	Pages	Year
* Lentjes, Marleen AH; Bhaniani, Amit; **Mulligan, Angela A**; Khaw, Kay-Tee; Welch, Ailsa A.	Developing a database of vitamin and mineral supplements (ViMiS) for the Norfolk arm of the European Prospective Investigation into Cancer (EPIC-Norfolk)	Public health nutrition	14	3	459-471	2011
Lentjes, Marleen AH; Keogh, Ruth H; Welch, Ailsa A; **Mulligan, Angela A**; Luben, Robert N; Wareham, Nicholas J; Khaw, Kay-Tee.	Longitudinal associations between marine omega-3 supplement users and coronary heart disease in a UK population-based cohort	BMJ open	7	10		2017
* Lentjes, Marleen AH; McTaggart, Alison; **Mulligan, Angela A**; Powell, Natasha A; Parry-Smith, David; Luben, Robert N; Bhaniani, Amit; Welch, Ailsa A; Khaw, Kay-Tee.	Dietary intake measurement using 7 d diet diaries in British men and women in the European Prospective Investigation into Cancer-Norfolk study: a focus on methodological issues	British Journal of Nutrition	111	3	516-526	2014
Lentjes, Marleen AH; Oude Griep, Linda M; **Mulligan, Angela A**; Montgomery, Scott; Wareham, Nick J; Khaw, Kay-Tee.	Face Validity of Observed Meal Patterns Reported with 7-Day Diet Diaries in a Large Population-Based Cohort Using Diurnal Variation in Concentration Biomarkers of Dietary Intake	Nutrients	14	2		2022
Lentjes, Marleen AH; Welch, Ailsa A; **Mulligan, Angela A**; Luben, Robert N; Wareham, Nicholas J; Khaw, Kay-Tee.	Cod Liver Oil Supplement Consumption and Health: Cross-sectional Results from the EPIC-Norfolk Cohort Study	Nutrients	6	10	4320-4337	2014

Authors	Title	Publication	Volume	Number	Pages	Year
Lewis, Lucy N; Hayhoe, Richard PG; **Mulligan, Angela A**; Luben, Robert N; Khaw, Kay-Tee; Welch, Ailsa A.	Lower Dietary and Circulating Vitamin C in Middle-and Older-Aged Men and Women Are Associated with Lower Estimated Skeletal Muscle Mass	The Journal of Nutrition	150	10	2789-2798	2020
Liggins, J; **Mulligan, A**; Runswick, S; Bingham, SA.	Daidzein and genistein content of cereals	European journal of clinical nutrition	56	10	961-966	2002
Loh, Yet Hua; Jakszyn, Paula; Luben, Robert N; **Mulligan, Angela A**; Mitrou, Panagiota N; Khaw, Kay-Tee.	N-nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)–Norfolk Study	The American journal of clinical nutrition	93	5	1053-1061	2011
Low, Yen-Ling; Taylor, James I; Grace, Philip B; Dowsett, Mitch; Folkerd, Elizabeth; Doody, Deborah; Dunning, Alison M; Scollen, Serena; **Mulligan, Angela A**; Welch, Ailsa A.	Polymorphisms in the CYP19 gene may affect the positive correlations between serum and urine phytoestrogen metabolites and plasma androgen concentrations in men	The Journal of nutrition	135	11	2680-2686	2005
Low, Yen-Ling; Taylor, James I; Grace, Philip B; Dowsett, Mitch; Scollen, Serena; Dunning, Alison M; **Mulligan, Angela A**; Welch, Ailsa A; Luben, Robert N; Khaw, Kay-Tee.	Phytoestrogen exposure correlation with plasma estradiol in postmenopausal women in European Prospective Investigation of Cancer and Nutrition-Norfolk may involve diet-gene interactions	Cancer Epidemiology Biomarkers & Prevention	14	1	213-220	2005
Low, Yen-Ling; Taylor, James I; Grace, Philip B; **Mulligan, Angela A**; Welch, Ailsa A; Scollen,	Phytoestrogen exposure, polymorphisms in COMT, CYP19, ESR1, and SHBG	Nutrition and cancer	56	1	31-39	2006

Authors	Title	Publication	Volume	Number	Pages	Year
Serena; Dunning, Alison M; Luben, Robert N; Khaw, Kay-Tee; Day, Nick E.	genes, and their associations with prostate cancer risk					
Luben, Robert; Hayat, Shabina; **Mulligan, Angela**; Lentjes, Marleen; Wareham, Nicholas; Pharoah, Paul; Khaw, Kay-Tee.	Alcohol consumption and future hospital usage: The EPIC-Norfolk prospective population study	PloS one	13	7		2018
Vorster, H; Jerling, J; Oosthuizen, W; Cummings, J; Bingham, S; Magee, L; **Mulligan, A**; Runswick, S.	Tea drinking and haemostasis: a randomized, placebo-controlled, crossover study in free-living subjects	Haemostasis	26		58-64	1996
* McKeown, NM; Day, NE; Welch, AA; Runswick, SA; Luben, RN; **Mulligan, AA**; McTaggart, A; Bingham, SA.	Original Research Communications-Nutritional status, dietary intake, and body composition-Use of biological markers to validate self-reported dietary intake in a random sample of the European	American Journal of Clinical Nutrition	74	2	188-196	2001
Monsivais, Pablo; Scarborough, Peter; Lloyd, Tina; Mizdrak, Anja; Luben, Robert; **Mulligan, Angela A**; Wareham, Nicholas J; Woodcock, James.	Greater accordance with the Dietary Approaches to Stop Hypertension dietary pattern is associated with lower diet-related greenhouse gas production but higher dietary costs in the United Kingdom	The American journal of clinical nutrition	102	1	138-145	2015
Mulligan, AA; Welch, AA; McTaggart, AA; Bhaniani, A; Bingham, SA.	Intakes and sources of soya foods and isoflavones in a UK population cohort study (EPIC-Norfolk)	European journal of clinical nutrition	61	2	248-254	2007
** **Mulligan, Angela A**; Hayhoe, Richard PG; Luben, Robert N; Welch, Ailsa A.	Positive Associations of Dietary Intake and Plasma	Antioxidants	10	2		2021

Authors	Title	Publication	Volume	Number	Pages	Year
	Concentrations of Vitamin E with Skeletal Muscle Mass, Heel Bone Ultrasound Attenuation and Fracture Risk in the EPIC-Norfolk Cohort					
* **Mulligan, Angela A**; Kuhnle, Gunter GC; Lentjes, Marleen AH; van Scheltinga, Veronica; Powell, Natasha A; McTaggart, Alison; Bhaniani, Amit; Khaw, Kay-Tee.	Intakes and sources of isoflavones, lignans, enterolignans, coumestrol and soya-containing foods in the Norfolk arm of the European Prospective Investigation into Cancer and Nutrition (EPIC-Norfolk), from 7 d food diaries, using a newly updated database	Public health nutrition	16	8	1454-1462	2013
** **Mulligan, Angela A**; Lentjes, Marleen AH; Luben, Robert N; Wareham, Nicholas J; Khaw, Kay-Tee.	Changes in waist circumference and risk of all-cause and CVD mortality: results from the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) cohort study	BMC cardiovascular disorders	19			2019
** **Mulligan, Angela A**; Lentjes, Marleen AH; Luben, Robert N; Wareham, Nicholas J; Khaw, Kay-Tee.	Weight change and 15 year mortality: results from the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) cohort study	European Journal of Epidemiology	33		37-53	2018
** **Mulligan, Angela A**; Lentjes, Marleen AH; Skinner, Jane; Welch, Ailsa A.	The Dietary Inflammatory Index and Its Associations with Biomarkers of Nutrients with	Antioxidants	13	8		2024

Authors	Title	Publication	Volume	Number	Pages	Year
	Antioxidant Potential, a Biomarker of Inflammation and Multiple Long-Term Conditions					
* **Mulligan, Angela A**; Luben, Robert N; Bhaniani, Amit; Parry-Smith, David J; O'Connor, Laura; Khawaja, Anthony P; Forouhi, Nita G; Khaw, Kay-Tee.	A new tool for converting food frequency questionnaire data into nutrient and food group values: FETA research methods and availability	BMJ open	4	3		2014
Ocké, MC; Larranaga, N; Grioni, S; Van Den Berg, SW; Ferrari, P; Salvini, S; Benetou, V; Linseisen, J; Wirfält, Elisabet; Rinaldi, S; Jenab, M; Halkjaer, J; Jakobsen, MU; Niravong, M; Clavel-Chapelon, F; Kaaks, R; Bergmann, M; Moutsiou, E; Trichopoulou, A; Lauria, C; Sacerdote, C; Bueno-de-Mesquita, HB; Peeters, PH; Hjartåker, A; Parr, CL; Tormo, MJ; Sanchez, MJ; Manjer, J; Hellstrom, V; **Mulligan, A**; Spencer, EA; Riboli, E; Bingham, S; Slimani, N.	Energy intake and sources of energy intake in the European Prospective Investigation into Cancer and Nutrition	European journal of clinical nutrition	63	4	S3-S15	2009
Ottaviani, Javier I; Britten, Abigail; Lucarelli, Debora; Luben, Robert; **Mulligan, Angela A**; Lentjes, Marleen A; Fong, Reedmond; Gray, Nicola; Grace, Philip B; Mawson, Deborah H.	Biomarker-estimated flavan-3-ol intake is associated with lower blood pressure in cross-sectional analysis in EPIC Norfolk	Scientific reports	10	1		2020
Park, Jin Young; Dahm, Christina C; Keogh, Ruth H; Mitrou, Panagiota N; Cairns, Benjamin J; Greenwood, Darren C; Spencer, Elizabeth A; Fentiman, Ian S; Shipley, Martin J; Brunner, Eric J; Cade, JE; Burley, VJ; Mishra, GD; Kuh, D; Stephen, AM; White, IR; Luben, RN; **Mulligan, AA**; Khaw, KT; Rodwell, SA.	Alcohol intake and risk of colorectal cancer: results from the UK Dietary Cohort Consortium	British journal of cancer	103	5	747-756	2010

Authors	Title	Publication	Volume	Number	Pages	Year
Patel, PS; Cooper, AJM; O'Connell, TC; Kuhnle, GGC; Kneale, CK; **Mulligan, A**; Luben, RN; Brage, S; Khaw, KT; Wareham, NJ.	Serum carbon and nitrogen stable isotopes as potential biomarkers of dietary intake and their relation with incident type 2 diabetes: the EPIC-Norfolk study	The American Journal of Clinical Nutrition	100	2	708-718	2014
Perez-Cornago, Aurora; Pollard, Zoe; Young, Heather; van Uden, Marloes; Andrews, Colm; Piernas, Carmen; Key, Timothy J; **Mulligan, Angela**; Lentjes, Marleen.	Description of the updated nutrition calculation of the Oxford WebQ questionnaire and comparison with the previous version among 207,144 participants in UK Biobank	European journal of nutrition	60	7	4019-4030	2021
Piernas, Carmen; Perez-Cornago, Aurora; Gao, Min; Young, Heather; Pollard, Zoe; **Mulligan, Angela**; Lentjes, Marleen; Carter, Jennifer; Bradbury, Kathryn; Key, Tim J.	Describing a new food group classification system for UK biobank: analysis of food groups and sources of macro-and micronutrients in 208,200 participants	European Journal of Nutrition	60		2879-2890	2021
Praagman, Jaike; Vissers, Linda ET; **Mulligan, Angela A**; Laursen, Anne Sofie Dam; Beulens, Joline WJ; van der Schouw, Yvonne T; Wareham, Nicholas J; Hansen, Camilla Plambeck; Khaw, Kay-Tee; Jakobsen, Marianne Uhre.	Consumption of individual saturated fatty acids and the risk of myocardial infarction in a UK and a Danish cohort	International journal of cardiology	279		18-26	2019
Roswall, Nina; Olsen, Anja; Boll, Katja; Christensen, Jane; Halkjær, Jytte; Sørensen, Thorkild IA; Dahm, Christina C; Overvad, Kim; Clavel-Chapelon, Françoise; Boutron-Ruault, Marie C; Cottet, V; Teucher, B; Kaaks, R; Boeing, H; von Ruesten, A; Trichopoulou, A; Oikonomou, E; Vasilopoulou, E; Pala, V; Sacerdote, C;	Consumption of predefined 'Nordic'dietary items in ten European countries–an investigation in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort	Public health nutrition	17	12	2650-2659	2014

Authors	Title	Publication	Volume	Number	Pages	Year
Mattiello, A; Masala, G; Peeters, PH; Bueno-de-Mesquita, HB; Engeset, D; Skeie, G; Asli, LA; Amiano, P; Jakszyn, P; Ardanaz, E; Huerta, JM; Quirós, JR; Molina-Montes, E; Nilsson, LM; Johansson, I; Wirfält, E; Drake, I; **Mulligan, AA**; Khaw, KT; Romaguera, D; Vergnaud, AC; Key, T; Riboli, E; Tjønneland A.						
Shannon, Oliver M; Ranson, Janice M; Gregory, Sarah; Macpherson, Helen; Milte, Catherine; Lentjes, Marleen; **Mulligan, Angela**; McEvoy, Claire; Griffiths, Alex; Matu, Jamie.	Mediterranean diet adherence is associated with lower dementia risk, independent of genetic predisposition: findings from the UK Biobank prospective cohort study	BMC Medicine	21	1		2023
* Shannon, Oliver M; Stephan, Blossom CM; Granic, Antoneta; Lentjes, Marleen; Hayat, Shabina; **Mulligan, Angela**; Brayne, Carol; Khaw, Kay-Tee; Bundy, Rafe; Aldred, Sarah.	Mediterranean diet adherence and cognitive function in older UK adults: the European Prospective Investigation into Cancer and Nutrition–Norfolk (EPIC-Norfolk) Study	The American journal of clinical nutrition	110	4	938-948	2019
Slimani, N; Deharveng, G; Southgate, DAT; Biessy, C; Chajès, V; Van Bakel, MME; Boutron-Ruault, MC; McTaggart, A; Grioni, S; Verkaik-Kloosterman, J; Huybrechts, I; Amiano, P; Jenab, M; Vignat, J; Bouckaert, K; Casagrande, C; Ferrari, P; Zourna, P; Trichopoulou, A; Wirfält, E; Johansson, G; Rohrmann, S; Illner, AK; Barricarte, A; Rodríguez, L; Touvier, M; Niravong, M; **Mulligan, A**; Crowe, F; Ocké, MC; van der Schouw, YT; Bendinelli, B; Lauria, C; Brustad, M;	Contribution of highly industrially processed foods to the nutrient intakes and patterns of middle-aged populations in the European Prospective Investigation into Cancer and Nutrition study	European journal of clinical nutrition	63	4	S206-S225	2009

Authors	Title	Publication	Volume	Number	Pages	Year
Hjartåker, A; Tjønneland, A; Jensen, AM; Riboli, E; Bingham, S.						
Slimani, N; Fahey, M; Welch, A; Wirfält, E; Stripp, C; Bergström, E; Linseisen, J; Schulze, MB; Bamia, C; Chloptsios, Y; Veglia, F; Panico, S; Bueno-de-Mesquita, HB; Ocké, MC; Brustad, M; Lund, E; González, CA; Barcos, A; Berglund, G; Winkvist, A; **Mulligan, A**; Appleby, P; Overvad, K; Tjønneland, A; Clavel-Chapelon, F; Kesse, E; Ferrari, P; Van Staveren, WA; Riboli, E.	Diversity of dietary patterns observed in the European Prospective Investigation into Cancer and Nutrition (EPIC) project	Public health nutrition	5	6b	1311-1328	2002
Spencer, Elizabeth A; Key, Timothy J; Appleby, Paul N; Dahm, Christina C; Keogh, Ruth H; Fentiman, Ian S; Akbaraly, Tasnime; Brunner, Eric J; Burley, Victoria; Cade, Janet E; Greenwood, Darren C; Stephen, Alison M; Mishra, Gita; Kuh, Diana; Luben, Robert; **Mulligan, Angela A**; Khaw, Kay-Tee; Rodwell, Sheila A.	Meat, poultry and fish and risk of colorectal cancer: pooled analysis of data from the UK dietary cohort consortium	Cancer causes & control	21		1417-1425	2010
Swann, Ruth; Perkins, Katherine A; Velentzis, Louiza S; Ciria, Cristian; Dutton, Susan J; **Mulligan, Angela A**; Woodside, Jayne V; Cantwell, Marie M; Leathem, Anthony J; Robertson, Claire E.	The DietCompLyf study: a prospective cohort study of breast cancer survival and phytoestrogen consumption	Maturitas	75	3	232-240	2013
van Erp-Baart, Marie-Agnes J; Brants, Henny AM; Kiely, Mairead; **Mulligan, Angela**; Turrini, Aida; Sermoneta, Colomba; Kilkkinen, Annamari; Valsta, Liisa M.	Isoflavone intake in four different European countries: the VENUS approach	British Journal of Nutrition	89	S1	S25-S30	2003
Vogiatzoglou, Anna; Heuer, Thorsten; **Mulligan, Angela A**; Lentjes, Marleen AH; Luben, Robert N; Kuhnle, Gunter GC.	Estimated dietary intakes and sources of flavanols in the German population (German National Nutrition Survey II)	European journal of nutrition	53		635-643	2014

Authors	Title	Publication	Volume	Number	Pages	Year
Vogiatzoglou, Anna; **Mulligan, Angela A**; Bhaniani, Amit; Lentjes, Marleen AH; McTaggart, Alison; Luben, Robert N; Heiss, Christian; Kelm, Malte; Merx, Marc W; Spencer, Jeremy PE.	Associations between flavan-3-ol intake and CVD risk in the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk)	Free Radical Biology and Medicine	84			2015
***** Vogiatzoglou, Anna; **Mulligan, Angela A**; Lentjes, Marleen AH; Luben, Robert N; Spencer, Jeremy PE; Schroeter, Hagen; Khaw, Kay-Tee; Kuhnle, Gunter GC.	Flavonoid intake in European adults (18 to 64 years)	PloS one	10	5		2015
***** Vogiatzoglou, Anna; **Mulligan, Angela A**; Luben, Robert N; Lentjes, Marleen AH; Heiss, Christian; Kelm, Malte; Merx, Marc W; Spencer, Jeremy PE; Schroeter, Hagen; Kuhnle, Gunter GC.	Assessment of the dietary intake of total flavan-3-ols, monomeric flavan-3-ols, proanthocyanidins and theaflavins in the European Union	British Journal of Nutrition	111	8	1463-1473	2014
Ward, Heather A; Kuhnle, Gunter GC; **Mulligan, Angela A**; Lentjes, Marleen AH; Luben, Robert N; Khaw, Kay-Tee.	Breast, colorectal, and prostate cancer risk in the European Prospective Investigation into Cancer and Nutrition–Norfolk in relation to phytoestrogen intake derived from an improved database	The American journal of clinical nutrition	91	2	440-448	2010
* Welch, AA; McTaggart, A; **Mulligan, AA**; Luben, R; Walker, N; Khaw, KT; Day, NE; Bingham, SA.	DINER (Data Into Nutrients for Epidemiological Research)–a new data-entry program for nutritional analysis in the EPIC–Norfolk cohort and the 7-day diary method	Public health nutrition	4	6	1253-1265	2001
Welch, AA; **Mulligan, AA**.	Dietary intake measurement: methodology	In 'Reference Module in				2021

Authors	Title	Publication	Volume	Number	Pages	Year
		Food Science'				
Welch, Ailsa A; **Mulligan, Angela**; Bingham, Sheila A; Khaw, Kay-tee.	Urine pH is an indicator of dietary acid–base load, fruit and vegetables and meat intakes: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk population study	British Journal of Nutrition	99	6	1335-1343	2008
Wirfält, Elisabet; McTaggart, A; Pala, V; Gullberg, Bo; Frasca, G; Panico, S; Bueno-de-Mesquita, HB; Peeters, PHM; Engeset, D; Skeie, G; Chirlaque, M D; Amiano, P; Lundin, E; **Mulligan, A**; Spencer, E A; Overvad, K; Tjønneland, A; Clavel-Chapelon, F; Linseisen, J; Nöthlings, U; Polychronopoulos, E; Georga, K; Charrondière, U R; Slimani, N	Food sources of carbohydrates in a European cohort of adults	Public health nutrition	5	6b	1197-1215	2002
Zamora-Ros, R; Knaze, V; Lujan-Barroso, L; Kuhnle, GGC; **Mulligan, AA**; Touillaud, M; Slimani, N; Romieu, I; Powell, N; Tumino, R.	Dietary intakes and food sources of phytoestrogens in the European Prospective Investigation into Cancer and Nutrition (EPIC) 24-hour dietary recall cohort	European journal of clinical nutrition	66	8	932-941	2012
Zamora-Ros, Raul; Agudo, Antonio; Luján-Barroso, Leila; Romieu, Isabelle; Ferrari, Pietro; Knaze, Viktoria; Bueno-de-Mesquita, H Bas; Leenders, Max; Travis, Ruth C; Navarro, Carmen; Sánchez-Cantalejo, E; Slimani, N; Scalbert, A; Fedirko, V; Hjartåker, A; Engeset, D; Skeie, G; Boeing, H; Förster, J; Li, K; Teucher, B; Agnoli, C;	Dietary flavonoid and lignan intake and gastric adenocarcinoma risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) study–	The American journal of clinical nutrition	96	6	1398-1408	2012

Authors	Title	Publication	Volume	Number	Pages	Year
Tumino, R; Mattiello, A; Saieva, C; Johansson, I; Stenling, R; Redondo, ML; Wallström, P; Ericson, U; Khaw, KT; **Mulligan, AA**; Trichopoulou, A; Dilis, V; Katsoulis, M; Peeters, PH; Igali, L; Tjønneland, A; Halkjær, J; Touillaud, M; Perquier, F; Fagherazzi, G; Amiano, P; Ardanaz, E; Bredsdorff, L; Overvad, K; Ricceri, F; Riboli, E; González, CA.						
Zamora-Ros, R; Knaze, V; Luján-Barroso, L; Romieu, I; Scalbert, A; Slimani, N; Hjartåker, A; Engeset, D; Skeie, G; Overvad, K; Bredsdorff, L; Tjønneland, A; Halkjær, J; Key, TJ; Khaw, KT; **Mulligan, AA**; Winkvist, A; Johansson, I; Bueno-de-Mesquita, HB; Peeters, PH; Wallström, P; Ericson, U; Pala, V; de Magistris, MS; Polidoro, S; Tumino, R; Trichopoulou, A; Dilis, V; Katsoulis, M; Huerta, JM; Martínez, V; Sánchez, MJ; Ardanaz, E; Amiano, P; Teucher, B; Grote, V; Bendinelli, B; Boeing, H; Förster, J; Touillaud, M; Perquier, F; Fagherazzi, G; Gallo, V; Riboli, E; González, CA.	Differences in dietary intakes, food sources and determinants of total flavonoids between Mediterranean and non-Mediterranean countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) study	British Journal of Nutrition	109	8	1498-1507	2013
Zheng, Ju-Sheng; Imamura, Fumiaki; Sharp, Stephen J; Koulman, Albert; Griffin, Julian L; **Mulligan, Angela A**; Luben, Robert; Khaw, Kay-Tee; Wareham, Nicholas J; Forouhi, Nita G.	Changes in plasma phospholipid fatty acid profiles over 13 years and correlates of change: European Prospective Investigation into Cancer and Nutrition-Norfolk Study	The American journal of clinical nutrition	109	6	1527-1534	2019

Appendix 2: Copies of statements from co-authors confirming my contribution to the papers submitted for PhD by Publication

Gonville and Caius College
University of Cambridge
Trinity Street
Cambridge CB2 1TA
17 October 2023

To whom it may concern

Angela Mulligan: PhD by publication: evidence of contribution
Nutritional influences on ageing and disease in UK population: the EPIC-Norfolk study

I am writing to confirm the original contribution of Angela Mulligan to the publications:

- Weight change and 15 year mortality: results from the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) cohort study. Eur J Epidemiol. 2017;33(1):41-57. https://doi.org/10.1007/s10654-017-0343-y &

- Changes in waist circumference and risk of all-cause and CVD mortality: Results from the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) cohort study. BMC Cardiovasc Disord. 2019;19(1):1–15. https://bmccardiovascdisord.biomedcentral.com/articles/10.1186/s12872-019-1223-z

I was the principal investigator of the EPIC-Norfolk population study from inception till my retirement in 2018. The submitted papers make use of the data available from the EPIC-Norfolk cohort. Angela Mulligan was responsible for much of the detailed nutritional assessment work on participants in this study including development of many of the tools and databases now in widespread use.

Based on her review of the existing literature, Angela Mulligan identified the questions to be explored on the relationship between changes in weight and fat distribution and health, outlined hypotheses to be tested in these two papers, designed the analytic approaches, including definition and construction of the new variables necessary, conducted all the statistical analyses, interpreted the results, and prepared the work for publication entirely independently. While necessarily based on a long standing population study, these publications are due to the original contributions from Angela Mulligan and her leadership of this work.

Kay tee Khaw

Kay-Tee Khaw MBBChir, FRCP, FFPHM, FMedSci, CBE
Professor Emeritus, University of Cambridge
Life Fellow, Gonville and Caius College

NIHR Moorfields Biomedical Research Centre
UCL Institute of Ophthalmology
11-43 Bath Street,
London EC1V 9EL
23rd August 2024

Dear Sir/Madam,

I would very much like to support Angela Mulligan's application for PhD by Publication at UEA entitled 'Investigating the impact of nutrition and body composition on mortality risk, diseases of ageing and inflammation in the EPIC-Norfolk cohort'. I am writing to confirm that Angela Mulligan had significant involvement on the following publication:

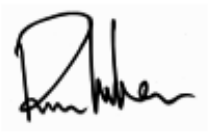
Mulligan, A.A., Hayhoe, R.P.G., Luben, R.N., Welch, A.A.
Positive Associations of Dietary Intake and Plasma Concentrations of Vitamin E with Skeletal Muscle Mass, Heel Bone Ultrasound Attenuation and Fracture Risk in the EPIC-Norfolk Cohort. Antioxidants 2021, 10, 159. https://doi.org/10.3390/antiox10020159

I am a Principal Research Fellow at UCL Institute of Ophthalmology and an Honorary Senior Visiting Fellow at the MRC Epidemiology Unit, University of Cambridge. Between 1994 and 2018, I was a member of the EPIC-Norfolk team at the University of Cambridge and worked closely with Angela over this period.

Angela worked on nutrition-related research in EPIC-Norfolk almost since its inception and is responsible for constructing a large part of underlying research data used in this area. She has contributed to many aspects of EPIC-Norfolk, involving both directly measured and questionnaire data from numerous phases and follow-up approaches.

Angela was the first author on the above paper and made the largest contribution to the publication. The material examined in the study used measurements made during the first and second health examinations including anthropometry and heel bone ultrasound; blood collection and nutritional biomarkers and dietary instruments including the seven day diet diary. Angela was directly involved with and made a significant contribution to these collections. She also developed the software and databases used for the analysis of nutritional data used in the study. Angela conceived the work together with colleagues, wrote the text, analysed and modelled the data and created tables.

Yours faithfully,



Robert Luben

University of East Anglia | NORWICH MEDICAL SCHOOL

Norwich NR4 7TJ

Telephone
01603 593120
Email
jane.skinner@uea.ac.uk

21/08/2024

To whom it may concern

Dear Sir/Madam

I am delighted to write in support of Angela Mulligan's application for award of PhD by Publication at the University of East Anglia, and to confirm Angela's very significant involvement in the following publication:

Mulligan, A.A.; Lentjes, M.A.H.; Skinner, J.; Welch, A.A. The Dietary Inflammatory Index and Its Associations with Biomarkers of Nutrients with Antioxidant Potential, a Biomarker of Inflammation and Multiple Long-Term Conditions. Antioxidants 2024, 13, 962. https://doi.org/10.3390/antiox13080962

Angela wrote the first draft of the manuscript and carried out all data preparation and statistical analyses. She co-devised the research question and the analytical approach in collaboration with her co-authors.

Yours faithfully,

JSkinner

Dr Jane Skinner
Lecturer in Medical Statistics

The Ipswich Hospital NHS | James Paget University Hospitals NHS | Norfolk and Suffolk NHS
The Queen Elizabeth Hospital King's Lynn NHS | Norfolk and Norwich University Hospitals NHS