SUPPLEMENT USE, VITAMIN C INTAKE AND BREAST CANCER RISK IN UK WOMEN

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DECLARATION

I confirm that the work submitted is my own, except where work which has formed part of jointly authored publications has been included. My contribution and the contribution of the other authors to this work has been explicitly indicated below. I confirm that appropriate credit has been given within the thesis where reference has been made to the work of others.

Chapter 6, relating to vitamin C supplement taking and family history of cancers, incorporates a large part of a jointly authored published paper.[1] I was jointly responsible for the design of the analysis, along with Janet Cade, Victoria Burley and Darren Greenwood, and I undertook the analysis and interpretation. Darren Greenwood provided statistical advice. I was the lead author responsible for writing and submitting the manuscript, and incorporated comments from the above co-authors. James Thomas managed the creation of the UKWCS supplement databases, and also enabled the calculation of vitamin C intake from all supplements used. I undertook an extensive final cleaning of the participant supplement database for these supplements.

Chapter 10, relating to vitamin C intake from diary recordings and risk of breast cancer in the UK Dietary Cohort Consortium, incorporates work from a jointly authored published paper.[2] The following co-authors contributed to the design, development and establishment of the consortium of five cohorts from the Universities of Oxford, Cambridge, Leeds and UCL: Marleen Lentjes, Darren Greenwood, Victoria Burley, Janet Cade, Cristina Cleghorn, Diane Threapleton, Tim Key, Benjamin Cairns, Ruth Keogh, Christina Dahm, Eric Brunner, Martin Shipley, Diana Kuh, Gita Mishra, Alison Stephen, Amit Bhaniani, Gabor Borgulya and Kay Tee Khaw. I provided supplement data from the UKWCS and EPIC-Oxford cohorts, and checked and recoded UKWCS covariates for the pooled dataset used in this thesis. Marleen Lentjes supplied EPIC-Norfolk supplement data. I was jointly responsible for the design of the vitamin C analysis, along with Janet Cade, Victoria Burley and Darren Greenwood, and I undertook the analysis and interpretation. Darren Greenwood provided statistical advice. I was the lead author responsible for writing and submitting the manuscript and the other co-authors were invited to review it. Marleen Lentjes, Janet Cade, Victoria Burley, Darren Greenwood, Ruth Keogh and Ben Cairns provided the majority of comments, most of which were incorporated into the manuscript.

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ABSTRACT

Background: When analysing relationships between breast cancer risk and vitamin C intake, few prospective studies have included vitamin C intake from supplements, or excluded general supplement users in dietary only analyses. For the first time these relationships are explored in UK women, and from diary recordings.

Methods: The UK Women's Cohort Study was used in prospective breast cancer risk analyses examining exposures from: general supplement use; fruit and vegetable intake; and dietary only vitamin C intake recorded at baseline using FFQs and additional questions for 33,000 women (~1,000 cases); vitamin C contained in supplements recorded by diaries at phase 2 for 11,000 women (239 cases); and total vitamin C intake from diet and supplement recorded by diaries in pooled UK nested case-control studies (851 cases 2727 controls).

Results: There was no evidence of dose-response relationships between breast cancer risk and vitamin C intake from diet, supplements or both, or from fruit and vegetable intake; risk estimates were non-significant and generally close to unity. There was some evidence that risks differed by menopausal status and supplement use. There were no significant associations for non-users of supplements or post-menopausal women by continuous estimate or intake category. Risks were raised for pre-menopausal women who were: frequent users of supplements containing low vitamin C (1-60mg/d) (HR=2.37; 95% CI: 1.32, 4.27; p=0.004); daily multivitamin users (HR=1.51; 95% CI: 0.90, 2.54); or general supplement users (HR=1.14; 95% CI: 0.91, 1.43), compared to non-users of these supplements. Women with a history of breast cancer were significantly more likely to be high dose vitamin C users (≥1000mg/d).

Conclusion: There was no evidence of significant associations between vitamin C intake per se and breast cancer incidence, even at high doses. The increased risk for pre-menopausal women taking supplements containing low dose vitamin C may be due to other ingredients.

TABLE OF CONTENTS

List of tables

List of figures

Abbreviations

AICR	American Institute for Cancer Research
BRAC1	Breast Cancer type susceptibility protein 1
BMI	Body Mass Index
BMJ	British Medical Journal
CNC	Centre for Nutritional Epidemiology in Cancer Prevention and Survival
DAG	Directed Acyclic Graph
DANTE	Diet and Nutrition Tool for Evaluation
DHA	Dehydroascorbic Acid
DNA	Deoxyribonucleic acid
DIDO	Diets in Nutrients Out
DINER	Diet into Nutrients for Epidemiological Research
EFSA	European Food Standards Authority
EPIC	European Prospective Investigation into Cancer
ER	Oestrogen Receptor
EU RDA	European Recommended Daily allowance
FFQ	Food Frequency Questionnaire
HR	Hazard Ratio
HRT	Hormone Replacement Therapy
IGF	Insulin-like Growth Factor
IQR	Inter-quartile range
NICE	National Institute of Clinical Excellence
NSHD	National survey of Health and Development
OR	Odds Ratio
PR	Progesterone Receptor
RCT	Randomised Control Trial
RDA	Recommended Daily Allowance
RNI	Recommended Nutrient Intake
ROS	Reactive Oxygen Species
RR	Risk Ratio
SHBG	Sex-Hormone-Binding Globulin
UKWCS	UK Women's Cohort Study
WACS	Women's Antioxidant Cardiovascular Study
WCRF	World Cancer Research Fund
WHI	Women's Health Initiative Study
WHO	World Health Organisation

CHAPTER 1

1 Introduction and Objectives

Breast cancer is the most common cancer in women in the UK, and in many other western countries.[3 4] The cumulative risk of being diagnosed with breast cancer in the UK was 6% by the age of 65, and 11% over a lifetime, based on cancers diagnosed between 1994 and 1997.[5] The latest estimate of life-time risk increased to 12.5% (.i.e. 1 in 8) based on breast cancers diagnosed in UK women in 2008.[6]

Whilst age, genetics, hormonal and reproductive factors are established breast cancer risk factors, there is evidence that lifestyle factors such as diet, weight and exercise can also contribute to risk (as discussed in chapter 3).[7] Initial research using retrospective recall methods indicated that fruit and vegetable intake probably reduced the risk of many cancers, including breast cancer.[8] Subsequently the 5-A-day fruit and vegetable initiative, based on the World Health Organisation (WHO) recommendations,[9] was introduced in the UK in 2001 to encourage healthy lifestyles with a view to reducing cancer risk in general, as well as the risk of other chronic diseases. Since then, however, an increasing number of null associations between breast cancer risk and prospective recordings of fruit and vegetable intake have been published; consequentially overall there is no convincing evidence of a protective effect.[7 10-13] Similarly, studies of antioxidant intake have often produced conflicting results between observation methods.[7] Furthermore, a recent Randomised Control Trial (RCT) using several types of antioxidant supplements found no significant associations with breast cancer incidence.[14] Although media reporting of conflicting studies may have caused many people to ignore dietary advice relating to cancer risks,[15] many health conscious women remain interested in such information. For instance, a study of UK women who were sufficiently health conscious to attend mammography screening for breast cancer, reported that the majority were interested in obtaining dietary and exercise advice at these clinics; many of these women were interested in advice about how to reduce risk of serious diseases.[16]

In addition to maintaining healthy lifestyle behaviours, many health conscious women tend to take supplements, moreover levels of use in general continue to rise in the UK (section 2.1) and Europe, as well as in the USA. Even though the majority of women are likely to use supplements for reasons other than specifically reducing their cancer risk, research is needed to determine whether some supplement types may increase the risk of cancers. The 2007 World Cancer Research Fund (WCRF) report has systematically reviewed research on cancer risk and diet and supplement use.[7] Some

of these and more recent studies are discussed in section 3.6, particularly in relation to vitamin C which is a common ingredient in supplements and a common micronutrient in fruit and vegetables. The majority of research on supplements has been undertaken in the USA; however it is important to evaluate the effects of supplementation on different populations, with different diets and levels of food fortification. No published research has assessed the effect of supplementation on cancer risk in the UK population.

The aim of this current research is to explore associations between the risk of developing breast cancer in UK women and intake of vitamin C from diet and supplements, and dietary supplements in general. Associations with any supplement use (chapter 7) and with vitamin C supplementation (chapter 8) one of the most popular supplements, are assessed in relation to breast cancer risk. The analysis of vitamin C intake from all supplement types categorised in relation to recommended daily allowance and high doses of vitamin C (see section 2.2.1 for definition) leads onto a brief analysis of risks relating to the use of multivitamins (chapter 8).Total vitamin C intake, which includes both supplement and dietary intake, is assessed (chapter 10) as is dietary vitamin C intake sub-analysed by users and non-users of any supplements (chapter 9). The analysis of non-users provides a clearer picture of the effects of dietary vitamin C intake on breast cancer risk, without the influence of the variety of different supplement types taken by many of the women. Breast cancer risks in relation to fruit and vegetable intake, the main source of vitamin C, are also explored in chapter 9.

Since UK women generally obtain sufficient nutrients from their diet, it is hypothesised that women who maximise their intake of vitamin C by taking high doses of this water-soluble supplement on a daily basis may have a higher risk of breast cancer due to pro-oxidation mechanisms or to the reduction of beneficial apoptosis. These mechanisms are discussed in more detail in section 2.2.4. Conversely, women who have a low vitamin C intake may have an increased risk of breast cancer particularly if they have high levels of damaging free radicals and generally have a low antioxidant status.[17] Consequently, the analysis of a population with a wide intake range may produce a U-shaped risk curve. It is also possible that vitamin C or other micronutrients could increase breast density which has been linked to breast cancer risk.

The analyses use pre-gathered prospective cohort data incorporating a wide range of intakes enabling important vitamin C dose-response relations to be examined, which would not be possible using RCTs where only one or two doses can be evaluated at a time. The thesis is original since no research on any of the above associations has been conducted on the UK population. Furthermore, the total vitamin C analysis utilises both supplement and dietary vitamin C intake recorded by diary as opposed to

questionnaire methods used in previous research in other countries. Evaluation of vitamin C intake by diary method in comparison to the questionnaire method is covered in chapter 5. Fruit and vegetable intake is also evaluated here.

Chapter 6 explores cross-sectional associations between taking supplements containing vitamin C and a personal or family history of cancer, primarily to identify whether these women are more likely to frequently take high dose vitamin C. Women who reported a family history of breast cancer, who may have had different health behaviours and cancer risks compared to other women, were identified and excluded in some sensitivity analyses of risks. Unfortunately numbers were too low to power risk sub-analyses by family history or by those estimated to be genetically at raised risk or high risk of breast cancer (defined in section 4.7.6.3 and 0). Other potential confounding characteristics of women are discussed in sections 3 4.7.5 and 4.7.5.2.

All the analyses use data from the UK Women's Cohort Study (UKWCS), either from the baseline FFQs (35,000 women), phase 2 diaries (12,500 women) or phase 2 questionnaires. The relationship between the available data and the thesis objectives are shown in Figure 1 of this introduction. Further details of the cohorts and tools used to capture the intake data can be found in sections 4.1 to 4.6 of the methods chapter. Unfortunately total vitamin C intake (diet plus supplements) could not be analysed for the full UKWCS cohort members at either phase; at baseline details of supplement types used were not electronically captured, and at phase 2 dietary intake recorded from diaries were only electronically captured for a small nested case-control analysis. However, total vitamin C was analysed by pooling nested case-control data from the UKWCS and two other UK cohorts: EPIC-Norfolk and EPIC-Oxford which are part of the European Prospective Investigation into Cancer (EPIC) and are also discussed in the main methods chapter, chapter 4.

1.1 Objectives

1. To evaluate vitamin C intake in UKWCS (Chapter 5):

    a. To compare the frequency of vitamin C supplement use in the UKWCS recorded by diaries with that recorded by the follow-up phase 2 questionnaire.

    b. To compare the baseline dietary vitamin C intake with fruit and vegetable intake in the UKWCS.

    c. To compare ascorbic acid blood plasma levels in a sub-sample of the UKWCS with derived vitamin C intake from diary recordings and also from Food Frequency Questionnaires (FFQs).

2. To determine what characteristics of women predict vitamin C supplement use at phase 2 in the UKWCS. To determine whether women who have a history of breast cancer are more likely to use high dose vitamin C supplements (Chapter 6).

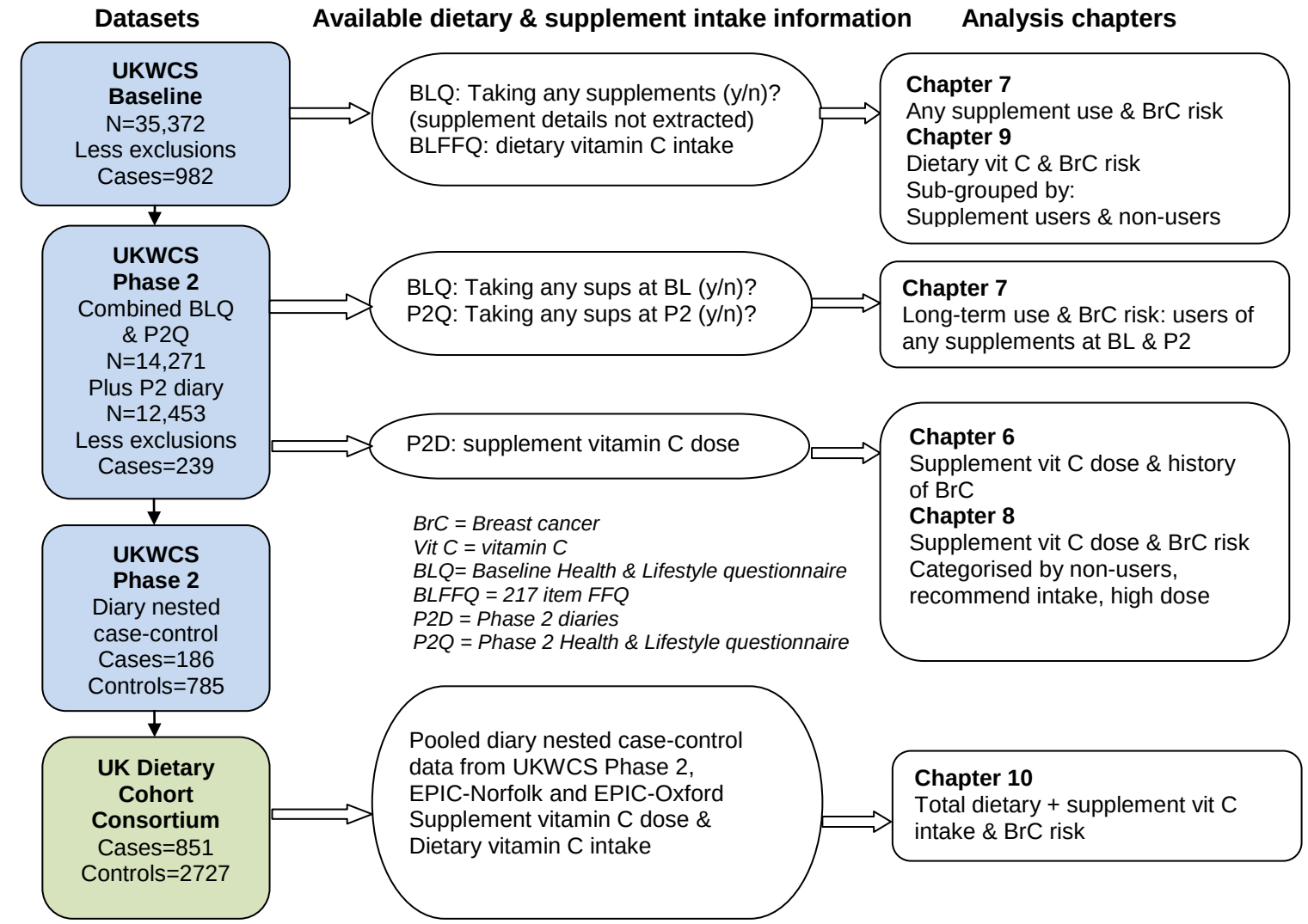
3. To determine whether there is an association between any supplement use and breast cancer risk at baseline in the UKWCS and whether the associations are modified by sub-factors. To determine whether the risk is different for long-term users: women who have taken supplements at both baseline and phase 2 of the UKWCS (Chapter 7).

4. To determine whether there is an association between vitamin C intake from supplements and the risk of breast cancer at phase 2 in the UKWCS, categorised in relation to the European Recommended Daily Allowance (60mg/d) and high dose (500mg/d) use (Chapter 8)

5. To determine whether there is an association between dietary vitamin C intake and the risk of breast cancer at baseline in the UKWCS, and whether it is modified by supplement use of any type (Chapter 9).

6. To determine whether there is an association between total vitamin C intake (supplements plus dietary) and the risk of breast cancer in the UK Dietary Cohort Consortium using pooled nested case-control data from UKWCS, EPIC-Norfolk and EPIC-Oxford (Chapter 10).

Figure 1 Flow chart of datasets, assessment tools & intake data available for use in risk analyses



The UKWCS and two of the other cohorts in the UK Dietary Cohort Consortium are some of the largest population-based prospective studies in the UK which were designed for examining associations between diet and chronic diseases, and are ideal studies to investigate the objectives above. Their large size increases the power of the analyses. Another major strength of these studies is their prospective nature which minimises reverse causality and recall bias (responder bias) which can affect results of retrospective case-control studies. Additionally the studies have captured many important health and lifestyle factors which may be potential confounders and can be adjusted for in the analyses. Furthermore, the majority of the analyses in this thesis use previously unexploited supplement data.

The UKWCS, used in all the analyses, has an extensive database of supplements used and covers a wider range of vitamin C intakes than most studies which have reported on similar objectives. Women from baseline and phase 2 in the UKWCS were followed-up for cancer incidences for over 11 and 7 years respectively, which is comparable to or better than most studies reviewed in section 3.7.

The UKWCS, instigated through the World Cancer Research Fund, in general comprised of health conscious women. The results are therefore directly applicable to similar women; who would be interested to know how lifestyle choices could affect their risk of breast cancer, and who may be prepared to alter their behaviours. In addition, the UK Dietary Cohort Consortium of other UK studies provides results that are more generalised to the UK population as a whole, as well as providing an opportunity to examine total vitamin C intake in relation to breast cancer risk using diary data.

First, supplement use and vitamin C in particular will be discussed in chapter 2, and then a review of literature about life-style factors affecting breast cancer risk, including diet, will be described in chapter 3, before systematically reviewing articles which examine the influence of vitamin C intake on breast cancer risk. Chapter 4 then describes the methods used in this thesis, which is followed by the results chapters (5-10) and the summary discussion in chapter 11.

CHAPTER 2

2 Supplement use and vitamin C

Supplements are proactively marketed in the developed world in a multi-billion pound industry, retailed through shops, mail order and the internet. One of the largest producers and consumers of supplements is the United States (US). Annual sales in the US since 2004 are estimated to have been over $20 billion per year, about 1% of the US health expenditure, providing employment for about 200,000 people directly and over a quarter of a million indirectly.[18] The number of different types of supplements marketed in the US may be between 29,000-50,000.[18]

Both in the US and Europe supplements are classified as food rather than drugs. In the US the Food and Drug Administration (FDA) regulates the supplement industry, with powers to take action relating to quality, unsafe levels, and false claims or misleading labelling. Although US manufacturers are allowed to make general health claims, any claims relating to treatment, prevention or cure for a specific disease or condition would be considered illegal.[19] Regulation has been similar in Europe under the Food Supplements Directive 2002.[20] However, the European Food Safety Authority (EFSA) has implemented regulations for approving or rejecting general health claims made on foods and supplements based on whether these claims are independently verified by research studies.[21] Additionally, safe or tolerable upper limits have been set by the EFSA for many supplements,[22] but many high dose products do not state warnings on their packages as required.

2.1 Supplement use in the UK

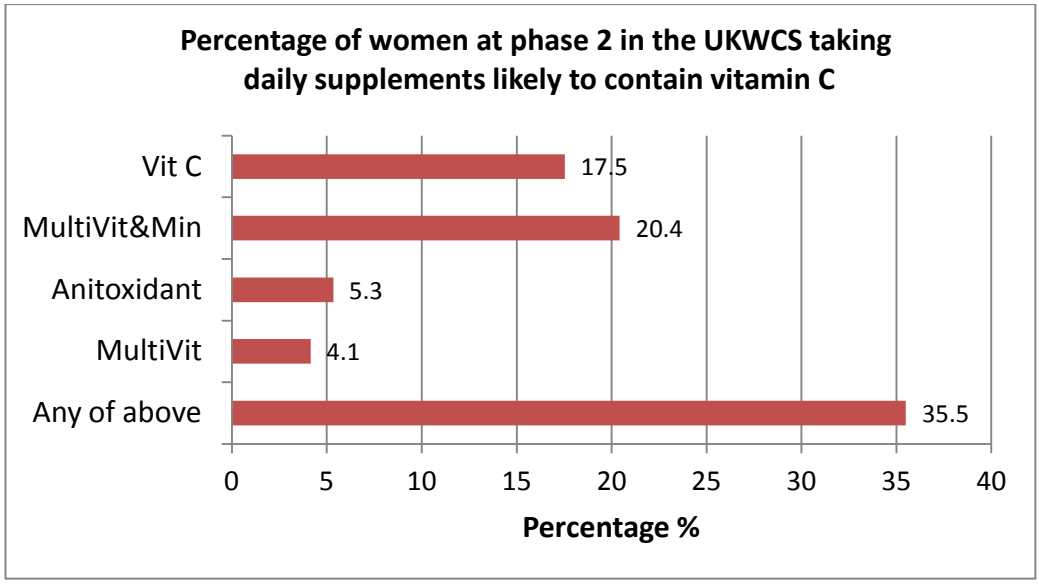
Sales of supplements in the UK in 2009 were estimated to be £396M.[23] Vitamin supplement use reported by UK women increased from 17% in 1986/1987 to 41% reported in 2008/9.[24 25] A 2008 UK survey reported users were most likely to be women above 55 years and of higher socioeconomic status.[26] An analysis of the UK Women's Cohort Study (UKWCS) found that users were significantly more likely to lead healthier lifestyles; to be more physically active; have a lower alcohol intake; a lower body mass index (BMI) and eat diets which met recommended dietary intakes.[27] Therefore they were less likely to need supplements than non-users.[27] Further support for this 'inverse supplement hypothesis' has been found in the UK,[25 28 29] and elsewhere.[30-35] Moreover, those classifying themselves as high strength supplement users in a recent UK survey were particularly health conscious.[26]

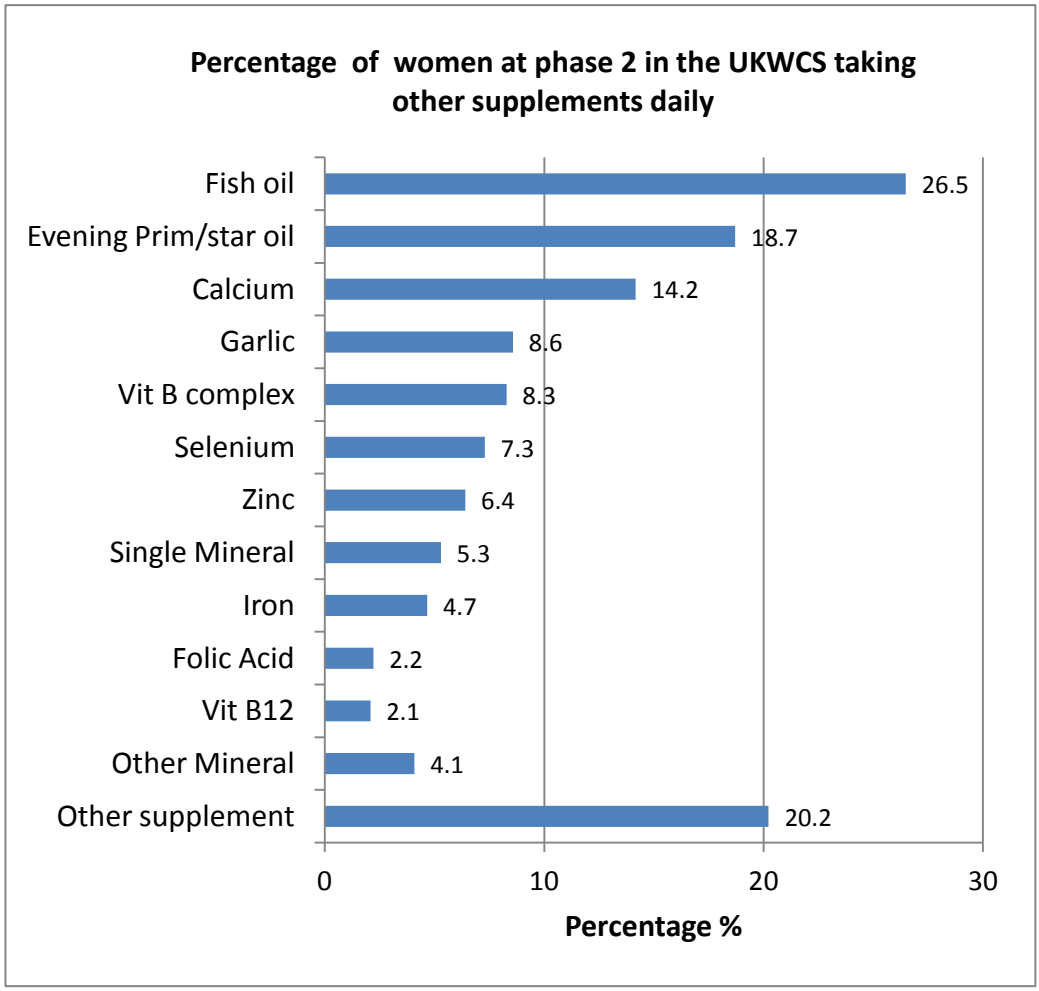
An exploration of reasons behind supplement use in a UKWCS sub-sample of 303 woman, incorporating the theory of planned behaviour and other potential predictors,[36] found supplement-taking was predicted by an individual's value of health and their perceived ability of supplements to reduce susceptibility to illness.[37] Supplement use was associated with a perception that supplements were particularly able to reduce development of colds, flu, arthritis and rheumatism, and also to have a protective effect on the development of heart disease, anaemia and menstrual problems.

General health and well-being was the most common reason (57%) given for taking supplements in the 2008 UK Random Location Omnibus Survey.[26] Ten percent of the 801 sample of both men and women were taking them to ward off colds, 11% of full time workers were taking them because they believed they had an unbalanced diet, 26% of over 45 year olds were taking them for a specific health reason, and 17% of women were taking them due to advice from a health practictioner.[26] High strength supplement users in particular considered the health implications of what they ate, actively looked for information about how to stay healthy and believed that they needed vitamin and mineral supplements to feel and stay healthy.[26] Additionally they were more likely to take other supplements than those not taking high strength supplements (52% vs. 43%). Thirty eight percent of those surveyed, an estimated 15% of the population, reported taking high strength supplements.[26] Sixteen percent of users bought supplements because of their high strength content; however only 68% of these users were fully aware that high doses may cause adverse side effects.[26] Only 6% of high strength users were likely to report they were in excellent health compared to 25% of non-users.[26]

The most commonly taken supplements in the 2008 UK survey were multivitamins (36%), cod liver oil (35%) and vitamin C (24%).[26] In the EPIC-Norfolk study only 4.7% of women took single supplement vitamin C, the most popular supplements being cod liver oil (24.5%) and multivitamins, with or without minerals (10.4%).[38] The percentages of women reporting daily use of supplements by questionnaire in the UK Women's Cohort Study (UKWCS), the main study in this thesis, are detailed by type in Figure 2. This shows vitamin C, the focus of this thesis, is one of the most commonly taken nutrients in supplements both as a single supplement and in combination with other nutrients.

Figure 2 Percentage of women reporting daily supplement use in the UKWCS phase 2 questionnaires by supplement type





2.2 Vitamin C

Vitamin C ($C_6H_8O_6$) exists as ascorbic acid (the reduced state) and dehydroascorbic acid (DHA, the oxidised state) in both food and the human body. Humans are one of only a few species that cannot synthesis vitamin C, and therefore obtain this micronutrient from fruit, vegetable and other plant food. It is widely present in plant tissues, for instance in peppers, oranges, lemons, strawberries, grapes, brussel spouts, broccoli, cauliflower and tomatoes.[39] The storage, processing, and cooking of foods can reduce vitamin C content substantially therefore fresh or steamed produce is recommended. Although natural and synthetic forms are chemically identical and have similar bioavailability, the added benefits of other bioactive components from food are lost if vitamin C is obtained predominately from supplements.

Vitamin C has a number of functions in the body, and supplementation is often necessary in malnourished populations such as the chronically sick and the elderly, as well as in developing countries. Low doses of vitamin C, 10mg/day, are needed to prevent scurvy,[40] and doses of 20mg/day are necessary for optimum wound healing.[41] Symptoms of scurvy, which is now uncommon in developed countries, include bleeding gums, easy bruising, impaired wound and fracture healing, joint pains, fatigue and depression.[39] Vitamin C acts as a cofactor for enzymes in the synthesis of collagen, carnitine, tyrosine and the neurotransmitters by maintaining metal ions within the enzymes in a reduced state.[42] Collagen is the main protein found in body structures such as skin, bones, teeth, cartilage, tendons, blood vessels and eye lens and cornea. Carnitine is involved in the transport of fatty acids into mitochondria for energy production. There is evidence that vitamin C suppresses the oxidation of low density lipoproteins (LDL) which are implicated in atherosclerosis.[43] Through electron donation ascorbic acid acts as a reducing agent i.e. an antioxidant, by scavenging oxygen and nitrogen free radicals and also regenerates the fat-soluble vitamin E antioxidant (α-tocopherol). The oxidised form of vitamin C, DHA, is recycled back to vitamin C by several enzyme systems within cells. Additionally vitamin C supplementation is found to increase the absorption of non-haem iron from plants, probably by reducing iron to its ferrous state (Fe^{2+}).[44]

Pharmacological uses of vitamin C, though not necessarily evidence based, include protection against cardiovascular disease, reducing histamine, boosting immune function, aiding iron absorption, protecting against cataracts, as well as use with the aim of reducing symptoms of the common cold and prolonging survival in cancer patients.[40] Ascorbic acid (E300) and its esters are also added to food as browning inhibitors, reducing agents, flavour stabilisers, dough modifiers and colour stabilisers.[42]

Suggestions that vitamin C is able to reduce the incidence of colds have been unsubstantiated in randomised controlled trials.[45] Additionally, food and supplement manufacturers often make claims for vitamin C in relation to protection of cells from premature ageing, antioxidant activity, antioxidant content and antioxidant properties, protection of DNA, proteins and lipids from oxidative damaging. However, the EFSA has recently reported that no evidence has been provided to them to establish that having antioxidant activity/content and/or antioxidant properties exerts beneficial physiological effects on humans.[46] In future manufacturers should not make these claims.

2.2.1 Vitamin C recommended intake

Table 1 shows current recommended vitamin C intakes, primarily to prevent scurvy and for optimum wound healing, and recommended safe upper levels set to minimise gastrointestinal disturbances.[47] Most supplements sold in the UK quote doses as a percentage of the EU Recommended Daily Allowance of 60 mg/day for adults. A meta-analysis indicated that daily intake of 60mg vitamin C would produce average plasma levels of ascorbic acid of 42.4 µmol/L.[48] A higher recommended daily allowance of 100 mg/d has been recommended to maintain average plasma concentrations of ascorbic acid at 50 µmol/L,[48 49] which has been suggested as optimum.[50]

Table 1 Current recommended vitamin C intakes

	Recommended daily intake	Recommended safe upper levels
UK	RNI 40 mg/day[51]	None set: insufficient data[52]
Europe	RDA 60 mg/day[53]	None set: insufficient data[22]
US	RDA 75 mg/day for women	2000 mg/day[47]

RNI = Reference Nutrient Intake

RDA = Recommended Daily Allowance

RDA = EAR + 2 SD_{EAR} (EAR: Estimated average requirement; SD_{EAR}: Standard deviation of the EAR)

The most clearly defined side effect of taking large doses of vitamin C is abdominal pain and osmotic diarrhoea due to unabsorbed vitamin C being metabolised by intestinal bacteria.[54] Although vitamin C does not accumulate to toxic levels like fat soluble vitamins, high intake may increase the risk of renal stones in some at risk individuals due to the increased excretion of the vitamin C metabolite oxalate. A recent review of clinical trial evidence concluded that doses of 2000mg/day are safe for most adults,[55] other than those genetically at risk.[56] Individuals with genetic iron disorders are at risk of iron overload and development of haemochromatosis or thalassaemia if

they consume high doses of vitamin C since it aids the absorption of dietary non-haem iron.[56] Rebound scurvy has been reported anecdotally, and was thought to occur after abrupt cessation of long-term high intakes of vitamin C due to established accelerated metabolism or disposal of ascorbic acid, but the results appear to have been misinterpreted,[41] and systematic conditioning appears to be minimal.[57] Evidence shows that plasma ascorbic acid concentrations do fall in well-nourished individuals in such circumstances, but to within normal levels.[57]

In its 2004 review of adverse effects of vitamin C, the EFSA suggested an upper limit of 1000mg/day,[58] however both the EFSA and the UK Expert Group on Vitamins and Minerals currently believe there is insufficient data to set an upper limit.[22 52] The EFSA acknowledged there was conflicting and insufficient evidence to set limits relating to breast cancer and vitamin C intake and welcomed future research on its safety.[22 58]

2.2.2 Vitamin C bioavailability, absorption, transport and excretion

The bioavailability of vitamin C is the effectiveness with which it is released from the source into the tissues of the body. Natural and synthetic vitamin C are chemically identical and the bioavailability of vitamin C from food sources and from supplements have been reported to be similar.[59] 'Slow release' or 'time release' vitamin C supplements and those which also contain bioflavonoids are promoted as having increased bioavailability, though evidence for this is lacking.

At low doses ascorbic acid is actively transported by sodium ions at the brush border membrane in the small intestine[60]. DHA, the oxidised form, diffuses into cells facilitated by glucose transporters, and is immediately reduced to ascorbic acid, creating a concentration gradient which drives the movement of the former.[60] At low doses of about 20mg/day absorption of vitamin C is about 98%;[61] at normal intakes of between 30-180mg/day absorption is about 70-95%.[54,62] Absorption falls to 50% at doses of 1.5g/day, 25% at 6g and 16% at 12g/day.[63] In plasma, vitamin C is transported mainly as water-soluble ascorbic acid, normal levels being about 30-70 µmol/L.

Vitamin C is stored in the body, although not to the same extent as fat soluble vitamins which have stable reserves and associated toxicities. Ascorbic acid is stored in white blood cells and is also distributed to all tissues of the body. Body stores can be more than 100 times greater than plasma content, the greatest concentrations being found in the adrenal and pituitary glands,[64] and the largest amount in the liver.[60] The ascorbic acid content of a range of leukocytes types generally reflect body stores, whereas plasma levels reflect recent vitamin C intake which fall more rapidly in depletion

studies. The maximum body pool of vitamin C has been estimated to be about 1500 mg.[62]

The bioavailability and therefore plasma levels of vitamin C as water-soluble ascorbic acid are determined by the rate of intestinal absorption and of excretion.[60] The relationship between vitamin C intake and plasma ascorbic acid is S-shaped; between doses of 30-100 mg/day plasma levels and increases linearly with increasing intake in healthy women, with plasma and circulating cell saturation occurring between 200-400 mg/day.[65] Excretion from the body in urine starts at intakes of about 100mg/day before tissue saturation is reached,[61,54] and continues to increase steadily if dosage is increased. However, oral doses of 500 mg and 1250 mg are not entirely excreted in urine by women;[65] most likely due to a decline in intestinal absorption at high doses.[63] Therefore, it appears that intakes as high as 200-400 mg/day are utilised in the body by healthy women, but whether these levels have optimum health effects is unknown. Urine excretion as well as absorption limitations means intakes above these levels may not be utilised and may be unnecessary.

Due to the body's limited ability to store vitamin C, measurements of plasma concentrations are thought to represent intake only in the preceding few weeks.[66] Another measurement problem is that it is unstable in blood and rapidly deteriorates if not stabilised by chemicals such as metaphosphoric acid and stored at low temperatures.[67]

2.2.3 Characteristics associated with vitamin C plasma concentrations

Low ascorbic plasma concentrations have been associated with older age, smoking,[48] high blood pressure and in women with high cholesterol.[67] Cross-sectional analyses of the EPIC-Norfolk study have reported inverse associations between plasma vitamin C and BMI, waist-to-hip ratio (WHR), diabetes, prevalent undiagnosed hyperglycemia and HbA1c plasma levels (a measure of blood glucose levels).[68] [69] Low plasma concentrations may reflect increased antioxidant requirements in those with higher oxidative stress, associated with abdominal obesity.[68] Furthermore, the increased blood glucose levels in those with hyperglycaemia and diabetes may inhibit the uptake of DHA by glucose transporters;[70] DHA may metabolize and be excreted if not taken up by cells. In addition, plasma vitamin C has been inversely associated with the inflammatory marker C-reactive protein (CRP),[71] though an ability to reduce inflammation is inconclusive.[72] In addition supplement use in general is also positively associated with ascorbic acid levels.[67] Lifestyle factors may explain the link between plasma vitamin C levels and adiposity, HbA1c and CRP plasma levels.[68] [69] Indeed, high

levels of plasma vitamin C may be a marker for healthy diet, lifestyle and socio-economic position across the life course which appear protective of a range of conditions; factors such as exercise, low fat and high fibre diets, housing conditions and education have been associated with increasing vitamin C plasma concentrations.[73]

Furthermore, in the UK low ascorbic acid plasma concentrations have been associated with increased mortality from all-causes, from cardiovascular disease (CVD), ischaemic heart disease in men and women, and cancer mortality in men but not women.[67] The latter difference between sexes may possibly be due to the lower baseline antioxidant status of men. The associations remained after excluding diabetics and cigarette smokers, and after excluding supplement users (except for ischemic heart disease in women). Unfortunately, it was not possible to adjust for socio-economic status (SES) or physical activity which were likely to confound the results.[67] Associations between cancer risk and ascorbic acid plasma concentrations are given in section 3.6.4.1.

2.2.4 Potential mechanisms of vitamin C and other antioxidants in relation to cancer

Sales of supplements such as vitamin C, vitamin E, vitamin A, selenium and other antioxidants have been promoted by focusing on their ability to eliminate free radicals which cause DNA damage and thereby increase risk of chronic diseases.[74] However, their mechanisms in relation to cancer have only been partially elucidated.[75] As discussed below antioxidants may exert different influences at different stages of cancer development. This can be split into three stages: initiation where DNA damage and repair occurs; promotion where cell proliferation, involving the mutation of tumour-suppressor genes and the formation of oncogenes, can be reversed by programmed cell death; and progression which leads to the change of cells from benign to malignant and involves angiogenesis (the increased supply of blood vessels to the tumour) and metastasis (the spread of the cancer to other areas of the body).[76]

Vitamin C, as ascorbic acid, can readily donate electrons to suppress activities of free radicals such as reactive oxygen species (ROS). High levels of ROS such as super oxide ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2), the hydroxyl radical ($OH^{\cdot}$) and nitric oxide radical ($NO^{\cdot}$) are thought to initiate cancer through oxidative damage to DNA. ROS are produced internally by the release of electrons from mitochondria, white blood cells, peroxisomes and the detoxification enzyme cytochrome P450.[76] It is possible that antioxidant supplementation may prevent DNA damage during the initial stage of cancer in individuals with high levels of ROS which are not eliminated by endogenous antioxidants such as superoxide dismutase.[76] [77] Salganik et al. (2001) suggest

populations may need screening to identify those with high and low innate levels of ROS in order to determine appropriate supplementation.[77] However, levels of 8-OHdG, a marker of DNA damage, do not appear to reduce with vitamin C supplementation, except for individuals with very low intakes.[78] Higher baseline levels of 8-OHdG have been found in individuals who had lower serum ascorbic acid levels, those who did not use antioxidant supplements, or had low fruit and vegetable intake, or did not exercise regularly.[79] However, two months of supplementation with vitamin C (500mg/day) or vitamin E (400 IU/day) in this US RCT did not affect levels of 8-OHdG.[79] These results may indicate that supplementation may need to be prolonged to be effective or that high vitamin C intake or high serum ascorbic acid levels may be a marker of a good diet and healthy life style rather than a true protective factor against the initiation of cancer.[79]

One study has found a moderate but significant increase in breast cancer risk in women who had short telomeres and who had low dietary and supplement intake of antioxidants.[80] Telomeres protect genes from degradation, but appear to be detrimentally shortened by oxidative damage; therefore it may be possible that antioxidant activity could protect telomere length.[80]

Conversely, antioxidants may decrease protective functions of ROS which are necessary to suppress the promotion or proliferation stage of cancer which leads to the formation of preneoplastic cells.[76] As part of our immune system, phagocytes require ROS to enable them to destroy invading microorganisms and cancer cells. Excess ROS induce beneficial apoptosis, programmed death of damaged cells, without which uncontrolled cell division may lead to the progression of cancer.[29, 77, 81] The inhibitive effects of antioxidants on programmed cell death may be one reason why some studies have not shown antioxidant supplementation,[7] or vegetarianism to be protective of cancer.[82] In individuals who are constantly exposed to carcinogens such as tobacco, antioxidants may eliminate ROS preventing beneficial apoptosis whilst the carcinogen is left to promote cancer cells.[77] This may explain why the antioxidant β-carotene, a form of vitamin A, increases the incidence of lung cancer in smokers, but decreases the incidence in non-smokers.[7 83]

On the other hand, vitamin C appears to beneficially modulate the expression of tumour suppressor genes such as p53 and p73 which initiate apoptosis of damaged cells.[84] Therefore the effect of vitamin C at the proliferation stage of cancer may depend upon the balance it offers between the detrimental elimination of ROS required for apoptosis and its beneficial promotion of suppressor genes.

Some anticancer drugs such as cis-platin generate ROS and eliminate cancer cells by mediating apoptosis. It has been reported that the antioxidant alpha-tocopherol, a form of vitamin E, can reduce the amount of ROS and apoptosis produced by cis-platin in breast cancer cells, thus reducing its effectiveness.[77]. Although vitamin C can regenerate vitamin E and may inhibit apoptosis itself, research on cell-lines have shown it can increase the sensitivity of cis-platin.[85] However, a recent systematic review of antioxidant supplementation during chemotherapy indicated in general they improved survival times and tumour responses, and also reduced toxicities to the chemotherapy.[86] Mixed results were produced using high dose vitamin C supplementation on terminal cancer patients.[87-89] Results of trials using intravenous vitamin C for cancer therapy also produced mixed results.[39]

It is unclear whether ascorbic acid and other antioxidants can inhibit processes in the final stage of cancer development involving angiogenesis and metastasis, although mechanisms have been proposed.[84] Since vitamin C promotes collagen formation this may help maintain the extracellular matrix and prevent tumour cells for spreading. On the other hand, collagen formation will support the growth of new blood vessels needed for tumours to develop.[84]

Vitamin C as ascorbic acid can also act as a pro-oxidant by reducing 'free' transition metals such as copper and iron, which via the Fenton reaction with hydrogen peroxide leads to the formation of highly reactive and damaging hydroxyl radicals ($Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^{\cdot} + OH^-$).[76 77 84] Potentially high intakes of haem-iron in red meat plus ascorbic acid could initiate cancer;[90] indeed this combination was associated with an increase risk of lung cancer in the Iowa Women's Health Study.[91] However, since free iron or copper are not normally available in vivo because they are bonded to proteins such as ferritin, transferrin and metallothioneins, this hypothesis is controversial. Nevertheless, Kabet and Rohan (2007) propose that high levels of stored iron, particularly found in menopausal women, can be released by mechanisms involving infection, inflammation, high alcohol intake and oestrogen metabolites which via the Fenton reaction could increase breast cancer risk.[92] Conversely, since both iron and copper are more abundant in cancer cells than in normal cells, this pro-oxidant mechanism could be used to target the apoptosis of tumour cells, particularly since ascorbic acid has been found to mobilise copper ions.[93 94]

As discussed above, the overall effect of various mechanisms involving vitamin C or other antioxidants on the development of cancer in general is still unclear. With regard to specific cancers, vitamin C may reduce the risk of gastric cancer by suppressing the formation of nitrosamine compounds; however its effect is reduced when infection by H pylori is present.[95 96] A meta-analysis of RCTs, however, did not find a significant

protective effect of antioxidant supplements on gastric cancer.[97] The influence that vitamin C may have on breast cancer specifically is less clear since, as seen in the next chapter in section 3.5, this cancer is predominately influenced by hormones. Vitamin C may have a greater influence on non-hormonal cancers, including non-hormonal breast cancers. Nevertheless, as seen in section 3.4.2, vitamin C may affect breast density which is associated with breast cancer risk.

CHAPTER 3

3 Literature review of breast cancer risks

3.1 Introduction

The main approach used for the time-to-event analyses in this thesis is based on the lifestyle model, and in particular on the assumption that lifestyles during mid-life may affect the risk of developing adult chronic diseases. Later chapters in this thesis use recordings of diet and/or supplement intake during one time period in mid-life to estimate their influence on cancer risk seven or more years later. Other lifestyle factors measured at mid-life which may affect breast cancer risk, such as exercise and alcohol intake, are discussed in sections 3.5 and 3.6 of this chapter and most of these are taken into account in the analyses. Some factors highlighted in the life course approach to chronic diseases which may also influence breast cancer risk can also be controlled for in the analyses, such as age at menarche or age of first birth; these are discussed in section 3.5. This combination of approaches has been used in the majority of studies evaluating diet and cancer risk discussed in section 3.6. Cancer processes which may be affected by diet are also mentioned in section 3.6.

Cancer can take decades to develop and may be due to independent cumulative exposure effects or may be due to a chain of linked events. Within the life course approach there are models which emphasise critical periods such as gestation, childhood, adolescence, young adulthood as well as adult life.[98] Interaction effects between exposures in different critical periods may also occur.[98] Exposures during gestation, childhood and adolescence may, for instance, influence hormonal mechanisms linked to breast cancer risk, as discussed in section 3.5. Such data have not been gathered in studies used in the current analyses. Indeed, few longitudinal studies have recorded information from the pre-natal stage, though famine studies and animal studies may illuminate hypotheses based on pre-natal programming of adult cancer.[99] [100] Long follow-up studies on humans are expensive, time consuming and prone to attrition, and as yet there is no intermediate breast cancer end-point that can be easily employed.

Cancer occurs both through genetic modification to the DNA sequence and epigenetic modifications, i.e. changes in gene expression which occur without altering the DNA sequence. DNA sequence mutations occur due to damage from external factors such as sunlight and radiation, and from internal factors. Damaging reactive oxygen species

(ROS) are produced by a variety of normal intracellular processes, some of which are mentioned in section 2.2.4. Furthermore, there is substantial evidence that ROS become elevated as a result of intense exercise[101] or hyperglycaemia[102]

Epigenetic changes are manifest in DNA methylation, histone (chromatin) modification and non-coding RNAs (ribonucleic acid). Such alterations are transmitted to daughter cells during cell division and appear to be involved in most steps of tumour progression and development.[103] Although currently unclear, maternal diet and other exposures *in utero* have the potential to cause epigenetic changes to the foetus, such as mammary gland development, which may increase susceptibility to breast cancer.[99] Twin studies show that epigenetic changes can also accumulate over the life course, with older twins showing greater differences in DNA methylation and histone acetylation levels.[104] Environmental and dietary factors such as tobacco smoke, ionizing radiation, UV radiation, alcohol and folic acid may contribute to the accumulation of epigenetic changes during aging which cause cancer.[103] Moreover, there is evidence that epigenetic changes originating *in utero* in animals can be passed on to future generations.[105] Unlike genetic changes, epigenetic changes are reversible which means new cancer prevention and treatment strategies could be developed in the future.[103]

This chapter will review the literature relating to breast cancer risks. Rather than reporting individual studies which can produce conflicting results it focuses in general on larger or more recent pooling studies and reviews, particularly systematic reviews and meta-analyses. Many articles referenced in this chapter appear to be influential, as evident from their large number of citations. Methodological issues including study design, measurement tools employed, measurement error, confounding factors, bias, length of follow-up and the initial health of study members, can all affect results. In relation to nutrition, conflicting results may arise due to dose effects, and to interactive and synergistic effects of nutrients. First, a brief description of breast cancer types and a summary of incidence rates, trends and survival are given.

3.2 Classification of breast cancer

Lobular carcinoma in situ (LCIS) and ductal carcinoma in situ (DCIS) are non-invasive or pre-invasive cancers, sometimes termed pre-cancerous, which have not spread elsewhere. Respectively they describe cancer cells present in the lobes in the breast glandular tissue, and cancer cells present in the milk ducts between the nipple and the lobes. LCIS does not cause symptoms and usually does not show up on mammograms.[106]

If cancer cells do spread outside of the lobe it is called invasive lobular breast cancer. Similarly if the cancer cells have spread outside of the ducts it is called invasive ductal breast cancer; 70-80% of breast cancers diagnosed are of this type.[107] When cancer is also found in the lymph glands, such as those in the armpit or surrounding the breast, then cancer is likely to spread to other parts of the body via the lymphatic system; nevertheless this is still classed as early stage breast cancer. Secondary breast cancer, also called metastatic breast cancer or stage 4 breast cancer, is a term for cancer that has spread to other parts of the body.

Rare breast cancers include inflammatory breast cancers which occur when the lymph channels become blocked, and Paget's disease which starts at the nipple area.

Breast cancers are also classified according to the presence and type of hormone receptor or oncogene. If the breast cancer cells have oestrogen receptors present then the tumour is said to be oestrogen receptor positive (ER+). Other receptor statuses relate to the presence of progesterone receptors (PR) and Human Epidermal Growth factor Receptor 2 (HER2). Triple negative cancers do not have these receptors; this is often found in basal type cancers which are associated with women who have BRCA1 germ line mutations.

Treatment decisions are based on the TNM stage and the grade of each breast cancer case. The TNM stage classification system describes the size of the tumour or whether the cancer remains in situ (T1-4, Tis(DCIS), Tis(LCIS)), whether cancer has spread to the lymph nodes (N0-3), and whether it has spread anywhere else in the body i.e. metastasized (M0 or M1).[108] The grade of cancer describes whether it is a slow or fast growing cancer. Fast growing cancers are more likely to spread and return after treatment than slow growing cancers.

3.3 Breast cancer incidence, trends and survival

Breast cancer is the most common cancer in women and the second most common cancer overall in men and women worldwide.[4] Globally, there were an estimated 1,383,000 cases of malignant breast cancer in women in 2008, making up 10.9% of all cancer cases and 22.9% of all female cancers (excluding non-melanoma skin cancer).[4] The world age-standardised incidence rates (ASR) in 2008 for female breast cancer were 39 per 100,000 and the cumulative risk to the age of 74 or more was 4.1%.[4] Breast cancer incidence is substantially higher in more developed countries (except Japan) compared to less developed countries (ASR = 66.4 vs. 27.3 per 100,000). In Northern Europe in 2008 it was 84.0 per 100,000 and the estimated cumulative risk to the age of 74 was 8.9%.[4] Figures for North America were 76.7 per 1000,000 and 8.4%

respectively.[4] The higher incidence in developed countries may be partially due to better procedures to detect breast cancer cases earlier e.g. through surveillance, as well as better procedures to accurately record cases. Some African and Asian countries submitted no data at all to Globocan for the global cancer estimate, and their estimates were based on data from neighbouring countries,[4] though the accuracy and quality of the latter may not be good.

In 2008 there were an estimated 332,770 breast cancer cases in the European Union and 45,570 in the UK. The European age standardised rate (EASR) for breast cancer incidence in the European Union (EU) countries in 2008 was 103.7 per 100,000, and the EASR for the UK was 119.1 per 100,000; the UK has sixth highest rate of the EU countries (Figure 3).[3] In the UK, 31% of all female cancers were breast cancers, making it the most common cancer.[3] The lifetime risk in 2008 in the UK has been estimated as 1 in 8. [6] Figure 4 shows that breast cancer incidence rates have more than doubled in Britain since the mid 1970s.[109] Rates increased substantially after breast screening was introduced in 1988, which found previously undetected cancers.[110] HRT use during the 1990s is also believed to have contributed to the increased rates.[111] Figure 5 shows that the sudden increase in rates was mainly confined to the 50-64 age group who were invited to join the breast screening program.[109]

The estimated deaths (458,000) attributed to breast cancer in women globally in 2008 was substantially lower than incidence rates, being 12.5 per 100,000. Globally 13.7% of female cancer deaths were attributed to breast cancer, it was the most common death from cancer in females and the fifth most common overall in men and women.[4] In 2008 there was an estimated 332,800 breast cancer deaths in the European Union and 89,800 in the UK; 16.6% and 16.0% cancer deaths in the EU and UK related to breast cancer.[3]

The percentage of women still surviving five years after their first malignant invasive breast cancer diagnosis varies widely between countries. Survival rates at 5 years, age-standardised to the International Cancer Survival Standard weights, for women diagnosed between 1990-1994, ranged from 80% or over in North America, Sweden, Japan, Finland, and Australia to less than 60% in Brazil and Slovakia, and below 40% in Algeria.[112] Most European countries were in the 70-79% range, however the UK survival rate was 69.7%.[112] The five year survival rates have improved in England over the last thirty years, from 52% for women diagnosed in the early 1970s to 82% for women diagnosed between 2001-2006.[109] Five year survival rates are lower the later

the stage at diagnosis, ranging from 98% for very early stage to 18% for metastatic breast cancers in Europe.[113]

Figure 3 Estimated European age-standardised incidence and mortality rates in 2008 by EU country[3 109]

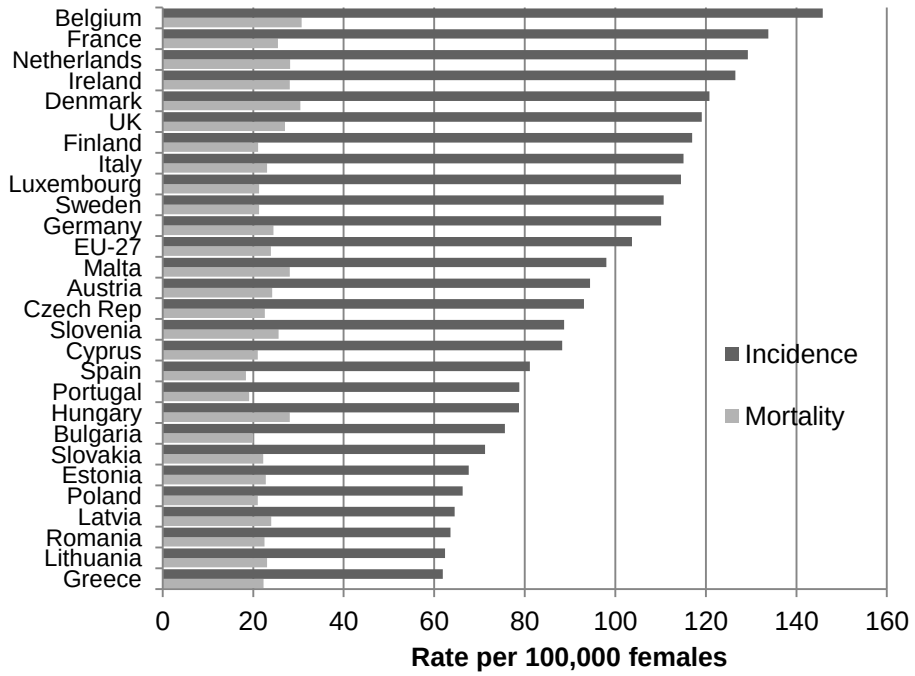
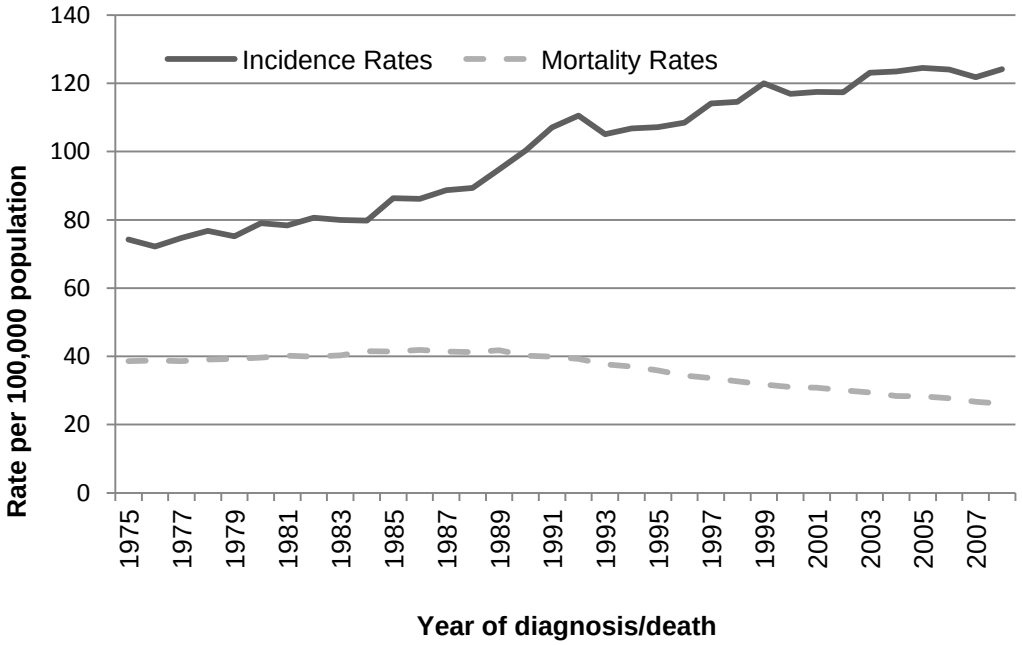
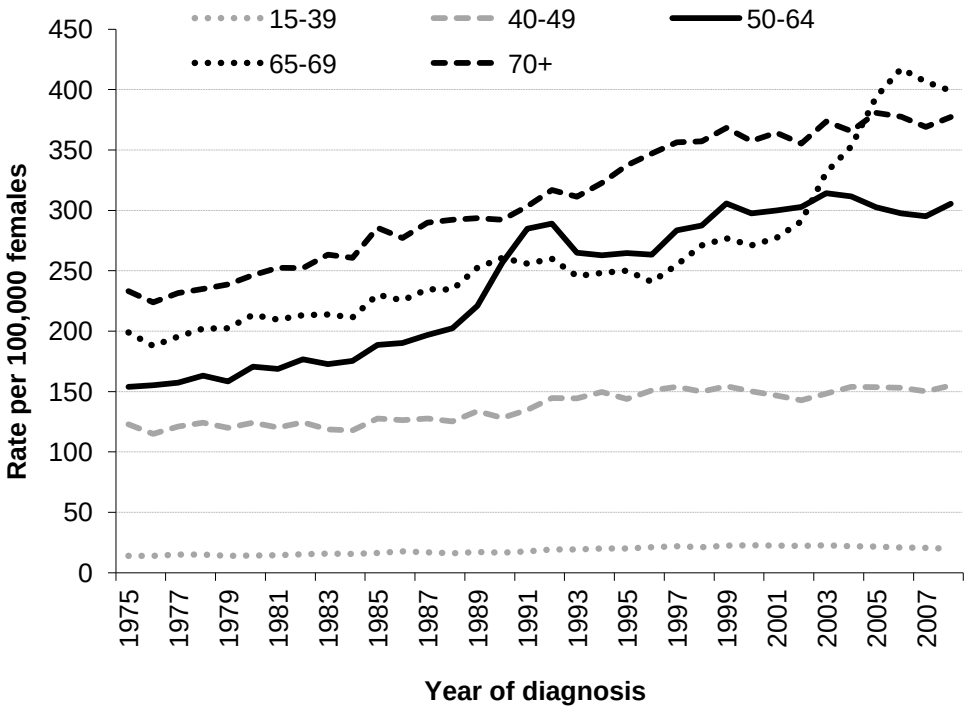


Figure 4 European age-standardised breast cancer incidence and mortality rates for Britain between 1975-2008[109]



Source: Cancer Research UK. Cancer Statistics - breast cancer http://info.cancerresearchuk.org/cancerstats/types/breast/incidence/

Figure 5 Age-Specific breast cancer incidence rates for Britain, 1975-2008[109]



Source: Cancer Research UK. Cancer Statistics - breast cancer http://info.cancerresearchuk.org/cancerstats/types/breast/incidence/

3.4 Major breast cancer risk factors

3.4.1 Family history and genetics

Most women who have a family history of breast cancer do not develop cancer themselves. A 2001 meta-analysis estimated that women with one first degree relative (mother, sisters, daughters) who developed breast cancer have about double the risk of developing breast cancer compared to women with no affected relative.[114] Increased risks may be due to shared genetic and/or environmental factors. Risks are estimated to be nearly four-fold for women who have three or more affected relatives;[114] these cancers are more likely to be inherited. Risks were higher the younger the relatives were at diagnosis, particularly if diagnosis was before the age of 50.[114]

Risks are very high in women who have certain rare germ line mutations such as BRCA1, BRCA2, TP53, PTEN, STK11 and CDH1. These high-risk alleles are rare (~0.1% of the population are carriers) but account for about 16% of familial breast cancer risk,[115] and about 5% of all breast cancer cases. Women with BRCA1 and BRCA2 mutations have a 10- to 20-fold relative risk and a 30-60% risk of developing

breast cancer before the age of 60 compared to 3% in the general population.[115] 0 describes the criteria the NHS use for defining people at high risk. These genetic mutations are also linked to high risks of developing other cancers; for instance BRCA2 is also associated with ovarian and pancreatic cancer as well as prostate and breast cancer in men.[116]

Other rare mutations, such as ATM, BRIP1, CHEK2 and PALB2 confer moderate risks of two- to 4-fold of developing breast cancer; together these may only account for 2.3% of familial risk of breast cancer.[115] These are involved in biological pathways relating to BRCA1 and BRCA2 genes. Additionally, the genome-wide search of hundreds of thousands of single-nucleotide polymorphisms have found common low-risk alleles associated with increased breast cancer risks of less than two-fold. Although the frequency in the population of some of these alleles may be as much as 50%, those discovered so far are likely to account for only a small percentage relating to familial risk in total.[115] It is unknown how these may interact with the high-risk alleles.

3.4.2 Breast density

Breast density, distinguished by non-fatty mammary tissue, is strongly and independently associated with increased breast cancer incidence, and shows a dose-response relationship.[117] A 2006 systematic review estimated that dense breast tissue in more than 75% of the breast increases risk of breast cancer by over four fold compared to densities below 5%.[117] Mammographic breast density is also associated with other risk factors such as age, menopausal status, parity and body weight, and to a lesser extent family history, race, alcohol consumption and diet. [118] High saturated fat intake has been associated with lower breast density.[119] One study found an inverse relationship between breast density and fruit and vegetable intake, vitamin C intake, and also with olive oil.[120] Conversely, another study reported total vitamin C intake and supplement vitamin C intake was positively associated with breast density in premenopausal women, although not in post-menopausal women.[119] There is evidence that density can be altered using combined oestrogen-progesterone hormone therapy, although the evidence relating to the effects of endogenous hormones and growth factors is inconsistent.[121] Twin studies have demonstrated that breast density is heritable,[121] nevertheless it has been reported that carriers of BRCA1 and BRCA2 genes have a similar breast density to non-carriers.[122] It has been suggested that breast density should be incorporated into risk prediction models,[123] however it is unknown whether breast density is predictive in young women when high density breast tissue is more common.[117]

3.4.3 Previous diagnosis

Benign breast disease is a risk factor for malignant breast; women with proliferative breast disease without the production of abnormal cells (without atypia), have double the risk compared to the general population, whilst those with atypical hyperplasia have a more than four-fold increased risk.[124] Women with a previous *in situ* tumour (DCIS and LCIS) have double the risk of invasive breast cancer in the same breast compared to the general population.[125]

Previous invasive breast cancer can increase the risk of contra-lateral breast cancer by as much as 5-fold, although the spread of the initial cancer to the other breast within the first two years probably inflates this figure.[126] Contra-lateral breast cancer diagnosed two years after the primary breast cancer is linked to tumour size and age; being more common if the first cancer was diagnosed before the age of 40.[126]

3.4.4 Thoracic radiotherapy

Women who were treated with moderate to high dose chest radiation for childhood, adolescent or young adult cancer have a substantially increased risk (13- to 15- fold) of developing breast cancer at a young age.[127] Exposure to lower doses of radiation in infancy has also been associated with increased breast cancer risk; risks increased linearly with increasing dose.[128]

3.4.5 Risk prediction tools

Various models have been developed to estimate life-time risk of breast cancer in order to help clinicians decide whether surveillance is necessary for particular women; these include Gail-2; Claus Model; Claus Tables; BOADICEA; Jonker Model; Claus-Extended Formula, and Tyrer–Cuzick.[129] In addition to breast biopsy history, family history and/or genetic test results, parameters often included in these models are age of menarche; parity; age at first child; menopausal status; menopausal age; BMI; ovarian status; age at ovarian status; HRT use.[129] These will be discussed below. Whilst the models aim to calculate the risk for an individual, the risks used in the models for these various risk factors are based on population risks from epidemiology studies.

3.5 Hormonal and related factors affecting risk of breast cancer

3.5.1 Hormone lifetime exposure

Hormonal and reproductive factors such as early onset of menarche, late age of first birth, low parity and late menopause are established risk factors for breast cancer.[130 131]

Hormonally induced changes in mammary cells during pregnancy may provide protective effects.[132] In contrast, it is believed that early onset of menarche and late menopause increase risk via their influence on women's lifetime exposure to endogenous oestrogens. High energy diets during childhood have been associated with early menarche thereby increasing exposure and risk.[133] [134] Risk increases rapidly during the reproductive years but then increases at a slower rate during menopause, which is reflected in the reduced production of oestrogen by the ovaries.[131] Due to fluctuations in hormone levels in the menstrual cycle of pre-menopausal women, lifetime exposure to oestrogens cannot be measured, therefore hormonal and reproductive events, plus the use of contraceptive pills and Hormone Replacement Therapy (HRT) that contain them are employed as proxy covariates in risk analyses.

3.5.2 Exogenous hormones in HRT and contraceptive pills

Oestrogen production from the ovaries diminishes as women approach the menopause, which can cause numerous problematic symptoms that can often be alleviated with Hormonal Replacement Therapy (HRT). However, there is substantial evidence that HRT increases breast cancer risk during its use, with increased risk disappearing about five years after cessation.[135] [136] It has been estimated that risk increases by 2·3% for each year of use in current or recent HRT users; this compares with an increased risk of 2.8% that would normally occur for each year that menopause is delayed.[135] The increased risk has been reported to be more pronounced in women with lower BMIs.[135] Post-menopausal oestrogen-plus-progesterone replacement therapy has been found to increase breast cancer risk to a greater extent than oestrogen alone replacement therapies;[136] [137] Additionally, there is evidence that mammographic breast density, which is linked to increased breast cancer risk, increases during HRT.[138]

In addition to oestrogen and progesterone, ovaries also produce testosterone in normal pre-menopausal women. Testosterone combined HRT has been used to treat women with removed ovaries, as well as to treat menopausal symptoms in normal post-menopausal women.[139] Adding testosterone to HRT has been found to inhibit the stimulatory effects of oestrogen on breast cell proliferation in monkeys.[140] However, there has been little research relating to women and this is inconclusive: although one study reported no increased breast cancer risk in women using the testosterone combined HRT,[141] another study reported increased risk.[142]

A modest increase in breast cancer risk was reported in a large meta-analysis of 54 studies relating to current or recent use of oral contraceptives (containing oestrogen

and progestin); this increased risk disappeared within 10 year of cessation.[143] There is evidence from an unadjusted meta-analysis of case-control studies that risk is more pronounced in multiparous women who used oral contraceptives before their first full term pregnancy than women who started use later.[144] Newer formulations of oral contraceptives have reduced oestrogen and progestin content; results of some studies indicate that the risk of breast cancer from the use of these may be lower than that from the older type of contraceptive.[145]

3.5.3 Endogenous hormones

There is consistent research evidence to show that high serum concentrations of endogenous oestrogen are associated with increased breast cancer risk in post-menopausal women; additionally there is evidence that androgens, prolactin, insulin hormones and insulin-like growth factors (IGF) may also influence risk.[146] This section describes the evidence that links serum concentrations of these hormones to breast cancer risk, much of which is from seminal work on the pooling of thousands of cases in nine prospective studies. Pooling not only increases the power to detect smaller effect sizes, but in contrast to meta-analyses ensures uniformity in analysis methods. The following sections describe how these hormones are related to lactation, body fatness, physical activity and height which are also associated with breast cancer risk.

The pooled analysis of nine prospective studies showed that post-menopausal women with the highest fifth of oestradiol serum concentrations had twice the relative risk compared to the lowest fifth for this endogenous oestrogen.[147] Other prospective studies such as EPIC have reported similar results.[132] [148] [149] Free oestradiol levels, including non-SHBG bound oestradiol which were free to enter breast cells had a larger effect than total oestradiol on risk.[147] Evidence suggests that oestradiol and other oestrogens stimulate mammary cell proliferation, thereby promoting cancer.[150] Oestradiol has been found to have a strong and significant association with oestrogen receptor positive breast cancer in post-menopausal women in some studies,[149] [151] although in other studies the increase was not statically significant. [148] [152] Although there is some evidence of an increased risk relating to higher oestradiol levels in pre-menopausal women, it is inconclusive, probably as a result of large fluctuations in levels during the menstrual cycle.[146] Despite the fact that some genes are associated with an increase in oestrogen levels there is no evidence they have a large enough effect to significantly increase breast cancer risk.[146]

The pooling of nine prospective studies also found breast cancer risk was more than doubled for post-menopausal women with testosterone concentrations in the highest

fifth compared to the lowest fifth.[147] Testosterone is more abundant in post-menopausal women than oestrogen, and is converted to oestradiol by aromatase; thereby indirectly increasing risk via increases in oestradiol. Nevertheless testosterone was found to be independently associated with breast cancer after adjustment for oestradiol, though rates were slightly attenuated.[147] A positive association was also seen in pre-menopausal women in four prospective studies.[146] Positive associations between other androgens with breast cancer have also been found.[147] In addition to being an oestrogen precursor, androgens can act directly on breast cells by binding to androgen receptors and may stimulate cell proliferation.[153] Conversely, other researchers propose that androgens such as testosterone are protective in respect to breast cancer risk,[139 154] since some studies found an inverse association; differences in results may depend on the methods for measuring testosterone, the calculation of free testosterone as well as oestrogen status.[139] Hypotheses have been proposed which may explain some, but not all of the inconsistencies in results: in the absence of oestrogens, for instance in post-menopausal women, androgens may stimulate growth of breast cancer cells via binding to oestrogen receptors; alternatively in the presence of high oestrogen concentrations, androgens may act as anti-oestrogens to inhibit oestrogen stimulation of growth of breast cancer cells via binding to androgen receptors.[155] Furthermore, in vitro androgens have been shown to stimulate or inhibit breast cancer cell proliferation depending on the breast cancer cell line.[155] In post-menopausal cancer patients, high testosterone levels have been significantly associated with positive oestrogen receptor status, large tumour size and high BMI.[156] Although this supports the original androgen excess theory,[156] the association in cross-sectional studies could also be explained by the tumours' effect on androgen production. The mechanisms by which androgens affect breast cancer risk remain unclear.

Serum levels of sex-hormone-binding globulin (SHBG) have been inversely associated with breast cancer risk,[147 157] risk reduced by approximately a third in a high verses low analysis of nine pooled cohorts.[147] SHBG not only reduces the bioavailability of oestrogens and testosterone to cells, but may also act directly on breast cancer cells by inhibiting oestradiol induced cell proliferation.[158]

Prolactin, the luteotropic hormone**,** was associated with a moderate increase in breast cancer risk in a pooled analysis of two prospective studies.[159] No difference in risks between pre-menopausal and post-menopausal women was found, probably since prolactin declines only slightly during the menopause.[159] Risks were higher for women with ER positive tumours.

In a large pooled analysis of seventeen studies insulin-like growth factor-1 (IGF-1) showed a significant association with breast cancer risk, in particular oestrogen receptor positive cancers (OR = 1.38 (95% CI 1.14-1.68), for both pre-menopausal and post-menopausal women.[160] Results of an earlier meta-analysis showed associations were limited to pre-menopausal women,[161] however this analysis did not include four large recent studies included in the pooled analysis. The association in the pooled study was independent of levels of oestradiol and testosterone, and was not modified by these.[160] IGF-1 was positively associated with height and age at first pregnancy and inversely associated with age at menarche and years since menopause,[160] which are risk factors for breast cancer. Dietary factors associated with increased IGF-1 are dairy protein and calcium.[162]

Other reviews have suggested a possible link between insulin or C-peptide (a marker of pancreatic insulin secretion) and increased breast cancer risk, although some studies showed no association.[163] [164] Hyperinsulinemia has also been associated with increased breast cancer risk.[165] Insulin may affect tumour growth indirectly through increasing bioavailability of IGF-1 and decreasing sex-hormone-binding globulin (SHBG). Insulin and IGF-1 may also have a direct effect by increasing breast cancer cell proliferation, though this has only been demonstrated in vitro.[158]

Despite evidence that the combined oral contraceptive containing oestrogen and progesterone confers more risk than oestrogen alone, the evidence for the influence of endogenous progesterone on breast cancer risk has been inconsistent and does not provide support for an oestrogen-progesterone hypothesis.

Shift work may affect breast cancer risk through hormones levels; a meta-analysis showed that shift work increased breast cancer risk by about 50%.[166] It is hypothesised that reduction in melatonin, which occurs in artificial light, may increase levels of hormones such as oestrogen, additionally melatonin may have a direct effect on tumour suppression.[166]

3.5.4 Pregnancy and lactation

In addition to a 7% reduction in breast cancer risk for every birth, a woman's risk is further decreased by 4.3% for every year in total that she breast-feeds.[167] This evidence is provided by a pooled analysis of 47 epidemiological studies from 30 countries.[167] The mechanisms involved remain unclear, however hormonal influences are likely to be involved, relating for instance to delayed ovulation and increased breast cell differentiation.[168]

3.5.5 Body fatness

Body fatness is an established risk factor for post-menopausal women, whereas a probable inverse relationship with breast cancer exists for pre-menopausal women. [7] [169] Several hypotheses involving hormones such as insulin, insulin-like growth factor and oestrogens that were discussed in previous sections have been proposed to explain the former. There is no clear mechanism, however, to explain the inverse relationship for pre-menopausal women. Waist circumference and waist to hip ratio are positively associated with breast cancer risk in postmenopausal women. [7] [169] The 2008 WCRF continuous update report stated that abdominal fat is a probable cause of post-menopausal breast cancer, as is adult weight gain.[169] BMI, which is derived from readily obtainable height and weight measurements, is often used as a marker of body fat and is associated with breast cancer risk. The updated meta-analyses of cohort studies in the 2008 WCRF continuous update report showed a 7 per cent decreased risk per 5kg/m2 for premenopausal women and an 8% increased risk per 5kg/m^2 increase in BMI for postmenopausal women.[169] Obese post-menopausal women in particular (BMIs over 30 kg/m^2) are at increased risk of breast cancer. Additionally, obese patients tend to have larger sized tumours, their cancers are more likely to metastasize due to lymph-node involvement and they generally have a poorer prognosis for survival.[170] [171] Weight loss is recommended for survivors and gastric bypass surgery improves cancer outcomes in obese patients, for both pre and post-menopausal women.[172] There is some evidence that women genetically at high risk could reduce their risk by avoiding weight gain post-menopausally.[173]

Levels of oestradiol are higher in obese post-menopausal women, which appear to explain why the risk of breast cancer is greater in post-menopausal women with high BMI; indeed controlling for oestradiol concentrations in a prospective study reduced the risk towards unity in the association between breast cancer and BMI.[174] The association between oestrogen production and obesity related breast cancers is further supported by the fact that these cancers are more often ER+,[175] and particularly in women who were not currently taking HRT. [176] Weight gain during adulthood is also associated with an increased risk of post-menopausal breast cancer,[169] and is also stronger in women who were not currently,[176] or previously using HRT,[177] or who had a later menarche.[176] Therefore the effects of weight gain on risk appear to be obscured in women who had early menarche or used HRT which would increase their cumulative exposure to oestrogen .[176]

Adipose tissue becomes the primary source of circulating oestrogens during menopause when the ovaries cease production. Aromatase in adipose tissue, mainly

found in breast, abdomen, thighs and buttocks, convert androgens to oestrogen.[158] Aromatase inhibitors are recommended to reduce peripheral production of oestrogens in women with advanced (or metastatic) breast cancer.[178]

Alternatively insulin resistance and IGF-1 concentrations associated with metabolic syndrome, together with the action of oestrogens may explain the link between breast cancer and obesity.[158] Since high levels of insulin are inversely related to SHBG, the bioavailability of oestrogens and testosterone are also likely to increase.[175 179 180] Intra-abdominal adipose tissue, in particular, influences the production of insulin and other hormones; waist circumference being a reasonable measure of abdominal adiposity. Hyperinsulinemia may also promote the synthesis and activity of IGF-1.[158] However, despite IGF-1 being positively associated with weight in overweight women, lower levels of IGF-1 are found in obese women and therefore cannot explain the increased risk of breast cancer in obese women.[160 181] Insulin resistance and hyperinsulinemia are seen in the early stages of type-2 diabetes, and both hyperinsulinemia and type-2-diabetes are associated with an increase in breast cancer risk.[163 165]

A third hypothesis proposes that various cytokines, polypeptides and hormone-like molecules secreted by adipocytes may influence risk via their autocrine, paracrine, and endocrine functions.[158] Since the majority of cells in the breast are adipocytes, their autocrine and paracrine activities are likely to affect breast epithelial cells. The adipokines secreted by adipocytes include leptin, adiponectin, necrosis factor-α (TNF-α) and interleukin (IL-6); the latter two being a marker of inflammation along with C-reactive protein. Furthermore, tumours stimulate the production of aromatase and oestrogens locally via the autocrine and paracrine actions of TNF-α and IL-6.[158] Chronic inflammation has been implicated in various steps of tumour formation including cell proliferation, angiogenesis and metastasis, and suppression of apoptosis has been associated with increased risk of breast cancer.[182] In vitro evidence suggests leptin may also promote cancer in a variety of ways including breast cancer cell proliferation, and via effects on ER and insulin signalling.[158] Additionally, case-control studies have reported an association between leptin and breast cancer.[164] Leptin suppresses appetite, and although high levels are found in obese individuals it is likely they develop leptin resistance.[158] Adiponectin is inversely associated with insulin resistance and obesity, and is also inversely associated with breast cancer.[183] Inflammation, which is linked to abdominal obesity, is also associated with increased reactive oxidative species which can cause damage to DNA.

3.5.6 Physical activity

There was sufficient evidence in the WCRF 2007 and 2008 reports to suggest that physical activity probably protects against breast cancer in post-menopausal women, whereas the evidence for pre-menopausal women is inconsistent and limited.[7 169] For post-menopausal women meta-analyses showed a 3% decrease in risk per 7 MET (Metabolic equivalent task) hours of recreational activity per week (equivalent to about 2 hours brisk walking).[7] Since physical activity is difficult to measure this may explain some of the inconsistencies in the evidence base. There are a number of biological mechanisms involving hormones whereby physical activity may reduce the risk of breast cancer in post-menopausal women, though they remain unclear.

In a recent review Neilson et al. proposed a biological model which links the influence of physical activity through BMI, oestrogens, insulin resistance and chronic inflammation, and also through less commonly considered biomarkers such as leptin.[164] Physical activity can reduce adiposity which will then result in reduced amounts of circulating oestrogen produced from the conversion of androgens by aromatase found in adipose tissue. Fat loss from exercise will also lead to a reduction of inflammatory markers, such as TNF-α and IL-6, and also leptin, which have been associated with breast cancer risk.[158] Physical activity also reduces insulin levels;[184] since this is inversely related to circulating SHBG the bioavailability of testosterone and oestradiol is also likely to decrease.[175 179 180] Additionally, regular physical activity can sustain insulin sensitivity and reduce the likelihood of developing type-2 diabetes,[184] which is associated with breast cancer.

3.5.7 Hormonal exposure *in utero* and childhood development

Increases in birth-weight above 4000g compared to below 2500g are associated with increased breast cancer risk, and most consistently with pre-menopausal breast cancer.[185] A 2007 WCRF meta-analysis showed an 8% increase in risk per kg increase in birth weight.[7] Trichopoulos hypothesised that *in utero* exposure to oestrogen influences the risk of breast cancer,[186] because oestrogen levels are at their highest during pregnancy and high maternal levels have been linked to high birth-weight.[187 188] IGF levels during pregnancy also substantially regulate foetal growth,[189] and may play a role in increased breast cancer risk.[185] These and other *in utero* factors may directly affect breast development, for instance by inducing epigenetic changes to the expression of oestrogen receptors, mammary stem cells or gland morphology.[99] Alternatively they may mediate breast cancer risk through other changes which increase risk in adult life; their mechanisms are as yet unclear.[190]

Birth length, adolescent height and adult height have been positively associated with breast cancer risk.[191] [192] Meta-analyses from the 2007 WCRF report showed for every 5cm increase in adult height there is a 9% and 11% increased risk of pre-menopausal and post-menopausal breast cancer respectively [7] Factors that lead to adult height may influence breast cancer risk. Birth length, which is associated with adult height, may be a proxy for exposure to oestrogen and growth factors *in utero.* Birth length has also been associated with age at menarche; one study showed that girls who were long and light at birth had earlier menarche, which is associated with an increased risk.[193] Early growth spurts in adolescence are also positively associated with early menarche, though it is unclear whether early growth spurts are an independent factor in breast cancer risk.[191] Nutrition may influence age at menarche via rapid post-natal growth,[193] or through diet during childhood.[133] [134] Despite being linked to earlier onset of menarche, body fatness during childhood has been inversely related to breast cancer in later life.[194] One hypothesis is that early breast differentiation induced via oestrogen by body fat may reduce breast malignancies later in life.[195] Additionally, as mentioned in section 3.5.3 IGF-1 concentrations have been positively associated with increased height and with breast cancer risk, but are inversely associated with age at menarche.[160] Furthermore, despite adult height being positively associated with breast cancer risk, taller women tend to have had a later menarche which can reduce breast cancer risk.[191] Despite some inconsistencies in these explanatory mechanisms, early life and adolescence are critical times for maturation of the hypothalamic pituitary ovarian axis, which regulates production of oestrogen and other hormones linked to breast cancer risk.[191]

3.6 Diet and breast cancer risk

The risk of breast cancer varies internationally; possible reasons for this, in addition to differing provisions for recording breast cancer incidence, include differences in genetics, environmental pollutants, and lifestyle factors such as reproductive events, exercise and diet. The observation that the risk of cancer for populations who migrate alters from their country of origin to their country of residence indicates that, at the population level, it is changes in lifestyle or environment which have a large influence on risk rather than genetics. Ecological evidence relating to breast cancer is apparent from women of Asian origin settled in the US whose breast cancer risk is substantially above that of their country of origin.[196] Given that one of the biggest changes in lifestyle for migrants is diet, this area of research has been given considerable attention.

On the whole the role of diet on cancer risk of individuals has been assessed using cohort or case-control studies, the latter being prone to recall bias and selection bias which may explain the frequent discrepancies in results between the two types of studies. Furthermore, self-reporting of food and supplement intake is prone to reporting bias in all types of studies, as well as measurement error, the latter being likely to attenuate any associations. Few dietary studies have used a RCT design, one weakness being the difficulty for randomised participants to adhere to new diets for a substantial length of time, although this is less of a problem with interventions using supplements. Conformity within the non-intervention group may also be poor, since motivated members may easily alter their dietary or supplement intake towards the intervention if this is known to them.

Several approaches have been used to assess the role of diet on cancer risk focusing on dietary patterns, individual or groups of foods, or individual nutrients. The majority of the analyses in this thesis uses the individual nutrient approach, assessing the impact of vitamin C from diet and supplements in chapters 8 to 10 using cohort designs. The effect of fruit and vegetable intake, the main source of vitamin C, is also analysed in chapter 9. Sections 3.6.4.1 and 3.7 of this chapter review the previous research on vitamin C. First the evidence of breast cancer risk in relation to dietary patterns and some foods, focusing in particular on fruit and vegetable intake, will be discussed in sections 3.6.1 and 3.6.2. Then evidence relating to macronutrients that have been linked to breast cancer risk will be discussed in section 3.6.3

3.6.1 Dietary Patterns

The advantage of this over the individual food or nutrient approach is that dietary patterns incorporate interactions between foods or nutrients which are likely to occur in the body. Because of the difficulty of characterising dietary patterns, research in this area is not as extensive as other approaches. A dietary pattern constitutes a variety of dietary components which may typify the intake of a population or sub-population. Some of those that have been examined are traditional diets such as the Mediterranean and the Asian diets, which are viewed as healthy in contrast to the Western diet. The Mediterranean diet, characterised by seafood, poultry, fresh vegetables and olive oil, has been associated with a lower risk of breast cancer in US women,[197] and in pre-menopausal women in the UKWCS, though the latter was non-significant.[198] Associations between a variety of healthy diet indices, based on healthy eating guidelines, and breast cancer risk have also been assessed.[199] The alternate Mediterranean Diet Score (aMDS) and also the Alternate Healthy Eating Index (AHEI) and the Recommended Food Score (RFS) have been inversely associated with risk of post-menopausal oestrogen receptor negative (ER-) breast cancers.[200] Inverse

associations between the Diet Quality Index-Revised (DQI-R) and also the Canadian Healthy Eating Index (CHEI) and BRCA cancers indicate that some dietary patterns may be protective for women at high risk.[201]

3.6.1.1 Healthy and unhealthy dietary patterns

Some studies have used factor analysis, principle components analysis or reduced rank regression to derive healthy and unhealthy dietary patterns; although these methods are less subjective than using diet scores, an element of subjectivity is still required during categorisation. A recent systematic review and meta-analysis of 18 case-control and cohort studies found a small decreased risk in breast cancer for women in the highest compared to those in the lowest categories of prudent/healthy dietary patterns.[202] No association was found for Western/unhealthy dietary patterns when both case-controls and cohorts were included, although a significant and modest increased risk was seen when only case-control studies were use; these however are prone to recall and selection bias.[202] The Western unhealthy dietary patterns were characterised by high intake of red and/ or processed meat, refined grains, potatoes, sweets and high-fat dairy. Conversely the prudent/healthy dietary patterns was typified by high intake of fruit, vegetables, poultry, fish low-fat dairy and whole-grains.[202]

3.6.1.2 Vegetarian compared to meat consumption dietary patterns

Compared to meat consumption diets, vegetarian diets are low in energy and protein and have been associated with lower levels of IGF-1;[203] increased levels of the latter have been positively associated with breast cancer risk as discussed in section 3.5.3.[160] Vegetarian diets also avoid the high temperature production of heterocyclic amines produced during the frying, grilling or barbecuing of muscle meats which are thought to increase cancer risk.[204] On the other hand, it is possible that nitrates and nitrites found in vegetables may be converted in the body into carcinogenic nitrosamines compounds.[205] These are also formed in meat during curing.[205]

There is evidence that Mediterranean diets are anti-inflammatory;[206] and as mentioned in section 3.5.5 this may influence breast cancer risk. In particular, there is some evidence that magnesium, dietary fibre, omega-3, polyunsaturated fatty acids, monounsaturated fatty acids, flavonoids, and carotenoids from food are associated with decreased levels of inflammatory markers in serum.[206] Additionally, there is evidence from cross-sectional studies that vitamin C is associated with the anti-inflammatory marker C-reactive protein, however there are conflicting reports from RCTs.[71 72]

A dietary pattern high in consumption of raw vegetables and olive oil, determined via factor analysis in the Italian ORDET cohort, was found to be protective against breast

cancer, particularly relating to HER-2 positive beast cancers or lean women of BMI below 25 kg/m^2.[207] Although vegetables are major components of the Mediterranean and prudent/health dietary patterns which are inversely associated with breast cancer, there was no evidence in the EPIC-Oxford cohort that a vegetarian diet free of meat or fish reduces the risk of breast cancer in UK women.[208] The non-vegetarian comparison group in this cohort also had relatively high intakes of fruit and vegetables and this may have reduced the ability to find a significant association. There was also no evidence of a significant association between breast cancer risk and vegetarians in the fully adjusted model for pre or post-menopausal women in a UKWCS analysis of dietary patterns, when comparing them to women who ate red meat at least once a week.[209] Vegetarians were defined pragmatically as women who consumed red meat, poultry or fish less than once a week. In contrast, an earlier UKWCS analysis using a stricter definition of vegetarians who ate no meat, reported that vegetarians had a lower breast cancer rate than women who had medium to high intakes of red or processed or total meat.[82] However in the analysis of pre-menopausal women, low meat consumers were found to have a 32% lower risk than vegetarians, even after adjusting for the different characteristics of these groups.[82] The authors suggest that the vegetarian group may consist of a larger proportion of women genetically at high risk of breast cancer who started a vegetarian diet in the hope that it may be protective.[82] Alternatively, vegetarians may lack dietary components that are protective, particularly if some foods are consumed in moderation. Dose-response relations relating to fruit and vegetable intake are mentioned in section 3.6.2.1 on food groups.

In the UKWCS, compared to women who ate red meat at least once a week, a dietary pattern of eating fish at least once a week (excluding the consumption of red or white meat once a week or more) was inversely associated with breast cancer incidence.[209] A reduced risk of 40% was found for post-menopausal women, but there were no associations relating to pre-menopausal women.

3.6.2 Foods

On the whole the WCRF 2007 report on Food, Nutrition, Physical Activity and the Prevention of Cancer focused on food rather than dietary patterns or individual nutrients, and reasoned that micronutrients may be simply markers for particular foods which contain other constituents that are associated with cancer development and progression.[7] Despite extensive systematic literature reviews and meta-analyses limited conclusions were reached about the influence of the foods considered in the WCRF 2007 report (summarised in Appendix A) and in the 2008 continuous update for breast cancer; evidence was limited due mainly to conflicting results from different

studies.[7] [169] Whilst retrospective case-control studies have produced evidence of protective effects for some foods, results from cohorts are often mixed. There was, however, convincing evidence that alcohol increased breast cancer risk in both pre- and post-menopausal women and suggestive evidence that fats increased risk in post-menopausal women. A review of prospective observational studies by Michels et al. (2007) relating to intake of fruit and vegetables, dairy products and macronutrients such as fats and carbohydrates found no consistent, strong or statistically significant evidence of an association between diet and breast cancer, other than through being overweight and gaining weight.[11] The evidence relating to breast cancer risk and the amount of fruit and vegetables and dairy products consumed are discussed in more detail here. Dietary constituents such as fats, carbohydrate, fibre and alcohol are discussed in the following macronutrient section.

3.6.2.1 Fruit and vegetables

Theoretically, there are various mechanisms by which fruit and vegetables could reduce cancer risk. As low energy-dense foods, they may help to reduce weight gain and obesity, which as seen in section 3.5.5 has been linked to increased breast cancer risk. Additionally their fibre content may help to regulate and increase the excretion of endogenous oestrogen,[210] thereby reducing exposure to one of the main hormones associated with breast cancer development (section 3.5.3). A diverse intake of fruit and vegetables can also provide a variety of vitamins and mineral including carotenoids, folate, vitamin C, D, E and selenium. Other bioactive compounds such as phytochemicals found in fruit and vegetables are non-essential components for humans but provide colour, flavour and preservation. Many of these bioactive compounds are believed to protect against cancer, for instance via their antioxidant properties. Unfortunately, storage and cooking processes usually reduce the nutrient content of fruit and vegetables, particularly in the case of vitamin C which is destroyed by heating or exposure to air by chopping; additionally, it is lost into the cooking water.[39] However, processing can also increase the bioavailability of some nutrients for absorption within the intestine; pureeing and adding oil increases the bioavailability of fat soluble lycopene found in tomatoes,[211] and chopping some foods can release enzymes that help the formation of other nutrients. Growing and transport conditions in addition to the timing of harvesting can also alter the nutrient content of foods.

The 2007 WCRF report judged that there was sufficient evidence to show that high intakes of fruit and non-starchy vegetables probably reduce the risk of cancers of the digestive track, such as mouth, larynx, oesophagus and stomach.[7]. However, a recent EPIC analysis found only a very small inverse association between intake of total fruits and vegetables and total cancer risk.[212] Furthermore, there is no conclusive evidence

they are associated with breast cancer, whether this be grouped as total fruit, total vegetables or fruit and vegetables.[7] [11-13] Meta-analyses which included mainly retrospective case-control studies reported a reduction in breast cancer risk with increasing intake of total fruit and vegetables by as much as 25%.[8] [213] However, results of a subsequent pooled analysis of cohort studies showed that fruit and vegetable consumption was not significantly associated with breast cancer risk.[12] The susceptibility of case-control studies to recall bias and selection bias may explain the discrepancy in results. Furthermore, the pooling of studies is superior to meta-analyses since it ensures a standardised approach to coding, grouping of intake categories, analysis and adjustment by confounders. Nevertheless, a limitation of both designs is that dietary measurement may vary systematically between studies, even if similar measurement tools are used. One aspect of measurement error for the FFQs is that recorded intake of fruit and vegetable tends to increase with increasing numbers of fruit and vegetable items on the FFQs. This was evident in the pooling of eight studies from Northern Europe and North America where fruit and vegetable items varied 4-fold between the studies.[12] A pooling of eight European cohort studies by EPIC attempted to overcome differences in FFQs by calibrating the FFQs used in all the studies with the same 24-hour recall instrument; furthermore the measurement errors of the tools were likely to be independent.[13] This analysis, however, also found no significant associations, although this may have been limited by the median follow-up of only 5.4 years.[13] A strength of this pooling project was the wide range of fruit and vegetable intakes found throughout Europe, which increased the power to detect a significant result.

The EPIC pooling project also examined specific vegetables in relation to breast cancer risk, but found no inverse associations.[13] Subgroups examined were: leafy vegetables, fruiting vegetables, root vegetables, cabbages, mushrooms and garlic and onions. Other groups of vegetables were examined in the pooling project of studies from Northern Europe and North America, but no associations were observed for green leafy vegetables, eight botanical groups and 17 specific fruit and vegetables.[12] Although preparation of brassica/cruciferous vegetables (broccoli, cauliflower and cabbage) produces isothiocyanates and indole-3-carbinol which may be anti-carcinogenic, evidence of a protective association between breast cancer and this type of vegetables has only been observed in case-control studies.[214]

Daily grapefruit intake, which is high in vitamin C content, was associated with a 30% increased risk of post-menopausal breast cancer in a US cohort study, where higher risks were observed for non-users of HRT [215] However this was not supported by

results of a second study.[216] Grapefruit, nevertheless, has been found to increase plasma oestrogen concentrations,[217] possibly through the inhibition of the CYP3A4 enzyme which is involved in the metabolism of oestrogen.

There are indications from cohort studies that the relationship between fruit and vegetable intake and breast cancer risk may be modified by hormonal receptor status. Fruit and vegetable intake, and healthy dietary indexes which recommend high intakes of fruit and vegetables amongst other items, have been inversely associated with risk of oestrogen receptor negative (ER-) breast cancers.[200 218] In a cohort study of African-American women significant associations between a prudent diet and specifically between frequent vegetable intake and oestrogen/progesterone receptor negative (ER-PR-) breast cancers have been observed.[219 220] There is also evidence that high consumption of raw vegetables can potentially reduce HER-2 positive beast cancers by up to 75%.[207]

3.6.2.2 Dairy products

Dairy products have been associated with IGF-1 levels in children in the UK.[221] US milk used in dairy products is likely to have higher levels of IGF-1 since, unlike milk in the UK and Europe, it is contaminated with bovine growth hormones which increase levels of IGF-1, as well as being fortified with vitamin D.[11] As discussed in section 3.5.3, IGF is positively associated with breast cancer,[160] therefore associations between breast cancer and dairy products in US studies may differ from European studies. However, in one review no differences in results were found from cohort studies by region;[11] many studies showed an inverse association between dairy products or milk and breast cancer risk, some being significant.[11 169] The 2008 WCRF continuous report, however, judged that no conclusions could be reached on whether dairy products influenced breast cancer risk, or on whether vitamin D did so either.[169]

3.6.3 Macronutrients and breast cancer risk

3.6.3.1 Fats

Fat intake has been the focus of many studies. In the 2007 WCRF report and its detailed breast cancer systematic literature review, meta-analyses of case-control studies showed weak but significant increases in breast cancer risk with increasing total fat, saturated fat intake or mono-unsaturated fat intake.[7] Conversely, inconsistent results were found for cohort studies, though a meta-analysis of 4 cohort studies showed a weak increased risk for saturated fat in post-menopausal women.[7] Since then a large EPIC pooling study of over 300,000 women from European cohorts

reported a significant 13% increased risk of breast cancer with increased saturated fat intake.[222]

In comparisons of dietary tools used, an initial analysis of EPIC-Norfolk found a 22% significant increase in breast cancer risk per quintile increase in energy adjusted saturated fat intake using 7-day diary data, but not using FFQ data when analysing the same women.[223] Bingham et al. (2003) suggest that measurement error may be the reason why many studies using FFQs find no associations. [223] Similarly the nested case-control analysis of the Women's Health Initiative Study (WHI) found a significant positive association using 4-day diaries and a non-significant association using FFQs.[224] Results of a recent pooled analysis of additional UK studies using diary data, however, did not support these findings.[225] Although diary recordings may be more detailed, they are not without their limitations, the main one being their short-term episodic nature. Additionally, since they are very time consuming, participants may alter their diet in order to record less items, furthermore the quality of recording may reduce over the diary time period; nevertheless this is the same for both cases and controls.

An RCT using an intervention group target of reducing dietary fat intake by 20% and increasing fruit and vegetable intake to at least five portions, and grain intake to at least six portions, did not find a significant reduction in breast cancer incidence overall after an average follow-up of 8 years.[226] Although there was a significant difference in fat intake between the groups, very few in the intervention group achieved the target of reducing fat intake by 20%. However, women in the intervention group who were in the highest quartile for fat intake at baseline showed a 20% significantly lower risk of breast cancer compared to similar women in the non-intervention group.[226] The intervention group also had a lower risk of developing progesterone receptor negative breast cancer. A recent review of studies of intentional weight loss supports the hypothesis that intentional weight loss can substantially reduce cancer risk.[227] Weight loss is associated with decreased endogenous oestrogen levels and increased levels of serum levels of sex-hormone-binding globulin (SHBG),[227] which have been consistently linked to breast cancer risk in menopausal women (section 3.5.3). Weight loss is also linked to decreased inflammatory markers,[227] which as discussed in section 3.5.5, is another mechanisms by which body fatness may be positively associated with breast cancer risk.

3.6.3.2 Carbohydrates

Carbohydrates as an energy source are available as complex polysaccharides such as starch found in breads, rice, peas, beans, root vegetables, potatoes and bananas, or

as simple carbohydrates i.e. sugars which are found in many processed foods and drinks, and also in fruit and vegetables.

A meta-analysis of cohort studies in the 2007 WCRF report, produced evidence of borderline significance of a positive association between carbohydrate intake and breast cancer risk in post-menopausal women (pooled RR per 50g/d=1.09; 95% CI: 1.00, 1.18).[7] The four studies included in the 2008 WCRF/AICR continuous update report relating to energy from carbohydrates and breast cancer risk provided insufficient evidence of an association.[169] Nevertheless, there was evidence of a positive association between percentage energy from carbohydrates with a high glycemic index (GI) and breast cancer risk in an Italian prospective study, not split by menopausal status.[228] Results for low GI food were not significant.[228] Glycemic Index (GI) is a measure of the effect of carbohydrates on plasma blood sugar levels, and glycemic load (GL) is the measure per 100g serving. Simple and refined carbohydrates, such as white bread, release sugar quickly and have a high GI, whereas whole grain products tend to have lower GI. Although GI and GL are positively associated with insulin levels, and insulin resistance and hyperinsulinemia has been positively associated with breast cancer incidence,[163] research into GI and GL have produced null or unexpected results with breast cancer incidence.[11 229 230]

3.6.3.3 Fibre

Fibre, the indigestible content of foods such as non-starch-polysaccharide carbohydrates which are present in some fruit and vegetable, cereals and legumes may also influence risk, via possible affects on hormone levels.[231] However the weak inverse association with fibre found in the 2008 WCRF meta-analysis of cohort studies did not reach significance for post-menopausal women (pooled RR=0.96,95% CI: 0.91, 1.01).[169] Results for pre-menopausal women were also inconclusive in the WCRF continuous update.[169] However there was some evidence of a reduction in risk with higher fibre intakes in an earlier analysis of pre-menopausal women in the UKWCS (high versus low RR= 0.48, 95% CI: 0.24, 0.96); fibre from cereals and possibly fruit appeared protective.[231] A very recent meta-analysis of 10 cohort studies, which grouped together both pre- and post-menopausal women, reported a 11% reduction in breast cancer risk from total dietary fibre which was significant.[232] Evidence, however, was limited about whether specific food sources of fibre, i.e. cereal, fruit or vegetable, were protective.[232]

3.6.3.4 Alcohol

The majority of cohort and case-control studies report a positive association between alcohol intake and breast cancer risk.[7] Meta-analyses and pooling studies estimate the risk to increase linearly by 7-10% per 10mg/d increase in ethanol intake.[7 169] This evidence was judged as convincing in the 2007 WCRF report and in the 2008 WCRF continuous update report for both pre- and post-menopausal breast cancers.[7 169] A few cohort studies have reported increased risk for hormone receptor positive tumours but not for ER-PR- breast cancer with increasing alcohol intake,[233-235] indicating that alcohol interferes with oestrogen metabolism. Additionally, it has been observed in some studies that high folate intake attenuates the association between alcohol intake and breast cancer risk.[236]

3.6.4 Micronutrients and breast cancer risk

Initial research using retrospective recall methods in case-control studies indicated that fruit and vegetable intake probably reduced the risk of many cancers, including breast cancer.[8] Subsequently the identification of the active component in fruit and vegetables has been the focus of many studies. Micronutrients such as vitamins, minerals and antioxidants in these foods have been considered to be potentially protective against cancer, indeed the meta-analyses of retrospective studies indicated some micronutrients were protective.[8] Possible protective mechanisms of vitamin C and other antioxidants have been put forward, which have been discussed in section 2.2.4.

Furthermore, the 2007 second WCRF report judged there was convincing or probable evidence from prospective studies that some micronutrients were associated with specific cancers (summarised in Appendix B).[7] For instance, it reported that the antioxidant selenium was associated with a reduced risk of lung, stomach and prostate cancer,[7] although more recent results from the SELECT trial do not support the latter.[237] Conversely, there was important convincing evidence that supplementation with the antioxidant β-carotene increased the risk of lung cancer in smokers.[238] However, prospective studies assessing dietary and/or supplement intake in relation to breast cancer incidence have produced many null or conflicting results. The 2007 WCRF report judged that the evidence was limited or non-conclusive, therefore no conclusion could be reached regarding the intake of micronutrients and breast cancer risk.[7] The 2008 WCRF continuous update report for breast cancer which reviewed studies published to the end of 2008 also made the same judgement.[169]

In relation to antioxidants and breast cancer risk most prospective studies have reported no associations for carotene, vitamin A or vitamin E intake,[239-246] although the Nurses Health Study reported protective effects for vitamin A, vitamin E intake and α-

and β-carotene for pre-menopausal women with a family history of breast cancer.[169 247] Similarly there was no overall convincing evidence of associations between the intake of vitamin C and breast cancer risk; these studies are discussed in sections 3.6.4.1 and 3.7.

In general, the 2007 WCRF report recommended that nutritional needs should be met through diet alone, when possible, rather than through supplementation. Furthermore, most studies published since the WCRF reported no reduced risk of cancer from micronutrient supplementation or reported evidence of harm.[14 248-254] A meta-analysis of four antioxidant RCTs published to October 2007 showed no association between antioxidant supplementation and breast cancer risk (RR=1.0; 95% CI: 0.90, 1.11).[255] A more recent factorial designed RCT based in the US using daily 500mg vitamin C, 600IU Vitamin E every other day and 50mg beta carotene every other day reported no effects of these on breast cancer incidence as well as on total cancer.[14] The French SU.VI.MAX trial used low dose antioxidant supplements (120mg vitamin C; 33iu vitamin E; 100µg selenium) and reported a reduced risk of total cancer in men but not women, which may be due to the lower baseline antioxidant status of men.[256] Breast cancer risk was not reported in this trial.

Most studies evaluating the relationship between supplement use and breast cancer risk have focused on individual supplements. Only a few studies mentioned in the 2007 WCRF review examined general supplement use in relation to breast cancer and these were case-control studies which may be prone to selection and recall bias.[257-259] No associations were found in the Danish and US studies reported in the 2007 review,[257 259] whereas a significant protective effect of supplements on breast cancer was found in a Taiwanese study (OR=0.40, 95% CI: 0.3, 0.7). Taiwanese women may generally have lower intake levels of nutrients than western women and therefore may require supplementation to reduce their risk.

A recent meta-analysis of five cohort studies found no evidence of a significant association between multivitamin use and breast cancer risk.[260] This included a Swedish study which reported an association between multivitamin use and increased risk of breast cancer.[261] The authors suggest that folic acid in the multivitamins may have promoted cancer, since there is no mandatory folic acid fortification of food in Sweden. Conversely, the US does fortify food with folic acid and the four US studies have not found an increased risk of breast cancer with multivitamin use.[250 251 262 263]

3.6.4.1 WCRF systematic literature review of vitamin C intake and breast cancer risk

Over thirty-five studies published to the beginning of 2006 relating to vitamin C intake and breast cancer risk were systematically reviewed in the 2007 WCRF report;[7] most were retrospective case-control studies, 12 were prospective cohort studies and 5 of these were nested case-control studies (the individual cohorts are detailed in Table 3 in section 3.7.2). The meta-analysis of 14 retrospective case-control studies of dietary vitamin C intake provided significant evidence of a protective association for breast cancer not split by menopausal status (RR=0.88, 95% CI: 0.84, 0.92 per 100mg/day), though heterogeneity was high (I^2 = 85%) between the studies. Meta-analyses of pre-menopausal (RR=0.90; 95% CI: 0.82, 0.98 per 100mg/day), and post-menopausal analyses (RR=0.86; 95% CI 0.79-0.93 per 100mg/day), supported this, as did the analyses comparing high verses low intake categories. In contrast, as seen later in Table 3 in section 3.7.2, cohort results were inconclusive; this was the case for analyses assessing dietary vitamin C only,[239 240 242 243 245-247 264-267] supplement vitamin C only,[241 242 245-247 267] or total vitamin C intake.[239 241 242 245 247] In the 2007 WCRF report no significant associations were produced for dietary vitamin C in the post-menopausal women meta-analyses of three cohort studies (HR=1.15; 95% CI: 0.92, 1.43 per 100mg/d), [7 245 246 265] or in the high versus low intake analysis of two US studies.[242 247]

One study included in the WCRF cohort meta-analyses did find substantial evidence that breast cancer risk increased with increasing vitamin C intake in relation to dietary only (RR=2.06; 95%CI 1.45, 2.91, per 100mg/d), supplementation only (RR=1.06; 95%CI: 1.01,1.13) and total vitamin C intake (RR=1.08; 95%CI: 1.02, 1.15).[245] This Danish nested case-control study is compared with the other studies later in this chapter.

A prospective nested case-control study included in the 2007 WCRF report assessed the relationship between vitamin C plasma levels and breast cancer and found no association.[268] Whether or not plasma levels are a better indicator of vitamin C intake than data from FFQs or diaries is discussed in section 5.4 in the evaluation chapter.

Differences in findings between study types may be due to recall bias in the retrospective case-control studies or to differences between assessment methods; FFQs were used in the cohort studies and interview-administered diet histories were used mainly in retrospective case-control studies. No relevant studies were published between the search for the 2007 WCRF report and that for the 2008 WCRF continuous update, although results are now clearly tabled in the latter.[169] The studies from the

WCRF reports are tabled and compared in section 3.7 of this thesis with the studies found from the new literature search undertaken in 2011 for this thesis.

3.7 Updated systematic literature review of vitamin C intake and risk of breast cancer

The purpose of this systematic review was to search for and evaluate cohort studies and RCTs relating to vitamin C intake and breast cancer incidence in women with no previous breast cancer. Only studies that were published since the beginning of 2006 until June 2011, and that were not included in the 2007 or 2008 WCRF, report were considered. New case-control studies were not evaluated since it was clear from previous meta-analyses that these types of studies show an inverse association between retrospectively reported vitamin C intake and decreased breast cancer risk; these findings may be due to recall bias.

3.7.1 Criteria for considering studies

Types of studies

Included: Cohorts, prospective nested case-control studies and RCTs. Reported in English.

Excluded: Retrospective case-control studies, cross-sectional studies

Types of participants

Included: women whose dietary or supplement vitamin C intake has been estimated, or who were part of an RCT vitamin C supplementation study and whose cancer status was known at time of dietary recording/intervention and at censor date. No age limit, any country

Excluded: women with previous breast cancer (i.e. cancer patients or survivors)

Types of exposure

Included: dietary or supplement vitamin C intake estimated using dietary assessment tools such as FFQs and diaries or intake provided in the intervention of a RCT supplementation study

Types of outcome

Included: incident invasive breast cancer

3.7.1.1 Search strategy for identification of studies

OVID MEDLINE (Jan 2006-June 2011) was systematically searched for relevant articles using the key words which may appear in the title, subject headings or abstract

of articles. These key words were: *vitamin C; ascorbate; ascorbic acid; antioxidant* in conjunction with *vitamin; micronutrients* in conjunction with *fruit and vegetable; breast* in conjunction with *cancer, neoplasm, tumour, malignant, carcinoma and adenocarcinoma.* The search was restricted to extract articles relating to diet only studies using key words: *food, diet, intake, nutrient, supplement, nutrient surveys, dietary supplement, and dietary record.* It was also restricted to include only those articles published during and after the last year of the WCRF 2007 search i.e. from the beginning of 2006 to the beginning of June 2011. Search criteria were also applied to restrict the search to epidemiological studies using terms recommended by the BMJ.[269] The full search strategies can be found in 0.

The initial search criteria found 62 articles, six of which were duplicates. The title, subject headings and abstracts for all the articles were downloaded into a reference database and labelled according to their relevance. Eight articles at this stage appeared relevant, relating to the study of the association between breast cancer risk and vitamin C intake.[244 270-276] Five were case-control studies and were excluded,[271 272 274-276] two of these were sub-analysed by genotype.[272 276] This left three cohort studies for the main review,[244 270 273] which are tabled in Table 2.

Articles citing or cited by relevant articles which were identified above or in the 2007 WCRF report were manually screened to determine whether they were also relevant. No further cohort studies were found; however, one RCT was identified which analysed vitamin C supplementation and breast cancer risk, though 'breast cancer' did not appear in the title or abstract.[14] Figure 6 shows the flow diagram of the studies included in this current review. A limitation of the search criteria was that articles with breast cancer analyses included in the main body would not be identified if only 'cancer', but not 'breast cancer' had been included in the title or abstract. Furthermore, articles that did not contain key words related to vitamin C in the title or abstract may have been missed. As described at the end of 0 some work was undertaken to check the latter, but no additional articles that were found met the full inclusion criteria.

Later, during a follow-up manual search for citations, a meta-analysis of vitamin C and breast cancer risk was found which was published online in July 2011. This review by Fulan et al. (2011), which had searched for publications to March 2011, found no studies in addition to those found in the search undertaken for this thesis.[277] Furthermore, it did not report finding one study (Roswall et al. 2010)[273] which was found in the current search. Nevertheless, since this recently published review had searched the PubMed, Embase and Cochrane databases, it was decided not to extend the current search to other databases.[277]

3.7.2 Search results summary

The four recent studies identified in the current search relating to breast cancer risk and vitamin C intake are summarised in this section and shown in Table 2. In later sections these are compared with the studies that were included in the 2007 WCRF report and the 2008 WCRF continuous update which are shown in Table 3.[7 169]

Two out of the three cohort studies retrieved undertook separate analysis for dietary vitamin C intake, supplement intake and also diet plus supplement i.e. total vitamin C intake in postmenopausal women.[270 273] Both of these studies also sub-analysed by hormone-receptor status of the cancers, the results of which are discussed in section 3.7.3.1.3. One was an analysis by Cui et al. (2008) of the Women's Health Initiative study, a large US cohort following 84,805 women over an average of 7.6 years with 2879 cases which did find an increased risk of breast cancer with intake.[270] The other study by Roswall et al. (2010) followed 26,224 Danish women over a median of 10.6 years with 1072 cases, and did not find any evidence of an association with vitamin C intake. [273] A previous analysis of this Danish cohort by Nissen et al. (2003) found an increase in breast cancer risk with dietary, supplement and total vitamin C intake, however this was a nested case-control study and therefore was subject to potential selection bias of the controls, and incidences were followed-up for a shorter period.[245]

The third study retrieved was an EPIC pooled analysis of cohort data from 10 European countries following about 520,000 women over a median of 8.8 years resulting in 7502 cases.[244] Only dietary vitamin C was considered in this analysis. It was substantially larger than any previous study undertaken relating to breast cancer and vitamin C intake, and had sufficient power to sub-analyse by supplement use, alcohol consumption, hormone use and smoking status, as well as by menopausal status. To account for differences in questionnaire design and follow-up procedures between the cohorts, a stratum method of Cox proportional hazards was used. This did not find any significant associations in the main pre- and post-menopausal sub-analyses.

The RCT, the Women's Antioxidant Cardiovascular Study (WACS) mentioned earlier in section 3.6.4, was based in the US and had a factorial design with intervention groups using 500mg vitamin C daily, 600IU Vitamin E every other day and 50mg beta carotene every other day for 9.4 years. Women who were at high risk of cardiovascular disease, post-menopausal and over 40 were recruited. The study reported no effects of the supplements, either individually or together, on total cancer, including breast cancer incidence or mortality.[14]

Figure 6 Flow diagram of studies identified in current literature search

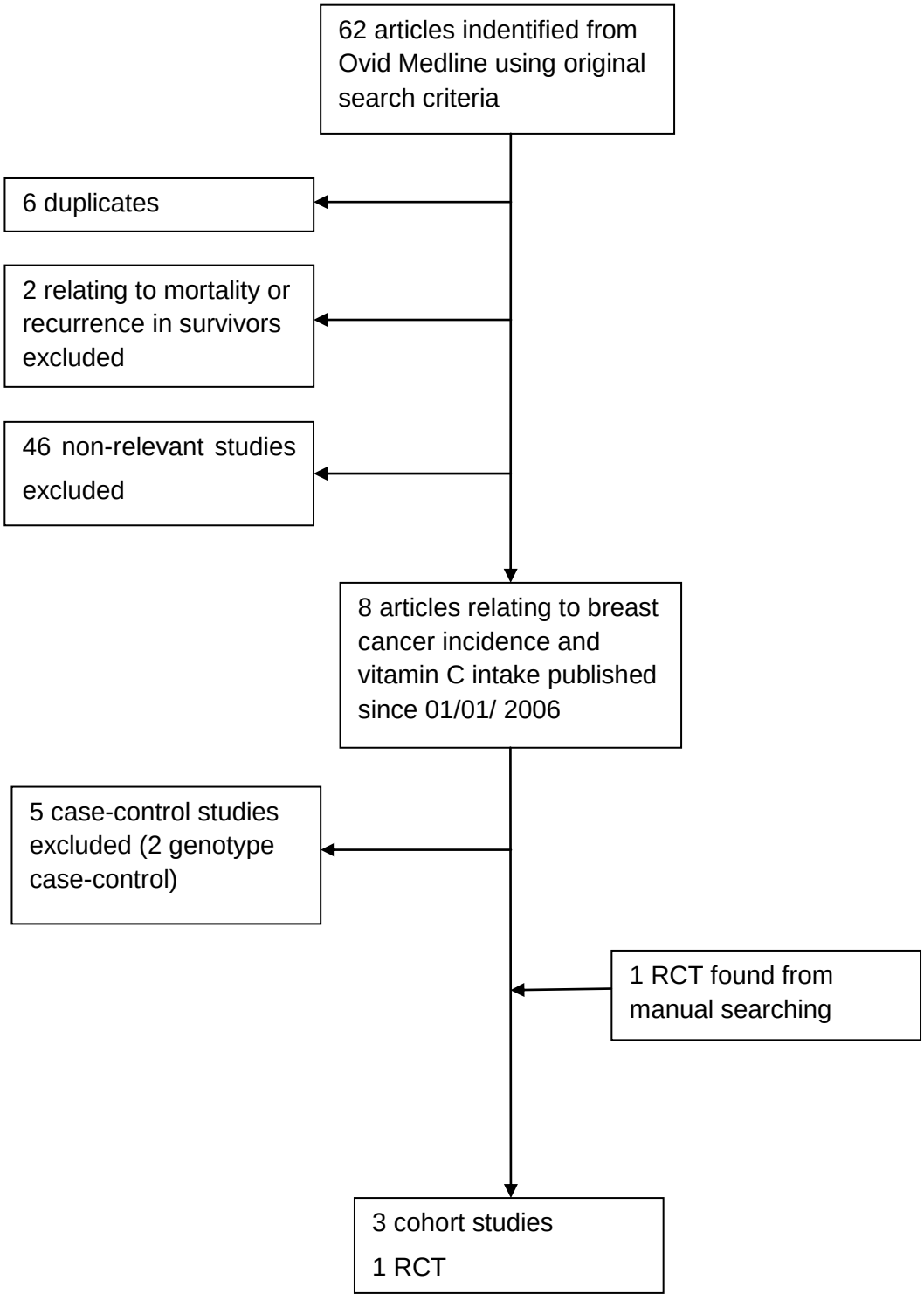


Table 2 Studies identified by a search for articles published after the 2007 WCRF report and 2008 WCRF continuous update

Author, year	Study	Design Baseline date & age	Follow up & cancer incidence	Meno-pausal status	Vitamin C risk ratios	Additional sub-group analyses
Roswall et al 2010[273]	Diet, Cancer and Health Cohort Denmark	Prospective N=26,224 1993-1997 Age 50-64	Median 10.6yrs 1072 cases	Post	High vs. Low intake Total 1.11 (0.88-1.40) p_{trend} = 0.38 Dietary 1.15 (0.92-1.44) p_{trend} = 0.96 Supplement 0.96 (0.77-1.21) p_{trend} = 0.41	ER and PR status Histology
Nagel et al 2010[244]	EPIC Europe wide	Prospective pooled cohorts N=520,000 1992-2000 Age 35-70	Median 8.8yrs 7502 cases	Pre Post	High vs. Low intake Dietary 1.12 (0.92-1.46) p_{trend} = 0.37 Non-supplement users 1.15 (0.88-1.51) p_{trend} = 0.69 Dietary 0.98 (0.87-1.11) p_{trend} = 0.79 Non-supplement users 0.92 (0.77-1.10) p_{trend} = 0.69	Alcohol consumption, hormone use, smoking status
Cui et al 2008[270]	Women's Health Initiative US	Prospective cohort N=84,805 1993-1998 Age 50-79	Av 7.6yrs 2879 cases	Post	High vs. Low intake Total 1.18 (1.04-1.34) p_{trend} = 0.009 Dietary 1.06 (0.92-1.22) p_{trend} = 0.23 Supplement 1.16 (1.04-1.30) p_{trend} = 0.03	ER and PR status
Lin et al 2009[14]	Women's Antioxidant Cardio-vascular Study US	RCT Factorial design N=7,627 Age >=40	Av 9 yrs 135 cases	Post	500mg/d supplement group compared to placebo group 1.11 (0.87–1.41)	600 IU/d vitamin E 50/2d β-carotene

Table 3 The WCRF reported studies on vitamin C intake and breast cancer risk

Author, year	Study	Design Baseline date & age	Follow up & cancer incidence	Meno-pausal status	Vitamin C risk ratios	Additional sub-group analyses
Cho et al. 2003[239]	Nurses' Health Study II US	Prospective cohort N=90,655 1991-1999 Age BL 26-46	8yrs: 714 cases	Pre	High vs. low intake Total 0.98 (0.75-1.21) p_{trend}=0.72 Diet 1.30 (1.00-1.69) p_{trend}=0.16	Smoking status
Nissen et al. 2003[245]	Danish Diet & Cancer Cohort	Prospective nested case-control 1993-1997 Age 50-64	Mean 4.7yrs: 418 Cases 394 control Both diet & sup: 228 Cases 246 controls	Post	Per 100mg/d (vit C sup users) Total 1.08 (1.02-1.15) Diet 2.06 (1.45-2.91) Sup 1.06 (1.01-1.13)	Non-vit C supplement users =1.54 (0.08-2.96)
Zhang et al. 1999[247]	Nurses' Health Study US	Prospective cohort 1980 N=83,235 Age 33-60	14yrs: 2697 Cases Pre 784 Post 1913	Pre Post Not split	High vs. low intake Total 1.01 (0.81-1.26) p_{trend}=0.59 Diet 1.01 (0.81-1.26) p_{trend}=0.82 Total 0.99 (0.85-1.14) p_{trend}=0.77 Diet 1.06 (0.91-1.22) p_{trend}=0.57 Supplement >1300mg/d vs. none 1.04 (0.77-1.42)	HRT use, Family history of breast cancer, Alcohol
Kushi et al. 1996[242]	Iowa Women's Health Study US	Prospective cohort 1986 N=34,387 N=18,910(sup) Age 55-69	Av 7yrs: 879 Cases (sup 570)	Post	High vs. low intake Total 0.88 (0.70-1.11) p_{trend}=0.46 Diet 1.06 (0.77-1.47) p_{trend}=0.88 Sup 0.77 (0.50-1.17) p_{trend}=0.20	Supplement only
Graham et al. 1992[265]	New York State Cohort US	Prospective cohort 1980 N=17401 Age 50-107	8yrs 344 Cases	Post	High vs. low intake Diet 0.81 (0.59-1.12) p_{trend}=0.20	

Table continued - The WCRF reported studies on vitamin C intake and breast cancer risk

Author, year	Study	Design Baseline date & age	Follow up & cancer incidence	Meno-pausal status	Vitamin C risk ratios	Additional sub-group analyses
Li et al. 2005[266]	Shanghai Breast Self-examination Trial	Prospective Nested case-control 1995-2001 Age 35-60+	Cases 130 (who also had proliferative fibrocystic breast conditions) Controls 1070	Not split	High vs. low Diet 0.8 (0.2 - 2.6) p_{trend}=0.6	
Horn-Ross et al. 2002[240]	California Teachers' Study	Prospective Cohort 1995-1996 Age 21-103	2 years Cases 711	Not split	High vs. low Diet 1.1 (0.8 - 1.3) p_{trend}=0.5	
Michels et al. 2001[243]	Swedish Mammography Screening Cohort	Prospective Cohort 1987-1990 Age 40-76	130 months Cases 1271	Not split	High vs. low Diet 0.94 (0.78 -1.14) p_{trend}=0.99	BMI: >25 vs. ≤25 kgm^{-2} & high linoleic acid intake
Verho-even et al. 1997[246]	The Netherlands Cohort Study on Diet and Cancer	Prospective Nested case-control 1986 Age 55-69	4.3 years Cases 650 Controls 1066	Not split (but likely post)	High vs. low Diet 0.77 (0.55 -1.08) p_{trend}=0.08 Vit C supplement yes vs. no 1.06 (0.79-1.43)	Low/High polyunsaturated fatty acids
Rohan et al. 1993[267]	Canadian National Breast Screening Study	Prospective Nested case-control 1982 Age 40-59	6 years Cases 519 Controls 1182	Not split	Diet per 119 mg/d 0.99 (0.83 -1.17) Supplement >250mg/d vs. none 1.46 (1.05-2.01) p_{trend}=0.98	
Giovann-ucci, et al. 1993[264]	Nurses' Health Study	Prospective Nested case-control 1986 Age 30-55	2 years Cases 392 Controls 786	Not split	High vs. low Diet 1.27 (0.82 -1.95) p_{trend}=0.25	
Hunter et al. 1993[241]	Nurses' Health Study	Prospective Cohort 1980 Age 34-59	8 years Cases 1439	Not split	High vs. low Total 1.03 (0.87-1.21) p_{trend}=0.67 Supplement >1300mg/d vs. none 1.12 (0.75-1.69) p_{trend}=0.98	

Table 4 Factors controlled for in the vitamin C studies published since the 2007 and 2008 WCRF reports

Author, year	Exclusions	Hormonal factors controlled for	Dietary factors controlled for	Other factors controlled for
Roswall et al. 2010[273]	Cancer diagnosis No lifetime menstruation Missing covariates and micronutrient intake	HRT Parity Age at first birth	Alcohol intake Total intake of vitamin E and B-carotene Dietary or supplement vitamin C intake mutually adjusted	Age (used as the underlying time scale for hazard ratio rather than adjusted for it as a covariate) BMI Education
Nagel et al. 2010[244]	Missing non-dietary data Top & bottom 1% energy intake	Age at menarche Age at full-term birth Parity Hormone therapy	Energy from protein and carbohydrate Saturated fatty acids (SFA) MFA, PFA intake Alcohol intake	Age (1 year intervals) Centre (stratified) Weight Height Smoking status Physical activity Education
Cui et al. 2008[270]	History of breast cancer Implausible total energy intake <600>5000kcal/d	Age at menarche Age at first live birth Parity Age at menopause Contraceptive use HRT use Hysterectomy Bilateral oophorectomy	Energy intake Alcohol intake Dietary folate intake Dietary vitamin C	Age Ethnicity Educational level Smoking Family history of breast cancer History of benign breast disease
Lin et al. 2009[14]	Self-reported cancer diagnosis (other than skin) Unwilling to avoid vitamin A,C or E use	Randomised	Randomised	Randomised

Table 5 Factors controlled for in the studies included in the 2007 and 2008 WCRF reports

Author, year	Exclusions	Hormonal factors controlled for	Dietary factors controlled for	Other factors controlled for
Cho et al. 2003[239]	Post menopausal. Cancer (other than skin) Implausible total energy intake Missing >70 items on FFQ	Age at menarche Oral contraceptive use Age at first birth Parity Menopausal status	Calorie intake Animal fat Alcohol	Age Height BMI Family history of breast cancer History of benign breast disease Smoking status
Nissen et al. 2003[245]	Previous cancer diagnosis Pre-menopausal Missing items on FFQ Missing potential confounders on lifestyle questionnaire	Age at first birth Parity HRT duration	Intake of vitamin A & E (tested for total energy intake) Alcohol intake	Cases and Controls matched on age School education History of benign breast disease BMI
Zhang et al. 1999[247]	Previous cancer diagnosis Implausible total energy intake <500 or >3500kcal/d Missing >10 FFQ blank	Age at menarche Age at first live birth Parity Age at menopause HRT use	Alcohol intake	Age Height Length of follow up BMI & BMI @18 yrs Weight change from 18yrs History of breast cancer in mother or sister
Kushi et al. 1996[242]	Post menopausal. Full or partial breast removal Cancer (other than skin). Missing >30 items on FFQ Extreme total energy intake	Age at menarche Age at first live birth Parity Age at menopause	Energy intake Alcohol intake	Age Educational attainment BMI @ BL & 18yrs Waist/hip ratio Family history of breast cancer among first degree relatives
Graham et al. 1992[265]	Previous cancer diagnosis			Age Education

Table continued - Factors controlled for in the studies included in the 2007 and 2008 WCRF reports

Author, year	Exclusions	Hormonal factors controlled for	Dietary factors controlled for	Other factors controlled for
Li et al. 2005[266]	Energy intake >4000 kcal/d		Fruit & vegetable intake	Age Matched on year of interview
Horn-Ross et al. 2002[240]	Previous cancer diagnosis Energy intakes <600 or >5000 kcal/d	Age at menarche Age at first live birth Menopausal status	Energy intake	Age Race Family history of breast cancer Physical activity BMI
Michels et al. 2001[243]	Previous cancer diagnosis (other than skin) Energy intakes <417 or >3729 kcal/d Missing or unreasonable covariate data	Parity Age at first live birth	Energy intake Intake of alcohol, fibre and mono-sat fatty acids	Age (5 year categories) Family history of breast cancer Height BMI Education
Verhoeven et al. 1997[246]	Baseline self-reported cancer (other than skin) at baseline Incomplete FFQ (>60 items blank)	Age at menarche Age at menopause Age at first live birth Parity	Energy intake Alcohol intake Vitamin A	Age History of benign breast disease History of breast cancer in mother or sister
Rohan et al. 1993[267]	History of breast cancer No mammogram in proceeding 12 months	Age at menarche Surgical menopause Age at first live birth	Energy intake	Matched on age (+/- 1yr, screening centre, date of enrolment) Age Years of education Family history of breast cancer History of benign breast disease
Giovannucci, et al. 1993[264]	Energy intakes <500 or >4000 kcal/d			Matched on age
Hunter et al. 1993[241]	Follow-up cases not confirmed by records	Age at menarche Age at first live birth Menopausal status	Energy intake Alcohol intake	Age (5 year categories) Length of follow-up History of breast cancer in mother or sister BMI History of benign breast disease

3.7.2.1 Quality of studies

The overall quality of the three cohort studies was good, with only some limitations. Quality was very good in relation to outcome measures which used methods employed by previous cohort studies: in two of the recent cohorts breast cancer incidence was provided from cancer registries;[244 273] and in the other cohort and RCT, self-reported cases were independently assessed from medical records.[270] Follow-up for the RCT was 93% complete. The average length of follow-up was between 7.6 and 10.6 years which is comparable with the previous cohort studies, the longest being the analyses of the Nurses' Health Study (14 years). A limitation, however, is that these follow-up times may not be long enough to detect the development of cancer which can take decades.

Quality was good in terms of controlling for confounding factors: similar to the majority of previous studies the cohorts adjusted for many known hormonal and non-hormonal factors linked to breast cancer as discussed earlier in this chapter, and seen in Table 4. There was additional adjustment for some dietary factors though these varied between cohorts and from previous cohorts. All of these, and previous studies, adjusted for alcohol intake and the majority adjusted for energy intake though this was not done in the main analysis of the Danish study;[273] the previous nested case-control analysis of this cohort made adjustment for energy in sensitivity analysis.[245] (A discussion of reasons for controlling for energy intake can be found in section 4.7.5.3.) The Danish study also adjusted for other antioxidants examined. In the supplement-only analyses adjustment was made for dietary vitamin C in the Women's Heath Initiative and the Danish studies,[245 270 273] but other supplement-only analyses did not do this.[242 247 267] Despite the adjustment for a large number of factors, residual confounding is still likely. The RCT was superior in this respect since potential confounding factors were randomised between intervention and non intervention group. However, there is no information on whether steps were taken to avoid subversion by staff during randomisation. Furthermore, some women in the non-intervention group may have consumed vitamin C supplements since they are readily available, even though they would have been asked to avoid using them.

There were some weaknesses in relation to exposure which is common to all cohort studies in this research area: the vitamin C dietary and supplement intake of participants of all the cohorts may not be very representative of women in the populations they were recruited from. Women who were prepared to complete the FFQs (which in general comprised of between 100-200 items) were likely to be more health conscious that the average woman, and therefore more likely to have a higher intake of vitamin C. Since all cohorts used FFQs, the ascertainment of intake is

reasonably comparable between cohorts though there were some differences between types used. However, there are limitations with the self-reporting nature of FFQs, the general ability of the FFQ to record average intake and the conversion of food intake into nutrient intake, some of which are discussed further in chapter 5. In relation to exposure the RCT was limited to 500mg/d supplement intake, meaning it may be less able to detect the most effective dose than cohorts. Compliance, defined as having taken at least two thirds of the study supplements, was 76% at 4 years and 68% at 8 years (average73%). Outside use of the supplements for at least 4 days per month was not different between the supplement and the placebo group, which ranged from 2% to 13% at both 4 years and 8 years.[14]

The results from the four recent studies for breast cancer risk in relation to vitamin C intake are discussed separately in the following sections by dietary, supplement and total intake (Table 2) and are compared to results of studies that were included in the 2007 WCRF report and the 2008 WCRF continuous update (Table 3).[7 169]

3.7.3 Dietary vitamin C intake

3.7.3.1 Post-menopausal women

All three of the recent cohort studies found no evidence of a significant association between dietary vitamin C intake and post-menopausal breast cancer risk.[244 270 273] As seen in Table 2 risk ratios for high versus low intake for all three studies showed weak but non-significant increases in risk. There was also no significant evidence of trends across the intake groups neither in the three studies, nor for the risk per 100mg/d increases calculated in two of the studies where risk ratios were close to unity.[244 273]

Similarly, three earlier analyses found no evidence of significant associations for post-menopausal women in the New York State cohort, the Iowa Women's Health Study and the Nurses' Health Study.[242 247 265] The analysis of women aged 55-69 in the Netherlands Cohort Study, likely to be of post-menopausal status, reported a small non-significant reduction in risk with increasing intake of dietary vitamin C (high versus lowest fifth OR= 0.77; 95% CI: 0.55, 1.08) and a significant trend in intake.[246]

In contrast, Nissen et al. (2003) in an earlier nested case-control analysis of the Danish Diet, Cancer and Health cohort (418 case and 394 controls) did find a significant doubling in risk per 100mg/d increase in intake for post-menopausal women (OR=2.06; 95% CI: 1.45, 2.91).[245] Unlike other studies mentioned, this was restricted to women who consumed vitamin C through both diet and supplements; women who did not consume vitamin C supplements were excluded.[245] This nested case-control study followed women for 4.7 years; a shorter period than the other studies. Unlike the other

studies in the WCRF meta-analysis, Nissen et al. (2003) adjusted for intake of the other antioxidants vitamin A and E. Other possible reasons for the difference between the results of two analyses of the Danish cohort could be due to selection bias of the controls in the earlier nested case-control study, or that both cases and controls may not have been representative of the whole cohort, or that the more recent analysis used age as the underlying time scale for the hazard ratio calculation rather than adjusted for it as a covariate.[245 273]

This risk ratio of 2.06 from the Danish study was used in the 2007 WCRF cohort meta-analysis of dietary vitamin C intake for postmenopausal women,[7] even though the other studies in the meta-analysis did not restrict their analysis to women who consumed vitamin C through both diet and supplements. Combined with the New York State Cohort and the Netherland Cohort,[246 265] a non-significant increase in risk per 100mg/d increase in dietary intake for post-menopausal women was produced in the meta-analysis (RR=1.15; 95% CI: 0.92, 1.43).[7] Updating the meta-analysis to include recent studies may produce results closer to unity; the risk ratio was close to unity for the EPIC pooling project, and since this was a very large study it would have a much greater weighting than the other cohorts. Indeed, the recent meta-analysis of dietary vitamin C by Fulan et al. (2011) produced non-significant odds ratios close to unity for post-menopausal women (RR=0.97; 95% CI: 0.81, 1.06). However this calculation included case-control studies as well as cohort studies.[277] Although it included the recent large EPIC pooling project and the Women's Health Initiative,[244 270] Fulan et al. (2011) had not included the Roswall et al. (2010) Danish study[273] in any of their meta-analyses.[277] Rather than continuous estimates, the Fulan et al. (2011) pooled high versus low results of the studies even though the cut off points for each study were different; this was an additional limitation of the meta-analyses.

3.7.3.1.1 Non-supplement users

Supplement users in general have healthier behaviours than non-users,[27] therefore excluding all types of supplement users from analyses will reduce potential confounding and provide a clearer picture of associations between dietary vitamin C intake and risk. The exclusion of all supplement users in the EPIC pooling project, which had sufficient power to undertake sub-analyses, did not produce any evidence of associations and the hazard ratios did not change substantially in the pre or post-menopausal analyses.[244]

Excluding vitamin C supplement users in the Danish nested case-control study reported by Nissen et al. (2003) did reduce the association substantially from a doubling in risk which became non-significant (OR=1.54; 95% CI: 0.80, 2.96 per

100mg/day).[245] The more recent analysis of the Danish study, however, did not exclude supplement users from the analysis.[273] An additional analysis of the Iowa Women's Health Study excluded women who took antioxidant supplements A,C and E and this increased the point estimate in the high versus low calculation from a non-significant decrease (HR=0.88; 95% CI: 0.70, 1.11) to a non-significant increase in risk (HR=1.06; 95% CI: 0.77, 1.47). Unlike the EPIC study all supplement users were not excluded.

3.7.3.1.2 HRT use

In the EPIC pooling project, post-menopausal women using exogenous hormones who were in the highest fifth of dietary vitamin C intake had a lower breast cancer risk than those in the lowest fifth (HR=0.88; 95% CI: 0.72, 1.07), though this was not significant. However there was evidence of a significant trend of decreased risk per 100/mg increases in intake, though the hazard ratio was close to unity and it was only marginally significant (HR=0.989; 95% CI 0.979, 0.999; p_{trend} 0.05).[244] This does not provide strong evidence of a protective effect. No other study appears to have undertaken this sub-analysis

3.7.3.1.3 Hormone receptor status

Both the Women's Health Initiative and the Danish Study undertook sub-analyses by hormone-receptor status of the cancers.[270] [273] They found no association between dietary vitamin C intake and breast cancer risk of ER+PR+, ER-PER-, ER+PR- or ER-Pr+ cancers. [270] [273] Results of analyses with supplement intake and total intake are found in sections 3.7.4 and 3.7.5.

3.7.3.2 Pre-menopausal women

Only one of the recent studies analysed dietary vitamin C intake for pre-menopausal women which was the pooled EPIC study of 10 European cohorts; this found no evidence of a significant association.[244]

Results included in the 2007 WCRF report from the first and second phase of the Nurses' Health Study produced some conflicting results; the first phase analysis found no associations,[247] however the second phase reported significant evidence of an increased risk between high and low intake (mean 200 vs. 69mg/d; HR=1.30; 95% CI: 1.00, 1.69) but there was no significant trend, the highest risk being in the middle intake group.[239]

Pre-menopausal women with a family history of breast cancer in the analysis of the Nurse' Health Study reported by Zhang et al (1999) did have a substantially reduced risk (high vs. low intake HR=0.37; 95% CI: 0.17, 0.80), however the number of cases in this sub-analysis was low (90) which may have influenced the results.[247]

3.7.3.2.1 Smoking status

The recent EPIC study and the Nurse' Health Study II were sub-analysed by smoking status.[239] [244] Although Negal et al. (2010) mentioned that the results of their study suggests pre-menopausal former and never smokers have a higher risk of breast cancer at higher vitamin C intakes, neither study has produced strong evidence that smoking status modifies risk.[239] [244]

3.7.3.3 BMI

None of the recent studies examined whether BMI modified the relationship with dietary vitamin C and breast cancer incidence. However the RCT did assess this for total cancer incidence and cancer death in relation to supplement intake; this is mentioned below in section 3.7.4.[14]

As tabled in the 2008 WCRF continuous update report,[169] a Swedish study of 59,036 women attending Mammography screening produced evidence of an inverse association between breast cancer incidence and dietary vitamin C intake in women who were overweight (>25kg/m^2) (high vs. low HR=0.61; 95% CI: 0.45, 0.82; p_{trend} 0.004).[243] Conversely there was an increase in risk with increased intake for women of normal or lower BMI (≥25kg/m^2) (high vs. low HR=1.27 95% CI: 0.99, 01.63; p_{trend} 0.02). This analysis was not split by menopausal status, though the women were aged between 40-76 years old therefore the majority would have been post-menopausal at the time the FFQs were completed.[243]

3.7.3.4 Menopausal status not specified

None of the three recent cohorts report risk un-stratified by menopausal status. As listed in the 2008 WCRF continuous update for breast cancer, there were six previous cohorts which reported risk un-stratified by menopausal status. [240] [243] [246] [264] [266] [267] As seen in Table 3 none of these reported a significant association between dietary vitamin C intake and breast cancer risk. The recent meta-analyses by Fulan et al. (2011) for cohort studies which was un-stratified by menopausal status produced a relative risk close to unity (RR=1.01; 95% CI: 0.95, 1.08) comparing high versus low intake; the heterogeneity between cohorts was low.[277]

One study (not tabled) compared dietary vitamin C intake in adolescence with breast cancer incidence between the ages of 40-65, and found no evidence of an association. [278] This nested case-control analysis of the Nurse's Health Study gathered the vitamin C data retrospectively; possible recall bias, and also the short 24 item food questionnaire were limitations of the study.

3.7.4 Supplement only vitamin C intake

Mixed results were produced in the three recent studies (the Women's Health Initiative, the Danish Diet and Cancer Cohort and the Women's Antioxidant Cardio-vascular Study RCT),[14 270 273] as well as in previous studies that assessed supplement only vitamin C intake.[242 245 247 267]

There was no evidence that supplementation with 500mg/d vitamin C had an effect overall on breast cancer incidence in the US double-blind RCT of initially cancer free post-menopausal women over the age of 40.[14] Additionally, in sub-analysis by BMI no evidence of significant risks of breast cancer were found. However, the sub-group analysis of women with normal or low BMI (<25kg/m^2) in the vitamin C supplementation group had a significantly increased risk of cancer death (RR=2.00; 95%CI: 1.12, 3.58), whereas there was no evidence of a significant association for overweight women (BMI ≥25kg/dm^2).[14] The author believes the results may be a chance finding due to small numbers involved (44 supplement group: 17 placebo group). An alternative explanation is that normal weight women may have a lower level of reactive oxygen species (ROS) than overweight women; ROS required for apoptosis of damaged cells may be suppressed by antioxidants in normal women.

Recently Cui et al. (2008) reported a significant trend ($p_{trend\ =}$ 0.03) of increasing risk with increasing vitamin C intake from supplements for post-menopausal women in the Women's Health Initiative; there was also significant evidence of a weak positive association in the high versus low intake calculation (HR=1.16; 95% CI: 1.04, 1.30).[270] Conversely, in the recent Danish analysis Roswall et al. (2010) reported no evidence of associations for post-menopausal women.[273]

However, in the Danish nested case-control analysis, Nissen et al. (2003) did report a significant but weak increased risk with increasing vitamin C intake from supplements (OR=1.06; 95% CI: 1.01,1.13).[245] Kushi et al. (1996) however, reported no significant associations in the Iowa Women's Health Study.[242] Neither was there evidence of an association in the Nurses' Health Study for pre-menopausal, post-menopausal or total women.[241 247] Information about the doses examined in these studies can be found later, in chapter 8. Only the Danish nested case-control study and the Iowa Women's Health Study were included in the 2007 WCRF meta-analysis per 100mg/d increase in supplement vitamin C which produced a risk ratio close to unity (RR=0.99; 95% CI: 0.98, 1.01).[7]

Rohan et al. (1993) reported evidence of a moderate increase in breast cancer risk in the Canadian Breast Screening Study for women consuming more than 250mg/d (OR=1.45: 95%CI: 1.05, 2.01) compared to women with no vitamin C intake from

supplements.[267] Results split by post-menopausal and other women were not statistically significantly different.[267]

In comparisons between total vitamin C users and non-vitamin C users no associations with breast cancer risk were found in the Netherland Cohort Study.[246] Duration of use was also not associated with risk in the Nurses' Health Study.[247]

Sub-analyses by hormone-receptor status for the recent Danish study and the Women's Health Initiative produced no evidence of significant associations for vitamin C intake from supplements. [270 273] No other studies have examined this relationship by hormone-receptor status.

The results of the above studies are detailed further by dose category in chapter 8 which determines whether breast cancer incidence in the UKWCS is associated with consumption of supplements containing vitamin C.

3.7.5 Total vitamin C intake

Two of the recent studies assessed total vitamin C intake.[270 273] A significant trend (p_{trend} = 0.009) of increasing risk by increasing total vitamin C intake was found in the Women's Health Initiative for post-menopausal women; there was also evidence between high versus low intake of a significant weak positive association (HR=1.18; 95% CI: 1.04,1.34).[270] However, in the Danish study there was no significant trend (p_{trend} = 0.38) and although the high versus low intake risk ratio showed a weak increase in risk this was not significant (HR=11; 95% CI: 0.88-1.40).[273]

Nissen et al. (2003) in the earlier nested case-control analysis of the Danish cohort found significant evidence of a moderate increase in risk comparing high versus low intake (OR=1.69: 95% CI: 1.12, 2.57) and also a weak increase in risk per 100mg/d increase in intake for post-menopausal women (OR=1.09: 95% CI: 1.07, 1.39).[245]

The Women's Health Initiative [270] was the most elderly cohort (average 64 years, oldest 79) of all the previous cohorts assessing total intake [239 242 245 247]; the positive associations could indicate that high intake of vitamin C may promote the progression of cancer in older people or at later stages of the disease. In contrast, the Nurses' Health Study, an equally large study, which had the longest follow-up (average 14 years) found no association with total vitamin C intake for women below the age of 60.[247]

3.7.6 Conclusion

The three recent cohorts and one RCT published since the 2007 WCRF report[7] produced mixed results; though on the whole they showed a tendency towards a weak increase in breast cancer risk with increased vitamin C intake, this was not statistically significant. These results, together with those from previous cohort studies detailed in the systematic literature review of the 2007 WCRF report and the 2008 WCRF continuous update report,[169] do not provide substantial evidence of associations between breast cancer risk and vitamin C intake. This is the case whether the relationship is examined as dietary intake, supplement intake or total intake.

3.7.6.1 How the current thesis may address areas where further research is needed

As seen from section 3.7.4 of this review, mixed results have been produced in analyses relating to vitamin C intake from supplements and breast cancer risk. The UKWCS phase 2 data provides an opportunity to assess the association between breast cancer incidence and vitamin C intake from supplements for this thesis. In chapter 8 this is assessed in relation to the European Recommended Daily Allowance (60mg/d) and high dose (500mg/d) use, as well as by continuous intake. Characteristics of women that predict vitamin C supplement use at phase 2 in the UKWCS are described first and an analysis determines whether women who have history of breast cancer are more likely to take high dose vitamin C supplements (Chapter 6).

As mentioned in section 3.7.3.1.1, previously very few studies have excluded supplement users from the dietary vitamin C analyses; these women generally have different health behaviours from non-supplement users, so their exclusion reduces confounding. The baseline phase of the UKWCS provides an opportunity within this thesis to sub-analyse dietary vitamin C intake derived from FFQ recordings by supplement users (58% of women) and non-supplement users (42%).

Other sub-analyses may be enlightening. Few of the previous cohort studies have sub-analysed by pre-menopausal status, one reason being there are less pre-menopausal than post-menopausal women. This thesis produces results by pre-menopausal status; however the power to find an effect may be limited in some analyses. Sub-analysis by hormone-receptor status could not be done since this information was not available for the UKWCS. Even though there is evidence that smoking status is associated with plasma C vitamin concentrations,[48] so far only the Nurses' Health Study II and the large EPIC pooling project have sub-analysed the relationship with dietary vitamin C by smoking status. [239] [244] However, since only 11% were current smokers in UKWCS,

there was limited power for sub-analysis, and this was not undertaken. HRT sub analysis has been undertaken in only one previous study,[244] and BMI has been sub-analysed in two studies.[14 243] Similar sub-analyses have not been undertaken in the UKWCS for this thesis, but with accumulating breast cancer cases there may be sufficient numbers in the future for these sub-analyses.

As mentioned in section 3.7.2.1, and discussed further in chapter 5, there are some limitations with the general ability of FFQs to record average vitamin C intake which have been used by the three recent cohort studies and previous cohorts. The UK Dietary Cohort Consortium, described in the methods chapter, provides an opportunity to assess vitamin C intake in relation to breast cancer risk using an alternative measurement method: diary data. It also provides an opportunity to assess total intake which has only be analysed previously in 5 cohorts (by 7 analyses). The current analysis and results of this are discussed in chapter 10 of this thesis.

The next chapter describes the methods relating to the UKWCS and the UK Dietary Cohort Consortium, and also describes the cleaning of vitamin C supplement data and discusses the choice of covariates used.

CHAPTER 4

4 Methods

4.1 Study design, study populations and datasets

All the analyses in this thesis used pre-gathered data from some of the largest population-based prospective studies in the UK which were designed to assess associations between diet and chronic diseases. Figure 1 in chapter 1 illustrates the datasets and the dietary tools used in the analyses. The majority of these analyses used previously unexploited vitamin C supplement data, results of which are reported in chapters 6, 8 and 10. Data from the UK Women's Cohort Study (UKWCS) was used in all the analyses, and was part of the consortium of UK nested case-control studies described below in section 4.1.2 and chapter 10.

For the first time these datasets have been used to explore breast cancer risk in relation to:

- any supplement use, and also dietary vitamin C intake from FFQs (split by supplement users and non-users) at baseline in the UKWCS
- vitamin C contained in supplements at phase 2 of the UKWCS
- total vitamin C intake (from diet and supplements) from diary records in the UK Dietary Cohort Consortium

The phase 2 UKWCS data also provided an opportunity to determine whether women who have a history of breast cancer were more likely to take high dose vitamin C supplements. Note that total vitamin C intake could not be assessed using the full UKWCS cohort because both dietary and supplement vitamin C intake had not been captured electronically at either baseline or phase 2. Instead, pooling of nested case-control data was required.

A major strength of these studies is that their prospective nature minimised recall bias and responder bias, which can affect results of retrospective case-control studies. Selection bias, however, may have been present in the prospective nested case-control studies, though this may have been minimal.

4.1.1 The UK Women's Cohort study (UKWCS)

4.1.1.1 Baseline dataset

The UK Women's Cohort Study was formed from about 500,000 responders to a direct mail survey from the World Cancer Research Fund (WCRF), targeted towards women living in England, Wales and Scotland.[279] The overall response rate to the initial mailing was 17%, and 75% of these were willing to participate further.[279] Sixty one thousand women aged between 35-69 years old were then invited to take part in the study. Since the cohort was originally designed to compare disease incidence in vegetarians, fish-eaters and red meat eaters, all eligible women who stated they were vegetarians or non-red meat eaters were asked to take part, but only a portion of the red meat eating majority were invited to do so. For each vegetarian, the next non-vegetarian in the list aged within 10 years of the vegetarian was selected for the cohort.[279] This structure provided a wide range of fruit and vegetable intakes, and as well vitamin C intakes, and helped to identify the effects of high consumption. The type of women recruited via the WCRF willing to complete long forms, in addition to the inclusion of a large proportion of vegetarians, meant that in general the cohort was likely to be more health conscious than UK women on average. This reduces the generalizability of the results.

In total 35,372 women (58% of those invited) provided data for the baseline; between 1995 and 1998 these women completed a 217-item Food Frequency Questionnaire (FFQ) and also additional demographic, health and lifestyle questions, totalling 24 pages (see 0).[279] The cohort was mainly white, well-educated, middle-class, middle-aged, married women.[279] Although 28% stated they were vegetarian, a more accurate but pragmatic definition classified 18% as vegetarian if they ate meat or fish less than once a week. At baseline 62% took some type of dietary supplement; however, the details of supplements taken were not captured electronically.

Baseline UKWCS dietary vitamin C data derived from the FFQ is evaluated in section 5.3. The risk of breast cancer risk in relation to any supplement use at baseline is reported in chapter 7 and in relation to dietary vitamin C intake at baseline is reported in chapter 8.

4.1.1.2 Phase 2 'follow up' dataset

All the initial participants were re-contacted between 1999 and 2004, on average four years after recruitment; 14,172 (40%) completed a follow-up health and lifestyle questionnaire similar to that used at baseline and 12,453 (35%) also completed a four day food diary.

Answers to questions on the health and lifestyle questionnaire relating to 17 different types of supplement were electronically captured for all phase 2 women (see section 4.5.2). More detailed supplement use was recorded in the 4-d diaries at phase 2 (see section 4.5.3). The cleaning of the vitamin C supplement data derived from supplement use detailed in the 4-d diary recordings is explained below in section 4.5.3.3. Phase 2 UKWCS supplement vitamin C data is evaluated in section 5.2 and the breast cancer risk in relation to supplement vitamin C intake at phase 2 is reported in chapter 8.

Unfortunately, the effects of dietary vitamin C intake could not be assessed for the whole cohort at phase 2 because FFQs were not included at phase 2 and in addition the dietary intake entered in diaries was only captured electronically for a small proportion of women in the nested case-control datasets (see section 4.1.1.3).

4.1.1.3 Phase 2 nested case-control dataset

Diaries provide an alternative method of measuring dietary intake; comparisons with FFQs relating to vitamin C intake are discussed in chapters 5 and 10. However, coding of diaries requires a large amount of resources. To reduce the amount of coding needed to examine the risk of breast cancer in relation to dietary intake recorded by diaries it had been necessary to create a nested case-control dataset within the phase 2 cohort. This initial UKWCS dataset comprised roughly 200 women from the total cohort women who had developed breast cancer after completing the diaries and these were matched to four or five controls. To increase the power of the analyses, the UKWCS nested case-control dataset, comprising 186 cases and 785 controls, was pooled the with four other UK nested case-control data by the Centre for Nutritional Epidemiology and Cancer Prevention (CNC) based in Cambridge to form the original breast cancer dataset of the UK Dietary Cohort Consortium. More information is given in section 4.1.2 about this consortium, and chapter 10 reports the results of breast cancer risk in this consortium in relation to dietary and total vitamin C intake recorded by diaries.

Note that, due to potential selection bias, 144 cases and 583 matched controls from the UKWCS, which were coded using DINER (Data into Nutrients for Epidemiological research), a system developed at Cambridge, were dropped from the dataset used for the published manuscript.[2] These controls were considered not to be a random selection since they were more likely to be meat-eaters and less likely to be vegetarians than the cohort as a whole. This did not change the overall results from those reported in chapter 10.

4.1.1.4 Laboratory analysed dataset

An additional 303 UKWCS women who completed phase 2 diaries and questionnaires also provided blood samples for laboratory analysis of micronutrient levels in a prior study.[280] This dataset was previously used to assess the impact of high non-starch polysaccharide intake on serum micronutrient concentrations.[280] Information on these women and the methods used to collect the plasma vitamin C data can be found in section 5.4.2.1 and 5.4.2.3. As described in section 5.4, this dataset was used within this thesis to compare plasma vitamin C concentrations with vitamin C intake derived from FFQs and with intake derived from diary data.

4.1.2 The UK Dietary Cohort Consortium

Breast cancer nested case-control data was pooled by the Centre for Nutritional Epidemiology and Cancer Prevention (CNC) at Cambridge to explore associations between breast cancer risk and dietary intake recorded by diary data from the five prospective UK cohorts described below. Information about the number of cases to controls, the mean time to diagnosis and the number of diary days for each cohort can be observed in Table 6. Data from all five cohorts were used in the analysis of dietary vitamin C intake and breast cancer risk. Only EPIC Oxford, EPIC Norfolk and UKWCS case-control datasets had detailed vitamin C intake from supplements and were pooled for the total vitamin C analyses. The risk of breast cancer risk in relation to dietary intake and total intake (supplement and dietary vitamin C) recorded by diary is reported in chapter 10.

4.1.2.1 Oxford arm of EPIC

A nested case-control group of women was taken from the EPIC Oxford study which was originally established to compare fish eaters, meat eaters, vegetarians and vegans.[281] In total, 65,429 men and women aged 20-79 years had been recruited during the 1990s using several methods: by direct mailing of the general public using GP listings in Oxfordshire, Buckinghamshire and Greater Manchester; by mailing members of The Vegetarian Society of the UK and also all surviving participants in the Oxford Vegetarian Study[282]; and by a small portion by GPs recruiting patients attending GP surgeries in Scotland. This resulted in a wide variation in intakes of major nutrients over the whole cohort.

4.1.2.2 Norfolk arm of EPIC

A nested case-control group of women was taken from the EPIC-Norfolk study which was made up of 23,003 men and women aged 45-75 years recruited between 1993 and1997 from people registered with 35 Norfolk GPs.[283]

Table 6 Characteristics of the five cohorts in the UK Dietary Cohort Consortium included in analyses of vitamin C and breast cancer risk

Cohort	Participants	Diary days	Years when food diary completed	Last follow up date	Mean time to diagnosis of cases	Cases	Controls	Mean(sd) dietary vit C intake	Mean(sd) total vit C intake
EPIC-Norfolk	General population in Norfolk	7 days	1993-1998	31.12.2006	6.0 yrs	365	1329	91 (50)	118 (167)
EPIC-Oxford	General population and vegetarians in the UK	7 days	1993-1998	31.12.2004	3.5 yrs	194	194	111 (61)	233 (436)
UK Women's Cohort Study (UKWCS)	Middle aged women in the UK	4 days	1999-2003	31.12.2006	2.4 yrs	186	785	117 (61)	239 (361)
Whitehall II	Civil servants in the UK	7 days	1991-1993	30.09.2005	7.8 yrs	70	275	101 (51)	_[a]
National Survey of Health and Development (NSHD)	Nationally reprehensive cohort of women who were born in one week in March 1946 in England, Wales and Scotland.	5 days	1989	31.12.2006	10.8 yrs	36	144	66 (37)	_[a]

[a]Whitehall and NSHD did not have detailed diary data of vitamin C intake from supplements

4.1.2.3 The UKWCS

The UKWCS women who were included in the UK Dietary Cohort Consortium have previously been described in section 4.1.1.3. [279]

4.1.2.4 The Whitehall II Study

A nested case-control group of women was taken from the Whitehall II study which recruited 10,308 male and female civil servants aged 35–55 years working in the London offices of 20 Whitehall departments in 1985–88.[284] People from a wide range of grades and salaries were recruited, including clerical and office support staff, middle-ranking executives, and senior administrative grades. The cohort was originally established to determine the extent to which psychosocial factors at work and outside account for social class differences in mortality and morbidity.

4.1.2.5 The National Survey of Health and Development

A nested case-control group of women was taken from the National Survey of Health and Development (NSHD); consisting of a nationally representative cohort of women born during one week in March 1946 in England, Wales and Scotland. [285]

4.2 Ethical considerations

Ethical approval was granted at the initiation of the cohorts; 174 local research ethics committees were contacted and permission to carry out the baseline UKWCS study was obtained.[279] Participants had consented to the use of information gathered at baseline, future phases and from cancer registries for research purposes provided that confidentiality was maintained.

4.3 Measurement of cancer outcomes

The cancer outcome in the analyses was incident malignant breast cancers. These were identified by codes 174 and C50 of the 9th and 10th versions respectively of the International Statistical Classification of Diseases, Injuries and Causes of Death (ICD). Cancer diagnoses are registered under ICD codes by local cancer registries and collated by the National Health Service Central Register (NHSCR). Cancer and death registrations for women in UKWCS are extracted quarterly by the NHSCR and made available to the University of Leeds at least once a year. This information is then linked to the UKWCS identification codes and any personal identifying information is deleted before the rest is made available for data analysis. At this time the distribution of the cancer dates are checked to ensure that a typical number of cases have been recorded and that a large number are not missing, particularly in the most recent months.

Research showed that the NHSCR for England and Wales missed about 10% of all incident cases of malignant disease, relating to diagnosis in 1971–1989.[286] However, incident cases for the analyses in this thesis were gathered after this time, and may well be more accurate. Cancer registration is currently about 18 months or more behind cancer incidence; the latest cancer incident dates used for the time-to-event analysis in this thesis were July 2008.

4.3.1 Censor dates

The censor date (follow-up date) for the UKWCS baseline dataset used in this study was 01/01/2008. The median time to cancer incidence or time to censor date from the questionnaire was received was 11.2 years.

At phase 2 of the UKWCS the censor date for the dataset used was 01/07/2008. The median number of years follow up was 7.4 years from phase 2 diary dates.

The breast cancer censor dates and the median follow-up dates differ for each cohort in the UK Dietary Cohort Consortium; these are shown in Table 6.

4.4 Exclusions

In all time-to-event analyses women were excluded who had any registered malignant cancer (except for skin cancer) prevalent at FFQ date (for UKWCS baseline analyses) or at diary date (for UKWCS phase 2 and UK dietary Cohort Consortium analyses). Women diagnosed with breast cancer within 6 months after the FFQ or diary dates were also excluded. This was done to ensure that latent disease not formally diagnosed was not present; otherwise disease suspected by participants could have influenced their dietary habits.

Women with extremely high or low total energy intake (more than 6000kcal and less than 500kcal/d) were excluded in the baseline UKWCS dietary vitamin C time-to-event analyses.

4.5 Assessment tools used for recording dietary and supplement intake in the UKWCS

4.5.1 Baseline FFQ and Health and Lifestyle Questionnaire

At baseline the mean daily dietary vitamin C intake for all women who had completed a FFQ had been previously calculated from the list of simple and mixed food items on the FFQ using DANTE (Diet and Nutrition Tool for Evaluation), a Microsoft Access program developed by the University of Leeds Nutritional Epidemiology Group. The vitamin C

intake for each food item had been calculated by multiplying an assumed standard portion in grams by the number of portions consumed per day, then multiplying the product by the grams of vitamin C contained in 100g of the food item as listed in The Royal Society of Chemistry Food tables, version 5 and supplements in the 5th Edition of McCance and Widdowson.[287] This was then converted into mg/d. Standard portions had been calculated by averaging three data sources: portion sizes from a pilot study of the weighed food diaries of vegetarians and from published 1993 and 1994 national survey values.[288]

The baseline FFQ, shown in 0, was developed from one used by EPIC UK studies,[289] [290] by adding extra vegetable composition dishes to accommodate the higher proportion of vegetarians in the UKWCS to produce a 217 item FFQ [279]. Thirty one vegetables (excluding potatoes), 19 fresh fruit and five dried fruit items were listed in the FFQ. Nine of the fresh fruit were seasonal; no canned or frozen fruits were specifically listed. For each item participants chose from 10 pre-coded frequency of consumption categories ranging from never to 6 or more times per day, on average over the past year (an example is given in Figure 7). Vitamin C content was also derived from mixed food items listed including casseroles, lasagnes and curries. An evaluation of fruit and vegetable intake is given in section 5.3.3.1.

Figure 7 A section from the baseline FFQ relating to intake of seasonal fruit

Please estimate how often you eat the following foods, and please answer every question.

PLEASE PUT A TICK(✓) ON EVERY LINE

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
SEASONAL FRUIT **How often have you eaten these fruits, when they are in season?**										
Apricots	0	1	2	3	4	5	6	7	8	9
Melon	0	1	2	3	4	5	6	7	8	9
Nectarines	0	1	2	3	4	5	6	7	8	9
Peaches	0	1	2	3	4	5	6	7	8	9
Plums	0	1	2	3	4	5	6	7	8	9
Raspberries	0	1	2	3	4	5	6	7	8	9
Red currants/Black currants	0	1	2	3	4	5	6	7	8	9
Rhubarb	0	1	2	3	4	5	6	7	8	9
Strawberries	0	1	2	3	4	5	6	7	8	9

Whether women were supplement users or non-users at baseline had been electronically captured from yes/no responses to the question:

Q29 *Do you take any vitamins, minerals, fish oils or other food supplements?*

Women who did not answer this question but recorded information about the type of supplements taken on the questionnaire were also classed as supplement users. Full details of the supplements, however, were not captured electronically, thus vitamin C supplement use at baseline was not available.

4.5.2 Phase 2 Health and Lifestyle Questionnaire

Dietary vitamin C intake could not be calculated from the 20 page Health and Lifestyle questionnaire at phase 2 since it did not include a full FFQ.

General supplement use at phase 2 question could be ascertained from yes/no responses to the following questions:

Q14 *Have you taken any vitamins, minerals, fish oils or fibre or other food supplements in the last year*

Q15 *Do you presently use any dietary supplements?*

General supplement use at phase 2 for the analyses in chapters 6 & 9 was determined using answers from Q15. Additionally, if participants did not answer yes to Q15 but provided further details of the type of supplements taken weekly or more frequently, then these women were designated as being current supplement users.

If participants answered yes to Q15 they were asked to note which supplements they took from a list of 17 and to indicate how often they used them. As seen in Figure 8 these included vitamin C, selenium, iron and antioxidants; although antioxidants, vitamin A and vitamin E were not listed separately. Frequencies of use were categorised as: more than daily; daily; weekly; monthly; and less than monthly. For each of the different types of supplements the percentages of women taking them daily were calculated, these percentages are displayed in Figure 2 in section 2.1.

Figure 8 Questions relating to supplements on the phase 2 questionnaire

14. Have you taken any vitamins, minerals, fish oils, fibre or other food supplements at all in the last year?

Yes ☐ 1 No ☐ 2

15. Do you presently use dietary supplements

Yes ☐ 1 No ☐ 2

If yes, please indicate which supplements you use. Please look at the list of dietary supplements & list in the box below how often you use them. See example

1 = Vitamin C
2 = Vitamin B complex
3 = Vitamin B12
4 = Folic Acid
5 = Antioxidants
6 = Multivitamins with minerals
7 = Multivitamins without minerals
8 = Other single vitamins
9 = Iron
10 = Calcium
11 = Selenium
12 = Zinc
13 = Other Minerals
14 = Fish Oils (Cod / halibut liver oils)
15 = Evening Primrose/Starflower Oils
16 = Garlic supplements
17 = Other supplements

Supplement number	How often do you take the Dietary Supplement – please tick ✓				
	More than once a day	Daily	Weekly	Monthly	Less than once a month
e.g. 3	1	2 ✓	3	4	5
	1	2	3	4	5
	1	2	3	4	5
	1	2	3	4	5
	1	2	3	4	5
	1	2	3	4	5
	1	2	3	4	5
	1	2	3	4	5
	1	2	3	4	5
	1	2	3	4	5
	1	2	3	4	5

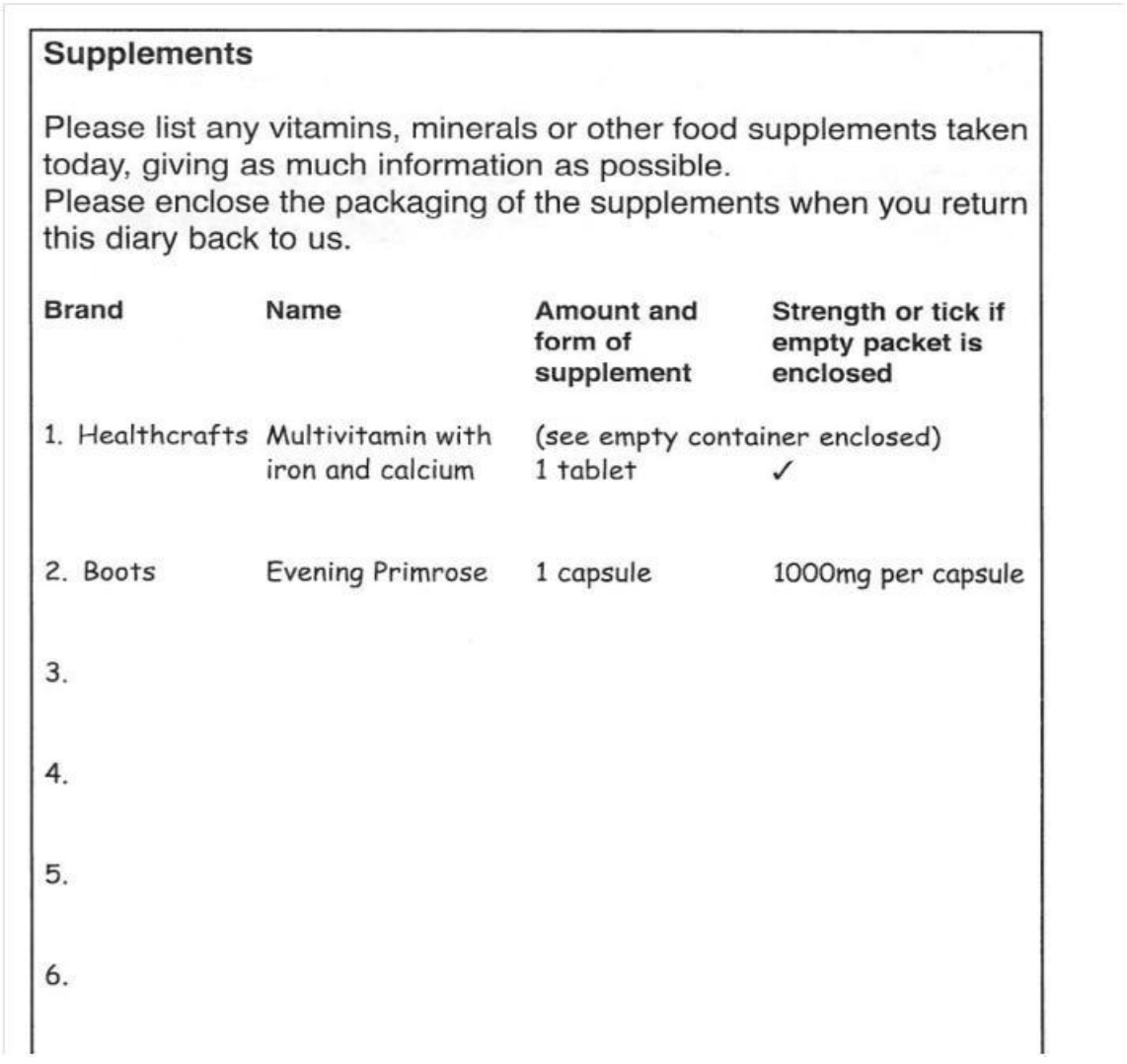
4.5.3 Phase 2 Diary

4.5.3.1 Supplement intake captured from phase 2 diaries

At phase 2 over 13,000 participants completed food diaries for 4 days with a separate page for each day to record individual supplements taken: Brand; Name; Amount taken; Dosage (the example provided to participants is shown below in Figure 9). Between 1999 and 2004, upon return of the completed diaries, this information was captured onto a Microsoft Access database of supplements taken by participants, and was matched at entry via a drop-down menu against supplements listed in a supplement ingredient database. This enabled the allocation of ingredient amounts to the supplements taken.

After cleaning (as explained in section 4.5.3.3), the daily vitamin C intake from all supplement types was calculated for each participant, and was averaged across the total number of diary days that vitamin C was taken.

Figure 9 Phase 2 diary supplement recording example provided to participants



Supplements

Please list any vitamins, minerals or other food supplements taken today, giving as much information as possible.
Please enclose the packaging of the supplements when you return this diary back to us.

Brand	Name	Amount and form of supplement	Strength or tick if empty packet is enclosed
1. Healthcrafts	Multivitamin with iron and calcium	(see empty container enclosed) 1 tablet	✓
2. Boots	Evening Primrose	1 capsule	1000mg per capsule
3.			
4.			
5.			
6.			

4.5.3.2 Supplement ingredient database

The supplement ingredient database is a Microsoft Access database created by the Nutritional Epidemiology department at the University of Leeds between about 1999 and 2004. In total there were 3,996 different marketed supplement types listed in the database and each was given a separate supplement identification code. This contained brand name, supplement descriptions, ingredient composition and units (normally milligrams (mgs) for vitamin C) which were obtained from product labels provided by participants, suppliers' websites or provided directly from manufacturers upon request. A small proportion of vitamin C supplements contained ascorbate buffered with metal irons such as magnesium, calcium, zinc or potassium; the milligrams for the whole compound had been recorded in the ingredient database and a conversion factor was later applied to establish the ascorbate content (this could be found online or calculated using relative atomic mass of the elements). No adjustment was made for 'slow release' or 'time release' vitamin C supplements and those containing bioflavonoids which are promoted as having increased bioavailability, although evidence for this is lacking. Additionally it was assumed that the bioavailability of vitamin C in tablets, capsules, powder and liquid form was the same.

The supplement ingredient database was inspected to determine the common types of supplements containing vitamin C in relation to the recommended daily allowance (EU RDA = 60mg/day[53]) and in relation to 500mg doses which are usually classed as high dose. As summarised in Table 7, a large proportion (46%) of supplements containing vitamin C up to the EU recommended daily allowance (EU RDA 60mg/day [53]) are branded as multivitamins in the UK. Supplements which include more than the EU RDA but less than 500mg/day are often named multivitamins (26%) or antioxidants (14%). Vitamin C supplements containing high doses of 500mg or more are usually single ingredient (68%), and only a small amount are name as antioxidants (6%) or multivitamins (1%).

Table 7 Percentage of supplements containing vitamin C in the UKWCS supplement ingredient database that are multivitamins, antioxidants or single vitamin C supplements, categorized by vitamin C content

	Supplements categorised by vitamin C content		
	≤60mg/d	>60<500mg/d	≥500mg/d
Branded as	N=561	N=408	N=219
Multi %(n)	46% (256)	26% (105)	1% (3)
Antioxidants[a] %(n)	13% (72)	14% (56)	6% (13)
Vitamin C (only)[b] %(n)	3% (16)	10% (40)	68% (150)

[a] or selenium with vitamins ACE

[b] names indicated supplements contain vitamin C only

4.5.3.3 Cleaning vitamin C supplement data in the database of supplements taken by participants

Although details of supplements recorded by participants in the 4-day diaries had already been entered into the database, over 15% of all supplement entries had no ingredients or doses allocated to them because no supplement identification code had been selected during entry. From participants' descriptions it appeared that the majority of these supplements did not contain vitamin C. In addition, it was unknown what proportion of supplement identification code allocations had to be deduced at entry because participants did not fully describe supplements used, or how many of these may have been incorrect. (Note that EPIC-Norfolk reported only 11% of their participants' supplement descriptions were direct matches to their supplement ingredient database at the point of coding and a substantial amount of allocations (41%) required an assumption[38]).

Any supplements listed in the UKWCS database of supplements taken by participants that were likely to contain vitamin C were checked and cleaned. Entries which indicated supplements containing vitamin C were found by systematically searching the participant database for the following descriptions: 'vitamin C', 'vitamin', 'ascorb', 'anti', 'A C E', 'glucosamine', multi', 'meno', 'A-Z', '50+', 'gold'. The participant's description captured in the database was compared with the description of the supplement allocated from the ingredient database; if different these were compared with the entries in the physical diaries. Over 400 diaries (3% of the total diaries) were checked as part of the cleaning process.

New fields in the database were created for corrected doses of vitamin C, or corrected or new supplement identification codes. New codes 9001-9007 were created to input mean doses for seven types of commonly taken supplements likely to contain vitamin C when dose and/or brand information was missing (see Table 8). The mean doses were calculated over the whole database by averaging complete entries which had supplement descriptions and identification codes relevant to these common supplements, and these were weighted by the number of women taking these types. (Note that EPIC-Norfolk also created generic supplement identification codes using weighted means, though these were more numerous and more specific[38]).

After this initial cleaning, the allocated vitamin C amount per day per participant was calculated by the database manager using the Access databases. This was checked and then uploaded into STATA. This diary-gathered data was then compared with participants' questionnaire answers relating to frequency of vitamin C use. The diaries of women who had no vitamin C dose captured were checked, if questionnaire answers

indicated they took vitamin C supplements daily. This cross-checking of assessment methods identified a further 400+ diaries which were manually checked; this resulted in vitamin C supplement intake recorded in diaries being identified and captured for an extra 87 women. (Detailed results of further comparisons between the two assessment tools are given in section 5.2).

Table 8 Mean dose of vitamin C by supplement type in the UKWCS supplement databases

Supplement type	Mean vitamin C dose	New code	Observations corrected
Selenium plus vitamins A, C and E	90mg	9001	48
Antioxidants	153mg	9002	19
Multivitamins	75mg	9003	71
Multivitamins and minerals	71mg	9004	62
Multivitamins and iron	57mg	9005	34
Vitamin C (no strength indicated)	609mg	9006	65
Vitamin C high strength (suggested by description*)	743mg	9007	16

*Note: 'high strength': those described as 'with bioflavonoids' or 'time released' or slow released', or if most of the suppliers products were 500mg or over.

A final check of 63 diaries (half a percent of the phase 2 dataset), randomly selected by computer and inline with the National Audit Office statistical sampling guidance,[291] indicated that 95% (+/-5%) of diaries have supplement usage correctly captured in STATA (both correct number of days and satisfactory doses). A 5% error has the potential to slightly reduce a true relative risk of 2 to an observed relative risk of 1.9.[292]

4.5.3.4 Dietary intake captured from phase 2 diaries

At phase 2 in the UKWCS, food and drink intake had also been recorded in the 4-d diaries. The front pages of the booklet detailed instructions on the information required, and included suggestions about using household measures such as tablespoons and cups if weight scales were not available. Participants were asked to describe food and drink consumed each day as shown in the example in Figure 10, and to list recipes and details of ready meals used and the portion consumed by the participant. Due to resource constraints only about a 1000 cases and controls in total were coded into the DANTE system developed at Leeds, or later into the DINER system developed at

Cambridge. These produced a dietary vitamin C intake per day per participant. DANTE had previously been validated against DINER using 100 diaries which showed excellent agreement; geometric mean energy intake from DANTE was just 2% lower (95% CI, 0%, 5%) than from DINER. [293]

In addition, dietary intake of micronutrients was also calculated by DANTE for 274 women who had plasma micronutrient levels laboratory analysed. Vitamin C intake derived from the UKWCS FFQ and also the phase 2 diaries are compared with plasma vitamin C concentrations in section 5.4

Figure 10 Phase 2 diary food and drink recording example provided to participants

EXAMPLE DAY - UP TO LUNCH

Date: 14 October 1998 Day of the week Friday		
Time of food or drink	**Description of food or drink consumed (include brandname where possible)**	**amount**
7.15 am	Filter Coffee	1 cup (200ml)
	semi-skimmed milk	3 tablespoons
7.30 am	Sainsbury's orange juice, un-sweetened	1 glass (150ml)
	Sainsbury's Bran flakes	40g
	semi-skimmed milk	180ml
10.30am	Plain chocolate digestives (large biscuits)	2
	Earl Grey tea (weak) no milk	1 cup
11 am	banana (medium sized)	95g
11.30 am	London herb company Lemon Zester tea	1
12.10 pm	Local bakery's wholemeal bread un-sliced loaf (cut thickly)	1 slice 47g
	Tesco sunflower margarine	thinly spread
	home-made mushroom risotto (see recipe)	About 1/3 of recipe
	green seedless grapes	32g
	Cox's Orange Pippin apple (medium)	82g
	Sainsbury's wholemilk fruit yoghurt (150g)	1 pot
	London herb company sweet berry tea	1
2pm	Warburton's Carrot cake – with cream cheese topping (see nutritional information)	1 slice – 75g (on packet)

4.6 Assessment tools for dietary and supplement intake in other cohorts of the UK Dietary Cohort Consortium

4.6.1 Dietary intake recorded by diaries

In the EPIC studies, the diaries allowed for the recording of the description, preparation and amount of foods eaten at main meals, snacks and between meal times over seven consecutive days. Detailed instructions on the information required in describing and quantifying each type of food or drink were printed on the front pages of the booklet.[283] Seventeen sets of colour photographs were included to help the participants describe the portion size of the food they consumed, as small, medium or large. The diaries were coded using DINER.

The Whitehall II study also used 7-d diaries, containing instructions and pages to record foods eaten during seven time periods (before breakfast, breakfast, mid-morning, lunch, tea, evening meal, later evening). Fifteen sets of black and white food photographs were included, showing three portion choices for a common food item.[294]

For the National Survey of Health and Development (NSHD) a 5–d diary was used. The instructions contained 66 examples of how to describe foods in terms of their preparation and quantity. The main part of the diary comprised of seven spaces for each day in which to record meals and between-meal snacks, a reminder section about any other snacks and drinks and a space in which to write recipes.[295] Fifteen sets of black and white food photographs were included, showing three portion choices for a common food item. The diaries were coded using DIDO.

4.6.2 Supplement use recordings in EPIC-Oxford and EPIC-Norfolk

Both cohorts used similar diaries to record supplement intake. Participants were asked to record their supplement use in a 7-day diary by answering an open-ended question, however this only provided room to record one supplement per day.

> *Please name any vitamins, minerals or other food supplements taken on each day of last week.*
> *Headings: Brand name; Amount taken*

In a later version of the diary more space was given for participants to list a number of supplements used and an example was provided.[38]

> *Please name any vitamins, minerals or other food supplements taken on each day of last week. Please write down all the details from each packet/container, and enclose label(s) giving ingredients and individual amounts where possible.*
>
> *Headings: Brand; Name; Amount taken per day – number of pills, capsules or teaspoons; Tick box(es) to show which day(s) supplements was taken last week –M T W T F S S*.

Using data from diary records and their supplement ingredient database, EPIC-Norfolk calculated the average vitamin C intake from supplements over the seven days of the diary for each participant in the nested case-control dataset. These averages were provided by Cambridge for pooling with the UKWCS and EPIC-Oxford.

The supplement use diary recordings for each participant in the EPIC-Oxford nested case-control dataset were photocopied and transferred to a spreadsheet. If details given did not state doses, then vitamin C amounts were allocated based on information from the UKWCS ingredient database. Although this database was established at the end of the period the Oxford diaries were completed, many of the supplements in the UKWCS ingredient database would have been available in this earlier period. The EPIC-Norfolk database was not available for use for this task.

4.7 Statistical methods

This section details the statistical methods used that are common to many of the following chapters containing results; for example time-to-event analysis, the selection of covariates for adjustment, coding of menopausal status, exclusion of outliers in sensitivity analysis, etc. Although conditional logistic regression is only used in one of the following chapters (10), it is appropriate to explain this here as an alternative to Cox's regression analysis.

Kappa statistics and Bland Altman plots are explained in chapter 5 which are used to evaluate diet and supplement vitamin C recordings in the UKWCS. In addition restricted cubic splines are explained in chapter 9 which are used to determine whether there are non-linear relationships between dietary vitamin C intake and breast cancer risk.

All statistical analyses used Stata SE version 10 and all tests calculated two sided p values and 95% confidence intervals.

4.7.1 Time-to-event analyses

Time-to-event analyses (also known as survival analyses) using Cox proportional hazards models were used in chapters 7 to 9 to determine whether dietary or supplement vitamin C intake were associated with breast cancer risk. The analyses measure the time from FFQ or diary completion date to breast cancer incidence or the date the participant was lost to follow-up because they died of other causes or, for women who did not develop breast cancer, the time to censor date (as detailed in section 4.3 for each dataset).[296] This enables the analysis to deal with recruitment which occurs at different times throughout the study. Therefore before running any risk analyses, a variable containing time to incidence or to censor date for each participant was created in Stata from FFQ completion dates for the baseline analyses, and for diary completion dates in the phase 2 analyses. Additionally a variable was required which flagged cancer incidence after the FFQ / dairy completion dates. As stated earlier women who were diagnosed with any cancer (except skin) before FFQ / diaries were completed, were excluded.

The proportion of women who did not have breast cancer at any time since recruitment can be plotted in a Kaplan-Meier curve. Two or more groups of women can be compared using this technique as shown in Figure 22 in chapter 7

This method produces an approximate estimate of a true time-to-event curve if both of the following assumptions hold:

1. Participants who are censored have the same risk of subsequently developing breast cancer than those who were not censored
2. The observations are independent of each other

Hazard ratios for time-to-event analyses in this thesis were calculated using Cox regression, also known as proportional hazards regression. Hazard ratios compare the hazard of one group with another group e.g. supplement users with non-users. In simple terms the hazard is the probability that an individual in the study with no prevalent breast cancer is diagnosed with breast cancer at any given time. The distribution of the hazard over the length of the study is called the hazard function. The hazard can change over the study period so to be more precise, the hazard function is the instantaneous risk (i.e. the probability over a small interval of time) of breast cancer for an individual given that the individual has been breast cancer free up to a particular time.[297] The analysis uses a conditional likelihood estimation procedure where at each point in time that an event occurs the value of the exposure variable, e.g. supplement use, for a participant who has incident breast cancer is compared to the value of exposure variable for all those without the disease.[296] Although the Cox regression

method makes no assumptions about the shape of the curve, the hazard ratio between the two groups should be a constant proportion over time. This proportional hazard assumption was tested using the cumulated hazard by plotting the minus log of minus log time-to-event against log of time. The curves should approximately be a similar distance apart all the way along or they should at least not cross. In addition there should be about ten cases per variable in the model.[298]

4.7.2 Conditional logistic regression analyses

The risk of developing breast cancer in the pooled nested case-control studies was calculated using conditional logistic regression. In this type of logistic regression method breast cancer cases are only compared to controls within the same matched set, making it conditional on matching.[296] Controls were randomly selected from women with matching variables who were still breast cancer free at the end of the study, representing the population at risk of the disease. They were matched within study centre by month of diary completion (± 3 months or as close as possible), so that follow-up times were comparable, thereby dealing with the varying recruitment dates. They were also matched on age (± 3 years). As an aid to this analysis each matched set had been numbered and this had been added to the dataset as a variable. It was also possible to control for variables other than those controlled through matching by adjustment in the model. Using between two and four controls for matching increased the likelihood that controls were representative of the cohorts.

Wacholder et al. (1992)[299] explained that overmatching, which can cause bias or reduce efficiency (the ability to demonstrate expose-outcome relationships) compared to an unmatched study, can occur in a number of situations if a matching variable is: 1) an intermediate on the causal pathway 2) is a surrogate for or a consequence of the outcome; or 3) is closely related to the exposure variable but not related to the outcome. It is unlikely that any of the matched variables would cause overmatching. For instance, age does not meet the first two points and, although age is related to breast cancer risk, as seen in the analysis of the nested case-control study in chapter 10 it is not closely related to dietary vitamin C intake.

4.7.3 Evaluating interactions

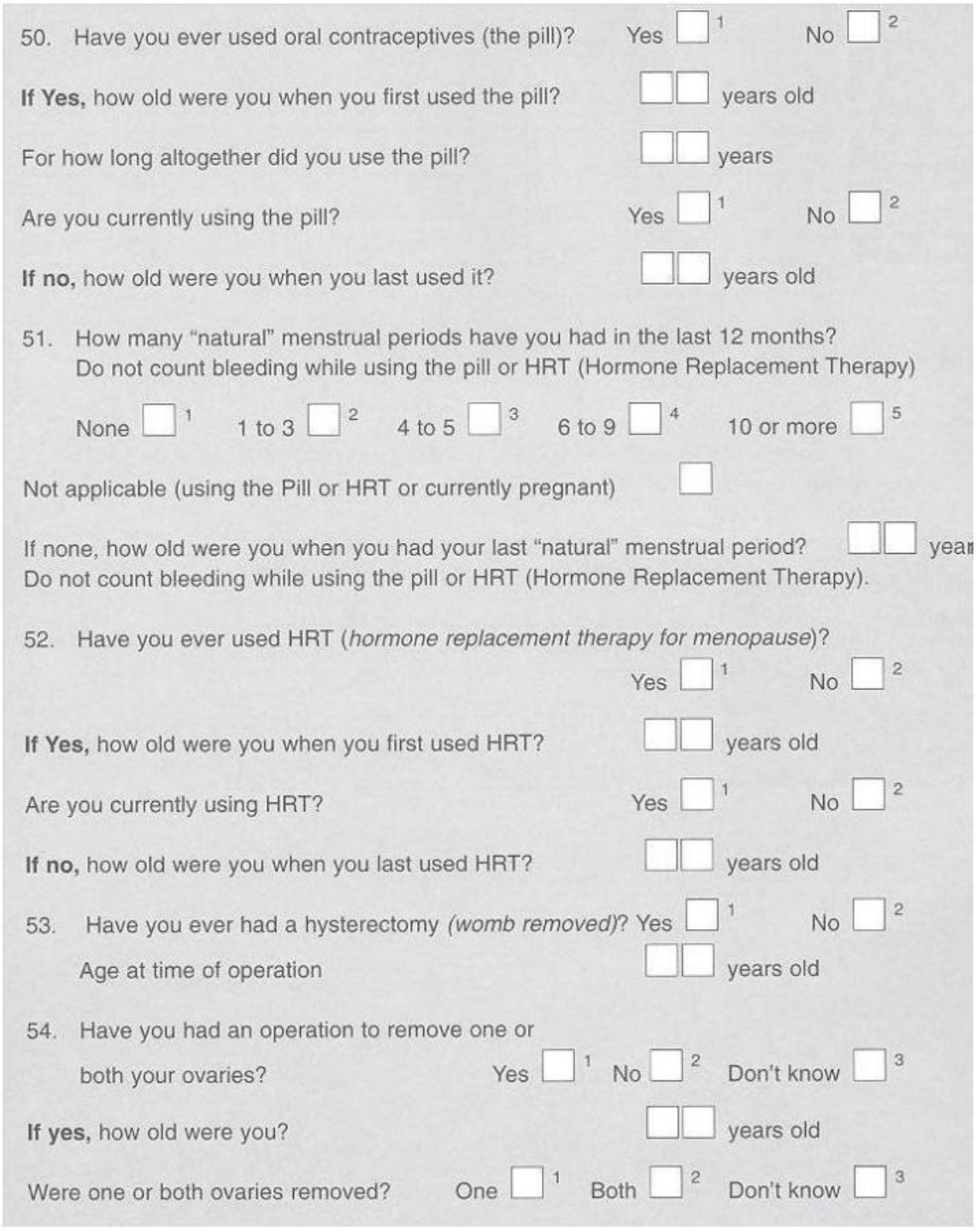
An interaction occurs between the effects of two exposures if the effect of one exposure on the outcome varies with the effect of the other. This is also called effect modification; for instance menopausal status has been found to modify the effect of an exposure on the incidence of breast cancer, examples of which can be found in the literature review chapter. Additionally, since supplement users are likely to have different health behaviours than non-users this variable was tested for interactions and split into strata in datasets when possible.

Other variables suggested as modifiers were defined *a priori*: BMI; amount of exercise; family history of cancer; and dietary group. Socio-economic status and HRT use was considered later. Chapter 7 tests whether these modify the effect of supplement use at baseline on breast cancer risk and analyses incorporating interaction terms were reported. However, these variables were not tested in relation to levels of vitamin C intake due to limited power and the possibility of spurious findings from multiple testing.

4.7.4 Coding of menopausal status

Menopausal status used in the time-to-event analyses relates to status at the time of exposure (dietary or supplement intake assessment) rather than time of diagnosis or censor date, due to difficulties of determining status later. This means that some women in the datasets who developed cancer when they were post-menopausal have been defined as pre-menopausal at baseline or phase 2 assessments. The questions used at baseline and at phase 2 to determine menopausal status are shown in Figure 11. The flow diagram in Figure 12 shows the rules used for coding menopausal status in the UKWCS for this thesis, which is similar to that used in the UK Dietary Cohort Consortium. For women who did not specify on the questionnaires whether they still had periods, it was assumed they were post-menopausal after the age of 50. The mean age (46 years) at which women were assumed to be post-menopausal after removal of ovaries was based on research by Farquhar 2005.[300] A peri-menopausal category was not used for the UKWCS analyses.

Figure 11 Questions used to determine menopausal status (phase 2 questionnaire)



50. Have you ever used oral contraceptives (the pill)? Yes ☐ 1 No ☐ 2

If Yes, how old were you when you first used the pill? ☐☐ years old

For how long altogether did you use the pill? ☐☐ years

Are you currently using the pill? Yes ☐ 1 No ☐ 2

If no, how old were you when you last used it? ☐☐ years old

51. How many "natural" menstrual periods have you had in the last 12 months?
Do not count bleeding while using the pill or HRT (Hormone Replacement Therapy)

None ☐ 1 1 to 3 ☐ 2 4 to 5 ☐ 3 6 to 9 ☐ 4 10 or more ☐ 5

Not applicable (using the Pill or HRT or currently pregnant) ☐

If none, how old were you when you had your last "natural" menstrual period? ☐☐ year
Do not count bleeding while using the pill or HRT (Hormone Replacement Therapy).

52. Have you ever used HRT (*hormone replacement therapy for menopause*)?
Yes ☐ 1 No ☐ 2

If Yes, how old were you when you first used HRT? ☐☐ years old

Are you currently using HRT? Yes ☐ 1 No ☐ 2

If no, how old were you when you last used HRT? ☐☐ years old

53. Have you ever had a hysterectomy *(womb removed)*? Yes ☐ 1 No ☐ 2

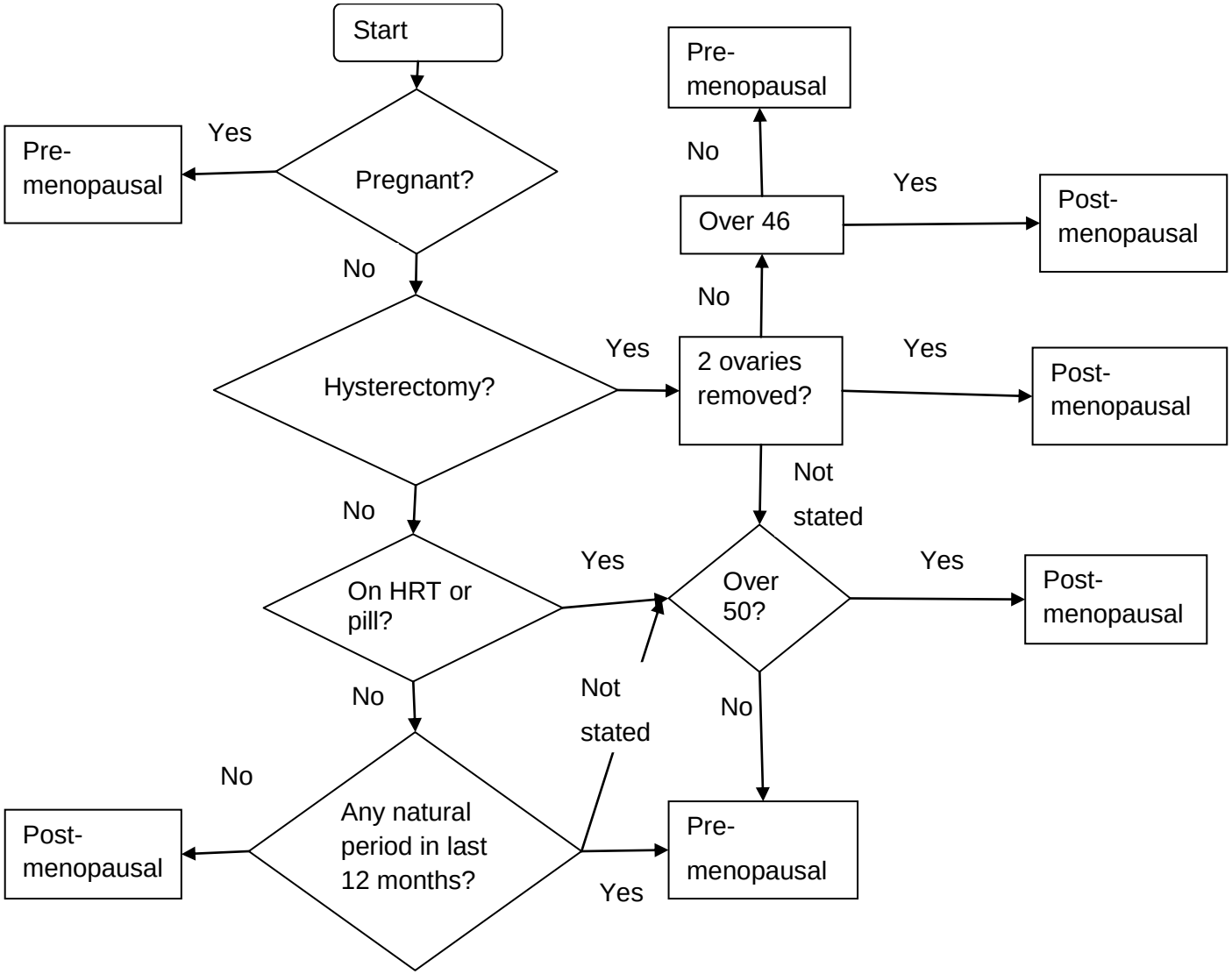
Age at time of operation ☐☐ years old

54. Have you had an operation to remove one or both your ovaries? Yes ☐ 1 No ☐ 2 Don't know ☐ 3

If yes, how old were you? ☐☐ years old

Were one or both ovaries removed? One ☐ 1 Both ☐ 2 Don't know ☐ 3

Figure 12 Flow diagram of coding for menopausal status in the UKWCS



4.7.5 Adjustments by potential confounders

Unlike RCTs, where behaviours and characteristics which may influence the risk of developing breast cancer are randomised between intervention groups, in cohort studies these potential confounders can seriously affect the results of risk analyses if they are not statistically controlled for. Therefore their selection was carefully considered for this thesis.

4.7.5.1 Model building

Potential confounders are variables that may be associated with both the exposure of interest i.e. vitamin C and the outcome variable i.e. breast cancer incidence. Univariate analyses were performed to examine the association of each potential confounder with breast cancer incidence and with vitamin C intake. Variables that were significantly associated with vitamin C as well as breast cancer incidence in the datasets were considered for adjustment. Those that did not meet this criteria but where there was strong prior evidence from previous studies, as detailed in section 2.2.3 and chapter 3, suggesting they were confounders, they were still considered for inclusion. These potential confounders were assessed by visual methods using diagrams called Directed Acyclic Graphs as detailed in section 4.7.5.2. Covariates which appear to be on the causal pathway between vitamin C intake and breast cancer should not be included, since these mediators are not true confounders and controlling for them would attenuate the effect of vitamin C.[301]

Confounders that had a substantial proportion of missing observations were not included in the main adjusted analyses (e.g. waistline had 7500 missing entries at baseline), particularly if other closely related variables were available e.g. BMI. For instance socio-economic status rather than education was controlled for at baseline since it had fewer missing observations. Important confounders which had a high proportion of missing variables in the UK Dietary Cohort Consortium analyses were considered for inclusion in sensitivity analyses i.e. cumulative breast feeding and age at menarche.

4.7.5.1.1 Assessing linearity with outcome

Continuous variables, such as age and BMI were tested to determine whether they had a linear or non-linear relationship with cancer incidence. As categorical variables, there were no assumptions about the shape of the relationship between the variable and the outcome. The null hypothesis that the relationship was linear, was tested using the Likelihood Ratio Test (LRT) by comparing the log likelihood from the model using the continuous variable with the log likelihood with the model using the variable split into

categories (e.g. BMI: underweight; normal; overweight; obese).[296] For BMI the LRT was significant meaning there was evidence against the null hypothesis and BMI was better modelled as a non-linear categorical variable which was subsequently used in the adjusted analyses. This was not the case for age.

4.7.5.1.2 Excluding highly correlated variables

If two highly correlated variables are included in an adjusted model they are likely to produce an underestimation of their association with the outcome, and are said to be collinear.[296] If included together neither may appear to be associated with the outcome even if one or both are strongly associated with it.[296] For instance it was inappropriate to adjust for both BMI and weight, or BMI and waist size, or weight and waist size since the Pearson's correlation between these (0.67 or higher) showed they were highly correlated. However since most of the variables were categorical it was difficult to determine whether the categorical variables were too highly related. Nevertheless, large increases in the standard error estimate in the risk analyses for two or more variables may indicate a problem.[296]

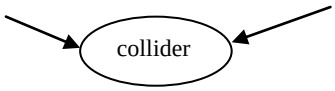
4.7.5.2 Assessment of potential confounders by Directed Acyclic Graphs

Directed Acyclic Graphs (DAGs) can provide a visual and logically rigorous way of summarising causal links between exposures and disease outcome, and can be used to check whether sufficient confounders have been selected for adjustment. Figure 13 and Figure 14 show causal pathways representing the first step in using the theory of DAGs to identify potential confounders relating to dietary and supplement vitamin C intake in unselected populations. The majority of these have been identified *a priori* from previous studies. All variables shown in Figure 13 and Figure 14, apart from 'health consciousness', were derived in the UKWCS datasets.

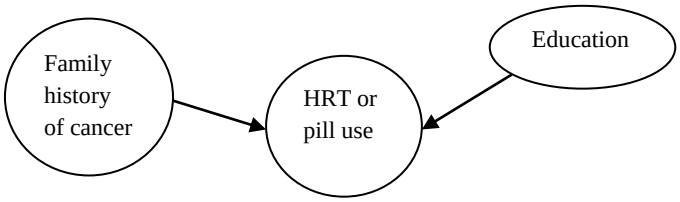
The arrows in the DAGs show the direction of influence from one variable to another, with the main causal pathway from exposure of interest to outcome highlighted in red. As explained by Greenland,[302,303] confounding is present if an association remains between the exposure of interest and outcome after removing all exposure effects from the exposure of interest. Therefore exposure effects of vitamin C should be removed on the diagram; on Figure 13 this includes removing the arrow from dietary vitamin C exposure to 'supplement use' in addition to removing the red arrow to breast cancer incidence. It can be argued that if low dietary vitamin C intake significantly promoted supplement use then the latter would mediate the effect of dietary vitamin C on breast cancer incidence; adjusting for it could create bias rather than reduce it. On the contrary, the odds of supplement use were found to increase significantly with

increasing dietary vitamin C intake; being health conscious was likely to have increased both.

Using these diagrams confounders can be detected by finding common influences on exposure and outcome. Identifying a minimum set of confounders for adjustment was done by tracing open backdoor paths from the outcome (breast cancer incidence) to the exposure (vitamin C intake); to aid their detection the first arrows in these backdoor paths were coloured blue in Figure 13. Paths containing colliders are not used for selecting confounding variables; these are closed backdoor paths where the path enters and exits a variable as follows:



An example of a collider is illustrated below; here pill use cannot have an effect on the other two variables which are conditions that existed before pill use occurred.



The Figure 13 and Figure 14 show that all open backdoor pathways pass through the variable ‘Health consciousness’; this variable, therefore, is the main common link between exposure and outcome. Given this and the fact it is not a collider, according to the DAG theory it would be sufficient in adjusted analyses to control for this one variable only.[303] Unfortunately, this latent variable was not readily available in the datasets, though it could have been created using factor analysis. Nevertheless, for each open backdoor path which passes through this variable there are other more readily derived variables that can be used for adjustment instead; additionally there is no direct path from this to the outcome.

As explained by Greenland,[303] at least one variable on each open backdoor path from the outcome breast cancer incidence to the exposure, should be controlled for in adjusted analyses. In these diagrams every variable linked directly by a blue line to the outcome can be traced back to vitamin C on open backdoor paths, therefore each of these variables could be used in adjustments, assuming there is evidence of their effects on breast cancer. For example, ‘cumulative breastfeeding’ is linked directly to the outcome, and can be traced back through ‘education’, ‘health consciousness’ and ‘fruit and vegetable intake’ to ‘dietary vitamin C intake’. It is questionable, though,

whether smoking status has a direct effect on breast cancer and whether it should be included as a confounder. However, a meta-analyses has reported smoking increases breast cancer risk in women with NAT2 slow acetylator genotypes, which occurs in about 50% of Causaians.[304]

Greenland posits that adjusting for more than the minimally sufficient set of confounders may cause confounding.[303] Thus in most cases it will be unnecessary to adjust for more than one variable on each open backdoor path. For instance it appears that education should not be included as a confounder in addition to 'parity' and 'cumulative breast feeding'. Furthermore, one can question whether 'BMI' 'weight', waist size' and 'total energy intake' should all be included as confounders since one open backdoor back passes through them all. A discussion about this is included in section 4.7.5.1.2.

Socio-economic status (SES) has been linked to health behaviours in the UK,[305] and women of higher SES are generally more health conscious. Conversely women of higher SES are likely to have fewer children which would increase their risk of breast cancer. However, it can be argued that SES should not be also included as a confounder since other variables on open backdoor pathways containing SES could control for its indirect effects. There may be possible biological mechanisms, however, by which low SES may have an effect on breast cancer risk, for instance via stress responses caused by unfairness and inequality. Although these have not been specifically researched in relation to breast cancer, low control in the workplace was not associated with increased breast cancer risk in the Nurses Health Study.[306,307] Nevertheless to avoid residual confounding SES has been included as a confounder in the UKWCS adjusted analyses when education has not been included.

Figure 13 A Directed Acyclic Graph (DAG) of factors associated with dietary vitamin C intake and breast cancer

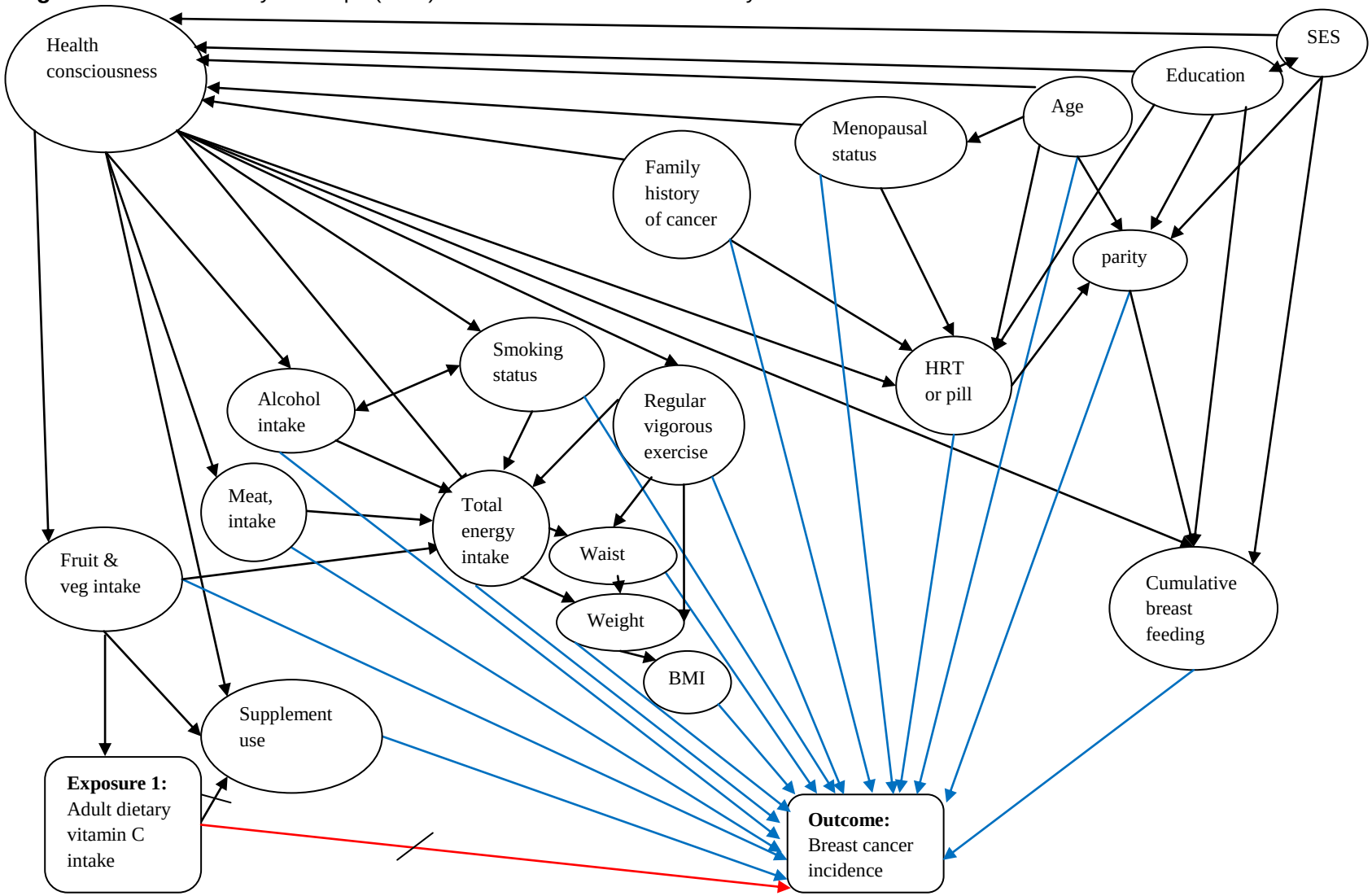


Figure 14 A Directed Acyclic Graph (DAG) of factors associated with supplement vitamin C intake and breast cancer

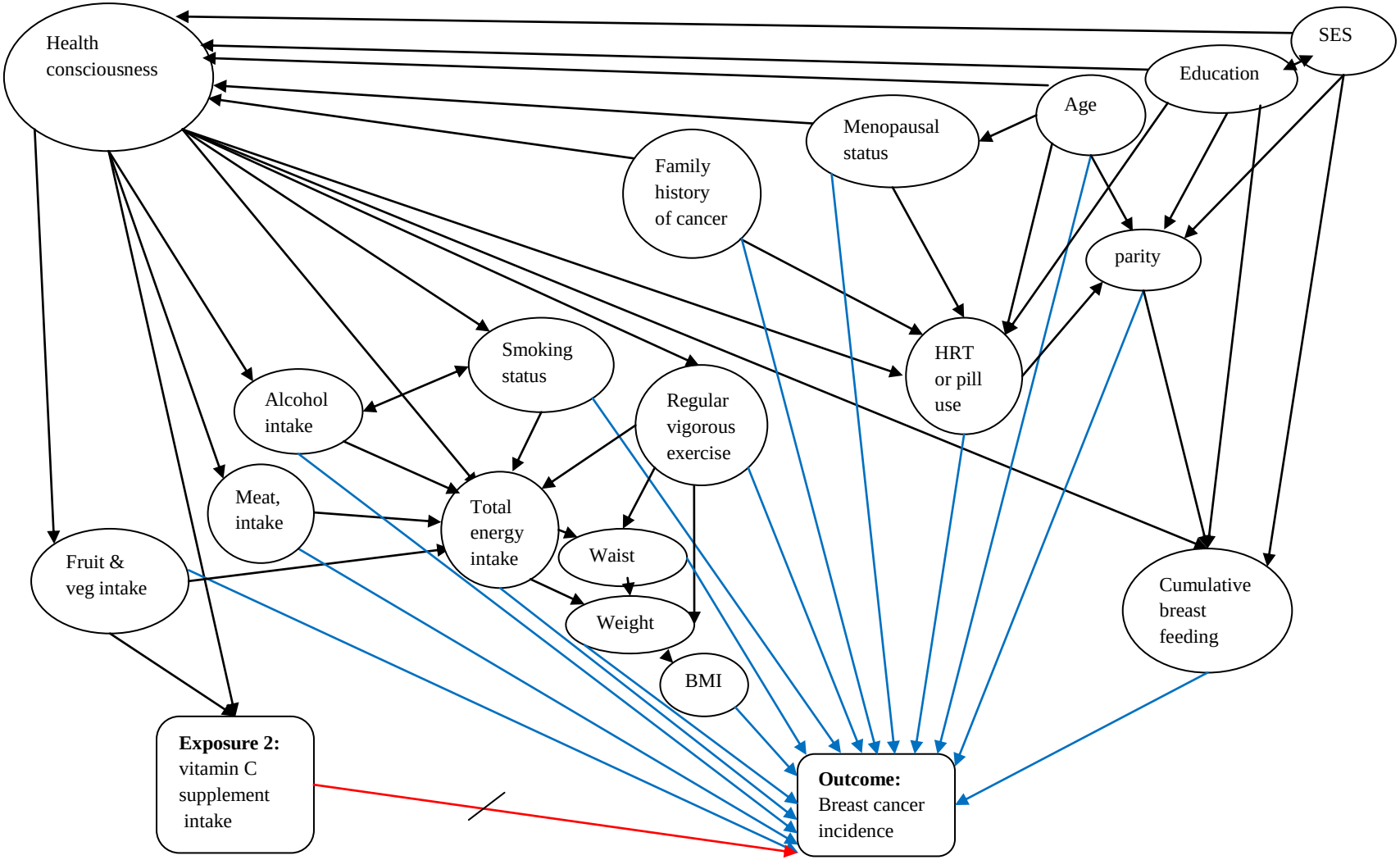
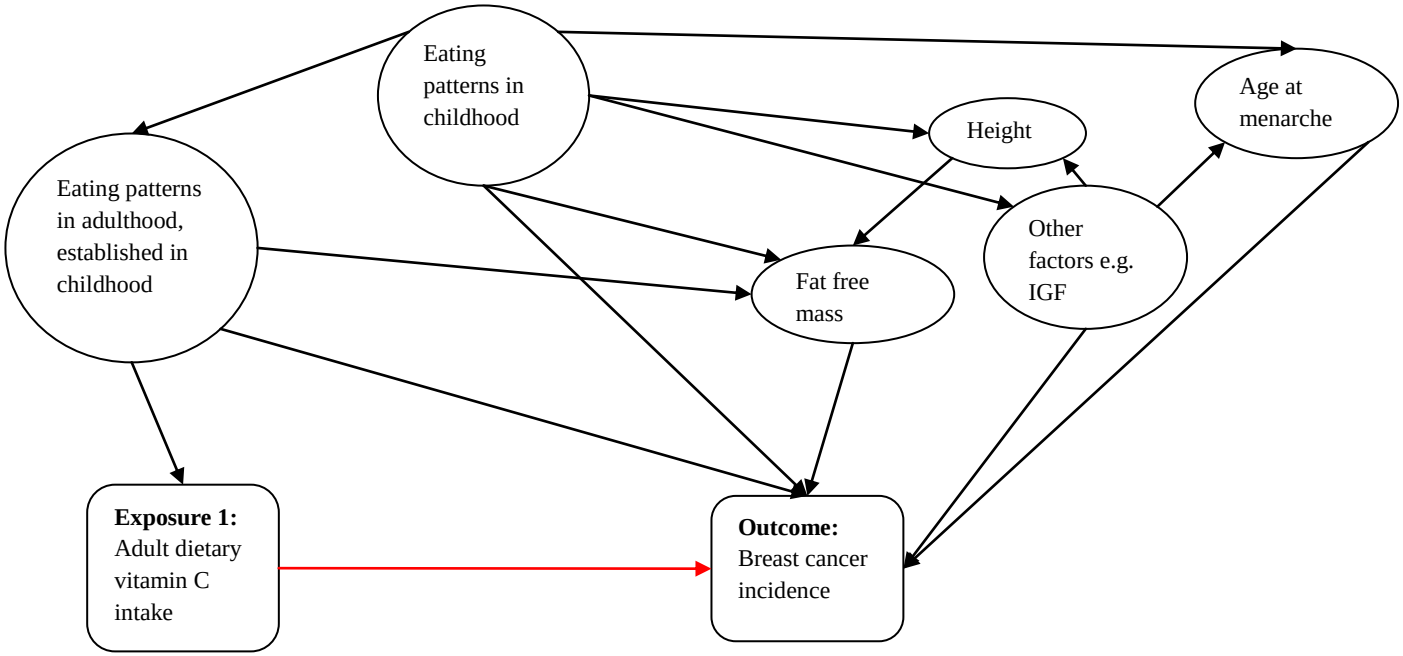


Figure 15 DAG linking eating patterns in childhood with the main exposure and outcome



Additional variables which were considered for adjustment that were not included in Figure 13 and Figure 14 DAGs were height, age at menarche and history of hysterectomies and oophorectomies. It has been established that height is associated with breast cancer.[7] Additionally, fat free mass has been independently associated with breast cancer incidence.[308] Eating patterns in childhood and adolescence influence age of menarche, height and fat free mass, therefore these variables may confound the main causal pathway between adult dietary intake and breast cancer via a variable relating to the maintenance of eating habits established in childhood (see Figure 15). Since height and age of menarche reflect childhood nutrition as well as the action of growth factors, they are often used as proxies for the early nutritional environment. Additionally, since height and FFM are correlated, height may be used as a proxy for fat free mass (FFM) which is not readily available. Note that the UK Dietary Cohort Consortium made the decision to use height and weight as a covariates rather than BMI which was used in other analyses in this thesis.

4.7.5.2.1 Other unmeasured variables relating to health consciousness.

There are various unmeasured factors which influence health consciousness or health behaviours. These include attitudes, norms, intentions and perceived behavioural control as presented in Fishbein and Ajzen's (1975) Theory of Planned Behaviour.[36] This model, together with three additional predictors of intentions (self-identity, health value and perceived susceptibility) and beliefs, underlying attitudes, intentions and perceived control has been used to predict supplement use in 303 UKWCS participants.[37] Results showed that intentions were the major predictor of dietary supplement use, and in turn these were influenced by behavioural beliefs: an individual's value of health and their perception that supplements might reduce susceptibility to illness.[37] Additionally, participants also believed media influenced their behaviour.

Personality is also likely to influence health consciousness, health behaviours and health outcomes. Indeed out of the Big Five personality dimensions (Neuroticism, Extraversion, Openness to Experience, Conscientiousness and Agreeableness)[309] Neuroticism and Conscientiousness, and its facets, have been shown to influence health behaviours and health outcomes.[310-312] These have not been measured for the UKWCS datasets used in this thesis, however a questionnaire has been designed to measure them in a sub-sample of UKWCS women.

Since many health behaviours have been adjusted for in the UKWCS it would be unnecessary to also control for these other factors that influence health behaviour

unless they have a direct influence on risk themselves. It is possible that the level of neuroticism could have a direct biological effect on the development of cancer.

4.7.5.3 Energy intake

Energy intake had been calculated from FFQ listings at baseline and from diary intake in the phase 2 nested case-control dataset, though this was not available for the full phase 2 dataset. There are various methods for controlling for energy intake, as discussed by Willet (1998).[301] A decision was made to produce two sets of adjusted risk analysis results relating to dietary vitamin C intake: using absolute vitamin C intake adjusted by energy intake; and the other using nutrient density intake (vitamin C intake/ metric mega joule energy intake), which was then adjusted further by energy intake in the risk analyses.

Nutrients such as dietary vitamin C intake tended to be weakly correlated with total energy intake even though they do not contribute energy;[301] this is because total food intake correlates with both. If it is hypothesised that vitamin C intake relative to body size has a biological influence on breast cancer risk, rather than absolute vitamin C intake, then given that energy intake tends to reflect body size, an adjustment for total energy intake would be appropriate. This assumes that a specific amount of vitamin C would have less effect on a larger, high energy consuming person than a smaller person.[301] These assumptions, however, cannot be applied to smaller people who are very physically active and are high energy consumers; for such women an analysis of absolute amounts of vitamin C may be more appropriate. Alternatively, adjusting for BMI or weight and physical activity in addition to energy intake may be suitable. Furthermore, if total energy intake is associated with breast cancer then an association between dietary vitamin C and breast cancer may be due to the former association, therefore adjustment for energy intake would be warranted.

An alternative approach to control for energy intake is through nutrient densities,[301] which are calculated by dividing total vitamin C dietary intake by total energy intake. Although its use tends to reduce between-person variations it may result in a nutrient density variable that is inversely related to breast cancer, if energy intake is related to breast cancer. Willet states that this inverse relationship with disease outcome is more likely when the nutrient is weakly correlated with total energy intake; furthermore he found a weak correlation between energy intake and vitamin C (Pearson's r=0.28).[301] Therefore, creating a vitamin C nutrient density variable would not necessarily control for energy intake. Willet suggests this can be overcome by adjusting nutrient density analyses by total energy intake.

To avoid over-adjustment, the intake of fat, meat and other specific energy containing foods were not adjusted for. However alcohol, which contains energy and for which there is convincing evidence of a positive association with breast cancer risk,[7] was adjusted for in addition to total energy intake in the analyses in this thesis. Some analyses were adjusted by a dichotomous variable which split the dataset into alcoholic drinkers and those drinking less than once a week or not at all; this may avoid over-adjustment of the energy contained in alcohol.

4.7.6 Sensitivity analyses

A variety of sensitivity analyses were carried out on the datasets e.g. excluding outliers or excluding women with a family history of breast cancer; details of the methods for these are given below in sections 4.7.6.1 and 4.7.6.3. Since sensitivity analyses were not decided *a priori,* they were not consistently used across the different datasets; therefore further sensitivity analyses on some of the results could be carried out later. For instance in addition to those excluded in other chapters, women with a family history of cancer at baseline could be excluded in sensitivity analyses for chapter 7 on general supplement use and breast cancer risk. Plans to exclude self-reported cancer in sensitivity analyses were not carried out. Risk results reported in chapter 8 on vitamin C supplementation did exclude self-reported prevalent cancers, however since self-reported cancers could be inaccurate, or could have been non-malignant, it may have been better to have included these women in the main analysis and excluded them in sensitivity analyses.

4.7.6.1 Excluding outliers e.g. for high dietary vitamin C intake

Various methods for dealing with outliers have been suggested and researched.[313 314] Many are only applicable to test univariate data or assume data is normally distributed or are designed to check for one outlier only. Grubbs method uses mean and standard deviations to calculate outliers,[315] however since these can be influenced by outliers themselves this univariate method may not be reliable. A simple alternative method which does not use the mean and does not assume a normal distribution is the use of box-plots.[313] This defines the lower and upper thresholds for outliers as 1.5 times the inter-quartile range (the upper quartile i.e. 75% percentile less the lower quartile i.e. 25% percentile). Figure 18 in section 5.3 shows that many dietary vitamin C data points in the UKWCS baseline dataset lie beyond the lines of the upper threshold. This method has been used in sensitivity analyses in this thesis to exclude outliers.

However, some outliers calculated using the inter-quartile range method may occur due to natural variation rather than error.[313] There is evidence that multivariate methods which used Mahalanobis distance calculations produced better agreement with

opinions of experts in the subject matter examined. [313] Unlike box-plots these methods do not sort all the data into ascending order but calculate the distance from each point to the geometric centre of all the scattered points; the processing time, however, is long for large datasets. Sensitivity analyses for dietary vitamin C intake were undertaken omitting outliers calculated using this type of multivariate method developed by Hadi and applied in Stata v10.[316,317]

4.7.6.2 Adjustment for vitamin E

The amount of vitamin E consumed from supplements recorded in the UKWCS phase 2 diaries was adjusted for in a sensitivity analysis of breast cancer risk and vitamin C intake from supplements in chapter 8. Vitamin E from supplements was cleaned and the mean daily intake was calculated in a similar manner to that described for vitamin C supplement data in section 4.5.3.3. Diary recordings of multivitamins which contained both vitamin C and E had already been cleaned earlier. The intake of vitamin A or other antioxidants in other supplements was not cleaned so could not be adjusted for.

Intake of vitamin E, along with vitamin A, which may affect cancer risk via their antioxidant or other properties, had been controlled for in the Danish nested case-control study which assessed the relationship between vitamin C intake and breast cancer risk.[245] No other previous studies had adjusted for these. By supplying electrons vitamin C reduces and regenerates the fat soluble vitamin E radical back to its active antioxidant form. Indeed, *in vitro* experiments have shown that vitamin E can act as a pro-oxidant in the absence of co-antioxidants such as vitamin C.[49] However, this may mean vitamin E may be on the causal pathway between vitamin C intake and breast cancer risk, and perhaps adjusting for vitamin E could create bias.

4.7.6.3 Excluding or adjusting for women with a family history of cancer

Since women with a family history of breast cancer may have a higher risk of breast cancer and may also be more likely to take supplements, these women could be excluded or adjusted in sensitivity analyses to provide better risk estimates for women without a family history. Since many women with a family history are not genetically at raised risk of breast cancer an attempt was made using the detailed family history data at phase 2 to identify women who may be classed at raised or higher risk (see section 4.7.6.3.2). The data at baseline, however, was not detailed enough to estimate those at raised or high risk of breast cancer.

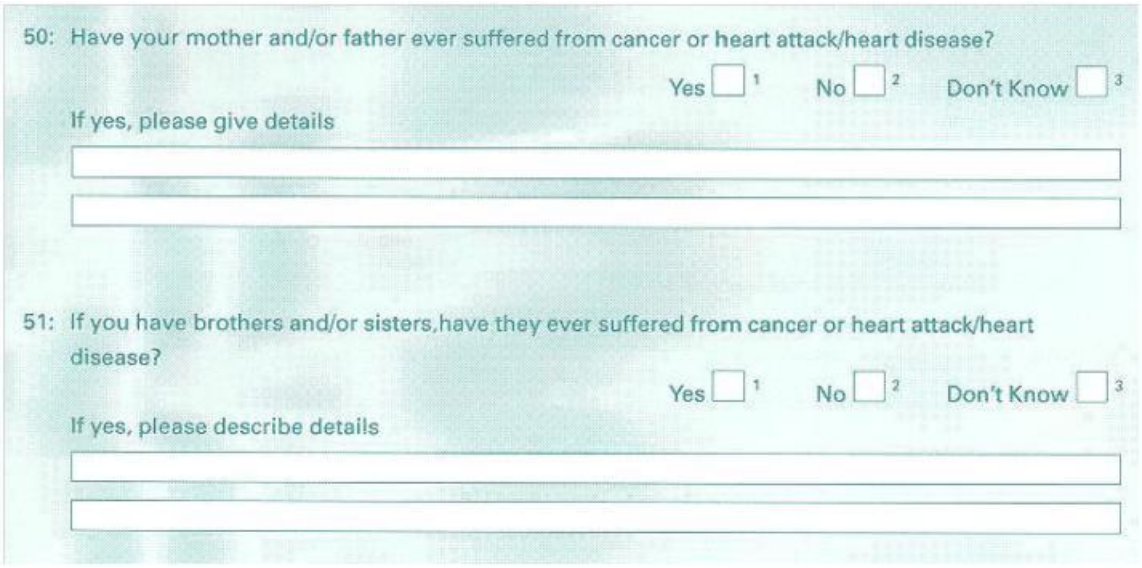
A review of 14 studies that evaluated both positive and negative self-reported family history cancers by comparing them with cancer registry records found that patient-

reported family cancer histories for first-degree relatives are accurate and valuable for breast and colon cancer risk assessments.[318] Negative family history reports for ovarian cancer was not so good, although the prevalence of this is low within families.[318]

4.7.6.3.1 UKWCS Baseline

At baseline a family history of breast cancer was defined as having a first degree relative; mother, father, brothers or sisters who had breast cancer. No information was given about aunts or uncles. Participants were asked to provide more details, but this was often incomplete. Since some participants had stated that a first degree relative had cancer but did not specify what type of cancer, additional adjustments/exclusions were made in sensitivity analyses for women who had a first degree relative with any type of cancer.

Figure 16 Questions asked at baseline relating to family history of cancer


50: Have your mother and/or father ever suffered from cancer or heart attack/heart disease?

Yes ☐ 1 No ☐ 2 Don't Know ☐ 3

If yes, please give details

51: If you have brothers and/or sisters, have they ever suffered from cancer or heart attack/heart disease?

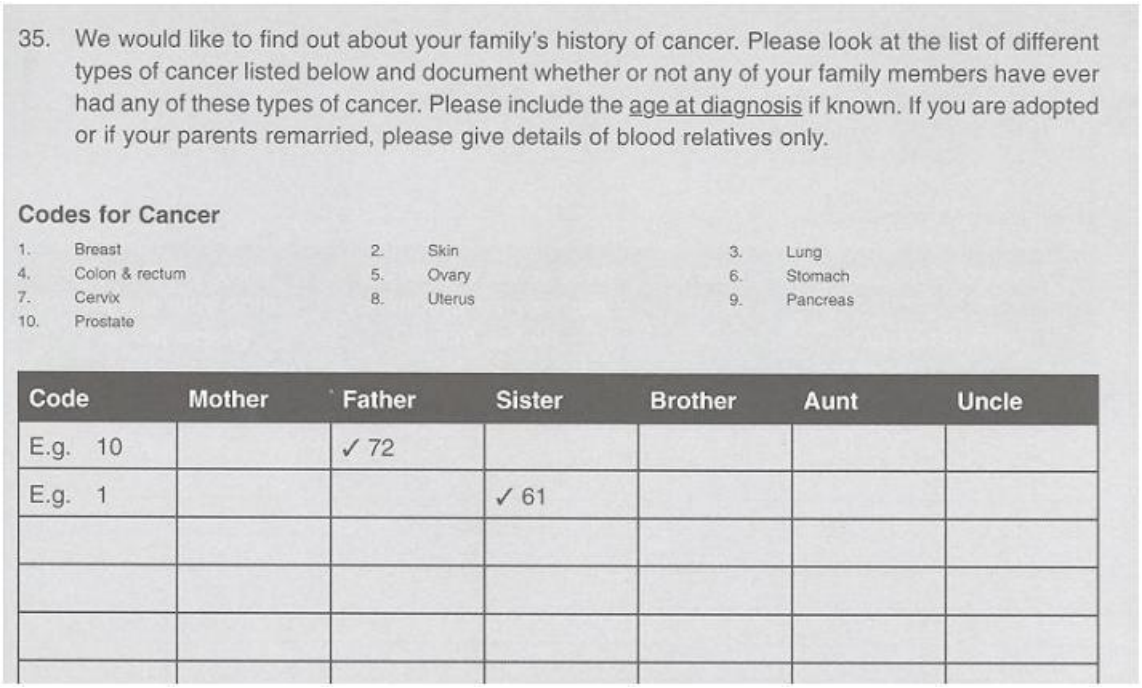
Yes ☐ 1 No ☐ 2 Don't Know ☐ 3

If yes, please describe details

4.7.6.3.2 UKWCS Phase 2

At phase 2 a family history of breast cancer was defined as having a blood relative diagnosed with breast cancer as reported by participants at phase 2 in the questionnaire; they were questioned about cancers in mothers, fathers, sisters, brothers, aunts, uncles. Unlike at baseline, participants were specifically asked which family members had breast cancer and their age at diagnosis (see Figure 17 compared to Figure 16); therefore the breast cancer family history information provided by participants at phase 2 was likely to be more accurate.

Figure 17 Questions asked at phase 2 relating to family history of cancer



35. We would like to find out about your family's history of cancer. Please look at the list of different types of cancer listed below and document whether or not any of your family members have ever had any of these types of cancer. Please include the age at diagnosis if known. If you are adopted or if your parents remarried, please give details of blood relatives only.

Codes for Cancer

1.	Breast	2.	Skin	3.	Lung
4.	Colon & rectum	5.	Ovary	6.	Stomach
7.	Cervix	8.	Uterus	9.	Pancreas
10.	Prostate				

Code	Mother	Father	Sister	Brother	Aunt	Uncle
E.g. 10		✓ 72				
E.g. 1			✓ 61			

Based on information from the NICE report on Familiar breast cancer issued October 2006,[319] it was possible to estimate a small percentage of the phase 2 UKWCS women who may have been at raised risk (2.1%) or high risk (0.8%) of developing breast cancer (see 0). Unfortunately the NICE guidelines could not be followed in some areas; therefore the categories are only estimates. In this report women with a 10-year risk of 3–8% between the ages of 40–49 or a lifetime risk of 17% or greater but less than 30% were classed as having raised risk of developing breast cancer. Women with a 10-year risk of greater than 8% between the ages of 40–49 or a lifetime risk of 30% or greater were classed as at high risk; it included those with a 20% or greater chance of a faulty BRCA1, BRCA2 or TP53 gene in the family.

4.7.7 Samples sizes

Sample sizes were calculated *a priori*. There were sufficient numbers of women in the whole of the UKWCS phase 2 cohort exposed to high doses of vitamin C supplementation greater or equal to 500mg/day when sub-analysed into pre/post-menopausal status in phase 2 of the UKWCS to detect relative risks of 1.3 or above in breast cancer risk analyses (e.g. with 404 pre-menopausal women detectable HR = 0.797 or 1.278).

Assumptions:
Average follow-up time = 7.5 years
Median time on the control treatment = 7 years
Power of study = 90%, Level of significance = 5%

However, in case-control analyses of total vitamin C intake from phase 2 UKWCS diaries with only 200 breast cancer cases it would only be possible to detect odds ratios for breast cancer of 0.486 or 1.804 in women exposed to vitamin C above 60mg/day supplements relative to unexposed subjects (see assumptions below). Furthermore there would be only 45% power to detect odds ratios of 1.4 for breast cancer in those taking vitamin C above 60mg/day relative to those not taking these. Moreover, the power would reduce further if the breast cancer cases were split into pre-menopausal and post-menopausal, or other sub-analyses were performed, or the exposure level was increased. To detect odds ratios of 1.4 with 90% power, 657 case patients with five matched controls per case would be required, meaning the UK Dietary Cohort Consortium studies had to be consolidated in order to sufficiently power the case-control analyses for total vitamin C intake.

Assumptions:
Number of matched breast cancer cases = 200
Ratio of matched controls per case = 5
Probability of vitamin C exposure above 60mg/day from supplements among controls = 0.20
Correlation coefficient for exposure between matched cases and controls = 0.1
Power of study = 90%, Level of significance = 5%

The next chapter evaluates the recording of dietary and supplement vitamin C intake in the UKWCS, then the following chapter determines whether women in the UKWCS with a history of breast cancer are more likely to take vitamin C supplements. The subsequent four chapters assess the relationships between breast cancer risk in UK women and general supplement use, vitamin C supplement use, dietary vitamin C intake and finally total vitamin C intake.

CHAPTER 5

5 Evaluation of diet and supplement vitamin C intake recordings in the UKWCS

5.1 Summary

Objective: Since measurement error of diet can attenuate or exaggerate associations between dietary intake and disease incidence and reduce the power to find these, it is important to evaluate how well dietary tools record dietary exposure. The objective of this chapter was to evaluate vitamin C intake derived from diet and supplement recordings in the UKWCS using a variety of assessment methods.

Methods:

Firstly, the frequency of taking supplements containing vitamin C recorded by diary was compared to the frequency of vitamin C supplement use recorded in the phase 2 questionnaire by 11,184 UKWCS participants. This was evaluated using kappa statistics and tabulations.

Secondly, fruit and vegetable servings derived from FFQ recordings were compared with summary cross-check questions recorded concurrently by 33,521 UKWCS baseline participants. Agreement between fruit and vegetable intake and vitamin C intake derived from the FFQ were also assessed. Spearman's correlations, tabulations, Bland-Altman plots and kappa statistics were used in the evaluations.

Thirdly, vitamin C intake derived from recordings from diaries and FFQs were compared to plasma ascorbic acid and plasma total vitamin C concentrations for 273 women who provided a blood sample at phase 2. This was evaluated using Spearman's correlations and regression analysis.

Results:

Daily vitamin C supplement use reported on the questionnaire captured most of the women recording high dose vitamin C (>500mg) by diary in the UKWCS, the majority of which was likely to be single ingredient supplements. The inclusion of daily reporting of multivitamin/mineral use, as well as vitamin C use, on the questionnaire, produced substantial agreement with diary recordings of supplements containing any vitamin C dose taken on three or four of the four diary days.

Although there were fair to moderate associations between the two methods of recording fruit and vegetable servings using Spearman's correlations and kappa agreement methods, high discrepancies were seen using Bland-Altman's plots. The associations and agreement between cross-check fruit and vegetable servings and FFQ derived dietary vitamin C was also fair to moderate, nevertheless the agreement between grams of fruit and vegetable recorded by FFQ and derived dietary vitamin C was good. Women who were vegetarians, or not supplement users or on a low fat or slimming diet or eating small portions were significantly more likely to have high discrepancies in vegetable reporting between the two methods. Non-graduates and women from lower SES groups were more likely to report high discrepancies for either fruit or vegetables. Correcting FFQ derived vitamin C using the cross-check question data appeared inappropriate.

In the final evaluation, FFQ derived dietary vitamin C correlations with plasma ascorbic acid, and also with dietary vitamin C calculated from diary recordings, were weak to moderate but in line with previous studies. Although the derived values of vitamin C consumed may be inaccurate, the relative ranking of individuals for the full UKWCS cohort based on a similar FFQ may be acceptable for assessing associations between vitamin C dietary intake and breast cancer risk.

Conclusion: The comparisons between FFQs, diary and plasma concentrations were in line with those from other studies and were considered suitable for further analysis. These results highlighted some weaknesses in methods used in the UKWCS and other studies for recording food and supplements containing vitamin C, which should be taken into account when interpreting results of associations between vitamin C intake and breast cancer risks in the UKWCS.

5.2 Comparison of diary and questionnaire methods to assess vitamin C supplement use at phase 2

5.2.1 Introduction

One aim of this thesis was to explore the associations between vitamin C contained in supplements and breast cancer risk, results of which are provided in chapter 8. To explore dose-response relationships, however, it is important to include vitamin C from all supplements taken, not only from single ingredient vitamin C supplements. As seen in section 4.5.3.2 of the methods chapter, a large proportion of supplements containing vitamin C up to the EU recommended daily allowance (EU RDA 60mg/day [53]) are branded as multivitamins in the UK. Supplements which include more than the EU RDA but less than 500mg/day are often named as multivitamins or antioxidants, whereas vitamin C supplements containing high doses of 500mg or more are usually single ingredient. Some of the previous studies which have examined associations between vitamin C supplementation and breast cancer appear not to have used an ingredient database to take account of the amount of vitamin C in all supplements, although doses for multivitamins appear to have been allocated in some studies.[242 267 270]

Other than checks described in section 4.5.3.3 which were made whilst cleaning the supplement database, unfortunately it was not possible to assess whether the dose of vitamin C had been correctly assigned to supplements recorded in the UKWCS phase 2 diaries. In this section, however, an attempt was made to verify the frequency of intake recorded in the diaries with that recorded in questionnaires, and to determine whether the agreement depended on the type of supplement recorded in the diaries and their recorded or derived vitamin C amount. Daily intake of vitamin C was assessed since this may have a greater influence on cancer risk than less regular intake because, as mentioned in chapter 2, excess vitamin C is excreted. It was expected that many women would have indicated use of vitamin C supplements on the phase 2 health and lifestyle questionnaires only if they had taken single supplement vitamin C, or if vitamin C was specified in the name. On the other hand it was expected that women would not indicate they took vitamin C supplements on the questionnaire if they took multivitamins or antioxidants, and other supplements which contained vitamin C but did not mention it in the name.

This analysis compares daily vitamin C use recorded by the phase 2 health and lifestyle questionnaire with supplements containing vitamin C which were recorded in the 4-day diary at phase 2 in the UKWCS.

5.2.2 Methods

5.2.2.1 Subjects

At phase 2 of the UKWCS, 12,453 women completed a health and lifestyle questionnaire and a 4-day food diary, both of which included sections on supplement use. Women who were to be excluded from the related time-to-event analyses in chapter 8 because of their cancer status were also excluded in this evaluation, leaving 11,184 women for the analysis.

5.2.2.2 Dietary assessment methods

For each of the four diary days, the women were required to record supplement brand, name, amount taken and dosage of any supplement taken (as shown in section 4.5.3.1). This information was electronically captured and matched against a database of supplement descriptions and ingredient composition obtained from product labels provided by participants, suppliers' websites or directly from manufacturers. The cleaning of the supplement usage database is detailed in section 4.5.3.3.

Additionally, the frequency of use of 17 types of dietary supplements had been captured from the phase 2 health and lifestyle questionnaire (as shown in section 4.5.2). In addition to vitamin C, the types listed included antioxidant supplements, multivitamins, and multivitamin plus mineral supplements. Frequencies were categorised as: more than daily; daily; weekly; monthly; less than monthly.

5.2.2.3 Statistical analyses

Descriptive statistics of the 11,184 women at phase 2 are included in chapter 8 which examines associations between vitamin C supplement use and breast cancer risk.

The agreement between the two methods of capturing frequency of vitamin C use, i.e. diaries and phase 2 questionnaire, was compared using kappa statistics.[320] Agreements were interpreted as suggested by Landis and Koch detailed in section 5.3.2.3.[321] The definition of frequent use of supplements containing vitamin C was relaxed to three or more of the four diary days, to allow for women who may have accidentally missed completing this section on one of the diary days. Tabulations between the two methods were also produced, including tabulations with vitamin C dose split between1-60mg/d; >60<500mg/d; ≥500mg/d. These are categories used in chapter 8 on vitamin C supplement use and breast cancer risk.

5.2.3 Results

5.2.3.1 Comparisons between vitamin C intake recorded by diary and supplements recorded by the phase 2 questionnaire

When vitamin C supplement use recorded on three or four of the four diary days were split into categories by dose at zero, 60 mg and 500 mg cut-off points, about a third of users were in each intake category. As observed in Table 9, of women who recorded on the phase 2 questionnaires they took daily vitamin C supplements, 18% recorded no use of supplements containing vitamin C in the diaries on 3 or more diary days, about a third took supplements containing less than 500mg/d vitamin C on 3 or more diary days, and half recorded in the phase 2 diaries they took supplements containing 500mg/d vitamin C or more on 3-4 of the diary days.

Table 9 Women who recorded daily use of vitamin C on the phase 2 questionnaire split by diary vitamin C intake category

Vitamin C supplement use recorded by diary split into categories by dose (taken on 3 or 4 of the four diary days)	Daily use of vitamin C on phase 2 questionnaires split by diary intake category		
	Yes	No	Total
No vitamin C	351 (18%)	7042 (76%)	7393 (66%)
1-60mg/d	200 (10%)	1293 (14%)	1493 (13%)
>60<500mg/d	418 (22%)	817 (9%)	1235 (11%)
≥500mg/d	947 (50%)	116 (1%)	1063 (10%)
Total	1916 (100%)	9268 (100%)	11184 (100%)

However, from the perspective of diary recordings, 2210 (56%) of the 3791 diary vitamin C supplement users documented use of supplements containing less than 500mg/d vitamin C in the diary but did not record vitamin C use in the questionnaire. As seen in Table 10 women (89%) who took 500mg/d vitamin C on three or four diary days also recorded on the phase 2 questionnaire that they took daily vitamin C supplements. As found from the inspection of the ingredient database in section 4.5.3.2., the majority of these supplements were likely to be single supplement vitamin C. About a third of women in the medium and high vitamin C intake categories reported on the questionnaire they took antioxidants. Forty percent of women in the highest vitamin C category also recorded the frequent use of multivitamins on the questionnaire, compared to 72% of women in the less than or equal to 60mg/d vitamin C category.

Table 10 Percentage of women in each vitamin C intake category who recorded daily intake of vitamin C, antioxidants or multivitamins on the phase 2 questionnaire

Vitamin C supplement use recorded by diary split into categories by dose (taken on 3 or 4 of the four diary days)		Percentage of women in each vitamin C intake category who recorded daily intake on phase 2 questionnaire of:		
		Vitamin C N=1916	Antioxidant N=1099	Multivitamin N=2714
No vitamin C	N= 7393 (66%)	5%	3%	8%
1-60mg/d	N= 1493 (13%)	13%	10%	72%
>60<500mg/d	N= 1235 (11%)	34%	34%	53%
≥500mg/d	N= 1063 (9.5%)	89%	28%	40%

Using kappa statistics, the measure of agreement between the two methods of capturing frequency of vitamin C supplement usage at phase 2 varies between fair to substantial depending upon what questionnaire data is included in the calculation (Table 11).

Table 11 Kappa agreements between frequency of vitamin C intake captured via diary and that captured via the phase 2 questionnaire

Questionnaire: Supplement taken daily	Supplement containing vitamin C from 4 days of diary keeping	Kappa (95%CI)	Agreement [321]
Vitamin C [a]	4 out of 4 days (daily)	0.39 (0.37-0.41)	Fair
Vitamin C	at least 3 out of 4 days	0.41 (0.39-0.43)	Moderate
Vitamin C or multivitamin [d]	at least 3 out of 4 days	0.70 (0.69-0.71)	Substantial
Vitamin C or multivitamin or antioxidant [e]	at least 3 out of 4 days	0.71 (0.70-0.72)	Substantial
Vitamin C or antioxidant [b]	>60mg, 3 out of 4 days	0.61 (0.60-0.62)	Substantial
Vitamin C	≥500mg, 3 out of 4 days	0.59 (0.58-0.59)	Moderate
Multivitamin/ mineral [c]	1-60mg, 3 out of 4 days	0.41 (0.40-0.42)	Moderate

[a]ticked takes vitamin C daily, or more than daily on the phase 2 questionnaire
[b]ticked takes vitamin C or antioxidant daily, or more than daily on the questionnaire
[c]ticked takes multivitamin or multivitamin and mineral daily, or more than daily on the questionnaire
[d]ticked takes vitamin C, multivitamin or multivitamin and mineral daily, or more than daily on the questionnaire
[e] ticked takes vitamin C, multivitamin or multivitamin and mineral daily, or more than daily,

Only fair agreement (kappa=0.39; 95%CI: 0.37, 0.41) occurs when comparing those women who have ticked daily vitamin C supplement usage on the questionnaire with those shown to have taken four days of supplements containing vitamin C during diary completion (Table 11). As observed in Table 12 77.7% of recordings are in the expected quadrant. According to the kappa statistics, the agreement increases if the comparison is relaxed to usage on three or four of the four diary days. The agreement becomes substantial if daily multivitamin use indicated on the questionnaire is also included in the comparison (kappa=0.70; 95%CI: 0.69, 0.71). This kappa statistic alters

only slightly if daily antioxidant supplement use on the questionnaire is then also included in the comparison ((kappa=0.71; 95%CI: 0.70, 0.72); as seen in Table 13 86.6% are in the expected quadrant). Excluding women who were ill or on a weight reducing diet during diary recording, and who may have taken vitamin C or antioxidants or multivitamins during this time, perhaps on a temporary basis, does not alter the kappa statistic (kappa=0.71; 95%CI: 0.70, 0.72).

Table 12 Cross-tabulation of daily vitamin C supplement use recorded by questionnaire with daily intake of supplement containing vitamin C recorded by diaries

Questionnaire: Daily vitamin C use	Diary: daily use of supplements containing vitamin C (4 out of 4 diary days)		
	No	Yes	Total
No	7332 (65.5%)	1936 (17.3%)	9268 (82.8%)
Yes	556 (5.0%)	1360 (12.2%)	1916 (17.2%)
Total	7888 (70.5%)	3296 (29.5%)	11184 (100%)

Kappa = 0.39 (0.37-0.41)

Table 13 Cross-tabulation of daily vitamin C or antioxidant or multivitamin supplement use recorded by questionnaire with intake of supplement containing vitamin C on 3 or 4 diary days

Questionnaire: Daily vitamin C or antioxidant or multivitamin use	Diary: use of supplements containing vitamin C on 3 or 4 diary days		
	No	Yes	Total
No	6497 (58.1%)	604 (5.4%)	7101 (63.5%)
Yes	896 (8.0%)	3187 (28.5%)	4083 (36.5%)
Total	7393 (66.1%)	3791 (33.9%)	11184 (100%)

Kappa = 0.71 (0.70-0.72)

Table 14 Cross-tabulation of daily vitamin C or antioxidant supplement use recorded by questionnaire and taking supplements containing vitamin C above 60mg for 3 or 4 diary days

Questionnaire: Daily vitamin C or antioxidant use	Diary: use of supplements containing vitamin C above 60mg on 3 or 4 diary days		
	No	Yes	Total
No	8054 (72.0%)	647 (5.8%)	8701 (77.8%)
Yes	832 (7.5%)	1651 (14.7%)	2483 (22.2%)
Total	8886 (79.5%)	2298 (20.5%)	11184 (100%)

Kappa = 0.61 (0.60-0.62)

Substantial agreement also occurs in comparisons between supplements containing over 60 mg/d of vitamin C recorded by diary on three or four days and daily vitamin C supplement or antioxidant usage indicated on the questionnaire ((kappa=0.61; 95% CI: 0.60, 0.62); 86.7% are in the expected quadrant in Table 14). Similar results are produced in comparisons between supplements containing 500mg or more of vitamin C recorded by diary on three or four days and daily vitamin C supplement usage indicated on the questionnaire (kappa=0.59; 95% CI: 0.60, 0.62). Similar results are also produced after excluding women who completed the diary between the winter months, November to end of February, who may only take antioxidants or the higher doses of vitamin C throughout the winter or when they develop winter colds (Kappa=0.58; 95% CI: 0.56, 0.58)).

However, there was only moderate agreement when recording of supplements containing low amounts of vitamin C (60mg/d or less) on three or four diary days were compared with daily multivitamin recordings on the questionnaire (kappa=0.41, 95%CI: 0.40, 0.42).

5.2.4 Discussion

Only moderate agreement occurred when comparing those women who ticked daily vitamin C supplement usage on the questionnaire with those shown to have taken supplements containing vitamin C on three or four days of the 4-d diary. This was unsurprising given the difference seen in the various tabulations. For instance, over a third of women consuming supplements containing vitamin C as per diary recordings were consuming ones less than or equal to 60mg, and the majority of women in this category recorded daily multivitamin use rather than vitamin C or antioxidant use on the questionnaire. The results clearly show that responses to a simple question about vitamin C supplement use on a questionnaire will not capture the recording of a large proportion of supplements containing vitamin C. Therefore, it was not possible to verify the frequency of diary recorded supplements containing vitamin C using only the vitamin C question on the questionnaire. Only after taking account of the reporting of daily use of multivitamin/minerals as well as vitamin C on the questionnaire, was substantial agreement reached between diary and questionnaire reports.

However, the results indicate that previous studies which have examined associations between vitamin C supplementation and breast cancer risk and allocated doses for single supplement vitamin C, multivitamins and antioxidants may have picked up the majority of supplements used containing vitamin C.[242 267 270] Nevertheless, the dose estimates that were allocated may have been somewhat inaccurate if no ingredient database was used. Furthermore, as seen from the inspection of the UKWCS

ingredient database in section 4.5.3.2, the majority of supplements in the medium vitamin C dose range are types other than those branded as vitamin C, multivitamins and antioxidants; these, however, may be less commonly consumed.

Although women recording supplements containing high doses of vitamin C (500mg/d or more) in the diaries usually reported vitamin C supplement on the phase 2 questionnaire, only half the women who reported the latter appeared to take high doses. Therefore, confirmative answers to vitamin C use on the questionnaire were not representative of high dose use in the diary, indicating that questionnaires should not be used for this purpose.

Although the majority of women (72%) using supplements containing doses of 60 mg/day or below (the EU recommended allowance) appear to report multivitamin use on the questionnaire, the overall kappa agreement between this diary category with recordings of daily use of multivitamins or multivitamins with minerals on the questionnaire was fairly poor. There are likely to be two reasons for this: the average multivitamin in the UK supplement data base contains over 70mg therefore some multivitamins will contain more than 60mg; and many women in the higher dose categories may also be taking low dose multivitamins so may have ticked the vitamin C and a multivitamin box on the questionnaire.

The questionnaire relates to a longer time period than the 4-d diary; this is likely to have lowered the agreement between the two tools. It is not clear from the section in the questionnaire whether the period of assessment for each supplement type listed relates to current use or use in the last year. The 4-d diary recordings are more likely to represent short-term episodic intake, particularly because high dose vitamin C is promoted for reducing seasonal common colds. Despite this, the agreements between the methods did not change when women who completed the diaries in the winter months were excluded. Neither did the agreement between methods change when women were excluded who were ill or on a weight reducing diet, and who may have been concerned that they lacked nutrients during this time and taken supplements temporarily. Furthermore, vitamin C supplementation was found to have the highest long-term use stability of any nutrient in a US study which compared supplement data collected by two questionnaires that were on average 2.4 years apart (kappa=0.64; 955 CI: 0.60, 0.67).[322] A similar agreement was found comparing 24-hr recall methods with the second questionnaire data, but comparisons with diary data were not undertaken.[322]

A previous study compared information transcribed from supplement packaging (the gold standard) with data from self-administered questionnaire, producing kappa

statistics for supplement type as 0.67 (0.53, 0.89) for vitamin C, and 0.68 (0.52, 0.84) for once a day multivitamins.[323] The questionnaires in this study requested more detail than those of the UKWCS, and the UKWCS diary recordings may not be as accurate as the gold standard, nevertheless some of the current results were in line with these results. The Spearman's correlation coefficients for dose of vitamin C in all supplements was 0.76 for this study.[323]

In the UKWCS the diary and questionnaire were completed within a short time period of each other; although this increases the likelihood that supplements were recorded on either tool, it may have also created recall bias and produced overstated comparisons.[324] Nevertheless, anomalies remain in the UKWCS data that may be due to recording or input error, which cannot be explained by changes in supplement use owing to seasonal differences, illnesses or weight-reducing diets. Despite the time that had been spent allocating supplement types from the ingredient database to supplements recorded in the diaries, and cleaning these, the methods used in recent research on the EPIC-Norfolk cohort to allocate vitamin C dose could potentially produce a more accurate vitamin C intake consumed from supplements.[38]

Despite diary assessment taking place over a narrow four day period, overall these results indicate that, after checking and cleaning supplements containing vitamin C, the validity of daily vitamin C supplement use is good when compared with the questionnaire assessment of vitamin C and multivitamin supplement use. This and the results of the final spot check indicate the data is acceptable for use in further analyses.

5.3 Comparisons relating to dietary vitamin C intake and servings of fruit and vegetable consumed at baseline

5.3.1 Introduction

Since measurement error of dietary exposure could distort the associations between vitamin C intake and breast cancer incidence in the UKWCS, it is important to evaluate the recording of food containing vitamin C in the UKWCS. At baseline self-reported FFQs were used to record diet since the cost and ease of administering them to over 35,000 women were lower than other methods. Additionally, the FFQs estimate intake over 12 months, being superior in this respect compared to diaries or biomarkers. This section focuses on the evaluation of UKWCS baseline FFQ recordings of fruit and vegetables, the main source of vitamin C.

Over-estimation of fruit and vegetables by FFQs is a known problem which has been previously assessed using data from 6,572 women at baseline in the UKWCS.[288] If estimated intake was systematically inflated for most women in the cohort then this may not impact on cancer risk ratios. However, the earlier analysis found that vegetarians and those on slimming diets were slightly more likely to have a large discrepancy between FFQ fruit and vegetable recordings and total portions of fruit and vegetables consumed given in two cross-check questions.[288] Women with higher education levels were less likely to over-estimate. The authors suggest that average portions of vegetables consumed in mixed dishes may have been over-estimated and socially desirable answers may have been given. Possible errors in FFQ derived fruit and vegetable or vitamin C intakes can be adjusted by applying a correcting factor for each subject using cross-check summary questions which ask for total fruit and vegetable portions consumed per day or per week.[288 325-327]

Expanding on the initial analysis of 6,572 women,[288] this baseline analysis of the full UKWCS cohort will compare fruit and vegetable intake derived from FFQ recordings with concurrent recordings by cross-check questions. Agreement between fruit and vegetable intake and vitamin C intake derived from the FFQ will also be assessed. The suitability of using the cross-check questions to correct for possible over-estimation of vitamin C derived from fruit and vegetables reported in the UKWCS FFQs will be discussed.

5.3.2 Methods

5.3.2.1 Subjects

General information about the recruitment of the 35,372 women who completed the baseline questionnaire is provided in section 4.1.1.1 of this thesis. Women who were to be excluded from the subsequent time-to-event analyses in chapter 9 were also excluded in this evaluation. This left 33,521 middle-aged women from the UKWCS who completed the baseline FFQ for this evaluation analysis.

5.3.2.2 Dietary assessment methods

At baseline the mean daily dietary vitamin C intake had been calculated from the list of simple and mixed food items on the FFQ. This FFQ was developed from one used in EPIC UK studies,[289 290] by adding extra vegetable composition dishes to accommodate the higher proportion of vegetarians in the UKWCS to produce a 217 item FFQ.[279] Thirty one vegetables (excluding potatoes), 19 fresh fruit and five dried fruit items were listed in the FFQ. Nine of the fresh fruit were seasonal; no canned or frozen fruit were specifically listed. As seen in section 4.5.1, for each item participants chose from 10 pre-coded frequency of consumption categories ranging from never to 6 or more times per day, on average over the past year. The cross-check questions were on a separate section of the baseline questionnaire:

Q7 *How many servings of vegetable or vegetable containing dishes (excluding potatoes) do you usually eat each week?*

Q11 *How many servings of fruit or fruit containing dishes do you usually eat each week?*

5.3.2.3 Statistical analyses

Descriptive statistics of the 33,521 women at baseline are included in chapter 9 on associations between dietary vitamin C intake and breast cancer risk.

After exclusions, histograms and box plots were produced of dietary vitamin C and total fruit and vegetable intake (see Figure 18). All graphs were skewed to the right and also showed twelve women with much higher intakes. No obvious errors were found between answers provided on the questionnaire and data electronically captured for these women. However, comparisons between the completion of the FFQ lists of fruit and vegetables and the number of servings given in the cross-check questions indicated that some women with very high intakes may have completed some sections incorrectly. For all but one participant, it was not obvious which parts were completed incorrectly, therefore no corrections or further exclusions were made at this stage.

The distributions of the variables were skewed, and so the median intakes and inter-quartile ranges (IQR) were calculated as well as the means for fruit and vegetables and for derived vitamin C intake split by fruit, vegetables, juice and potatoes. The Wilcoxon sign-rank test, for paired non-parametric data, was used to compare servings of fruit or vegetables measured by FFQ and by cross-check question for each woman. The correlations between total fruit and vegetable servings and also intake as grams and derived vitamin C were calculated using Spearman's correlation coefficients for non-parametric data (using pair-wise deletion of observations with missing values). This method is less influenced by outliers than Pearson's product moment correlations. The Spearman's method ranks data for each variable and calculates the Pearson's correlation between the ranks which is the number of standard deviations that one variable changes for a standard deviation change in the other. Unfortunately, correlations between two variables can be strong even if one is consistently much higher than the other variable, making the technique misleading.[328] Correlations measure the strength of the association between two variables but, unlike the Bland-Altman technique, cannot assess the extent of the agreement between them. The Bland-Altman technique can only be used to compare two methods with the same underlying units of measurement such as fruit and vegetable servings; the ratio of servings derived from FFQs to servings reported by the cross-check question was plotted against the mean of the two measures using log scales.[328 329] More specifically, this was achieved by plotting the exponential of the difference in the two log-transformed measures against the exponential of the mean of the log-transformed variables on log scaled graphs. The ratio was used instead of the difference between the two measures normally used in Bland-Altman plots, since the differences increased substantially as the servings increased. The ratios between the two variables should be relatively small for the agreement between the two measurements to be considered good. The 731 women who did not complete either cross-check question were excluded from the plot.

In a similar manner to previous research on the smaller sample of UKWCS, [288] the characteristics of women with FFQ derived servings that were more than five times larger than the cross-check answers were tabled.

For comparisons of variables with different underlying units, the variables were categorised into 5 groups of ascending intake and the agreement between the categorisation of participants using the two methods was compared using kappa statistics;[320] a technique normally used to measure agreement between two raters or measure reproducibility between two time points. Unweighted and weighted kappa statistics, κ and $\kappa_{w,}$ were produced.

For instance, the kappa statistic compared the observed portion of agreement between fruit intake in grams and fruit servings with the portion of agreements that would be expected by chance. Given that 5 categories were used, the chance of misclassification was quite high which tended to decrease the value of kappa. Rather than assigning no agreement, classification occurring in adjacent categories was counted as partial agreement and was weighted accordingly. The following linear weighting was used:

$$K_{wi} = 1 - (i /(n - 1))$$

where *i* is the number of categories of difference between the two methods for each participant, and *n* is the number of categories. This gave weightings of: 1; 0.75; 0.5; 0.25, and 0 depending on whether the classifications differed by none, 1, 2, 3, or 4 categories respectively.

Agreements were interpreted as suggested by Landis and Koch (1977):[321]

K =0 none, no better than chance

K <0.2 slight agreement

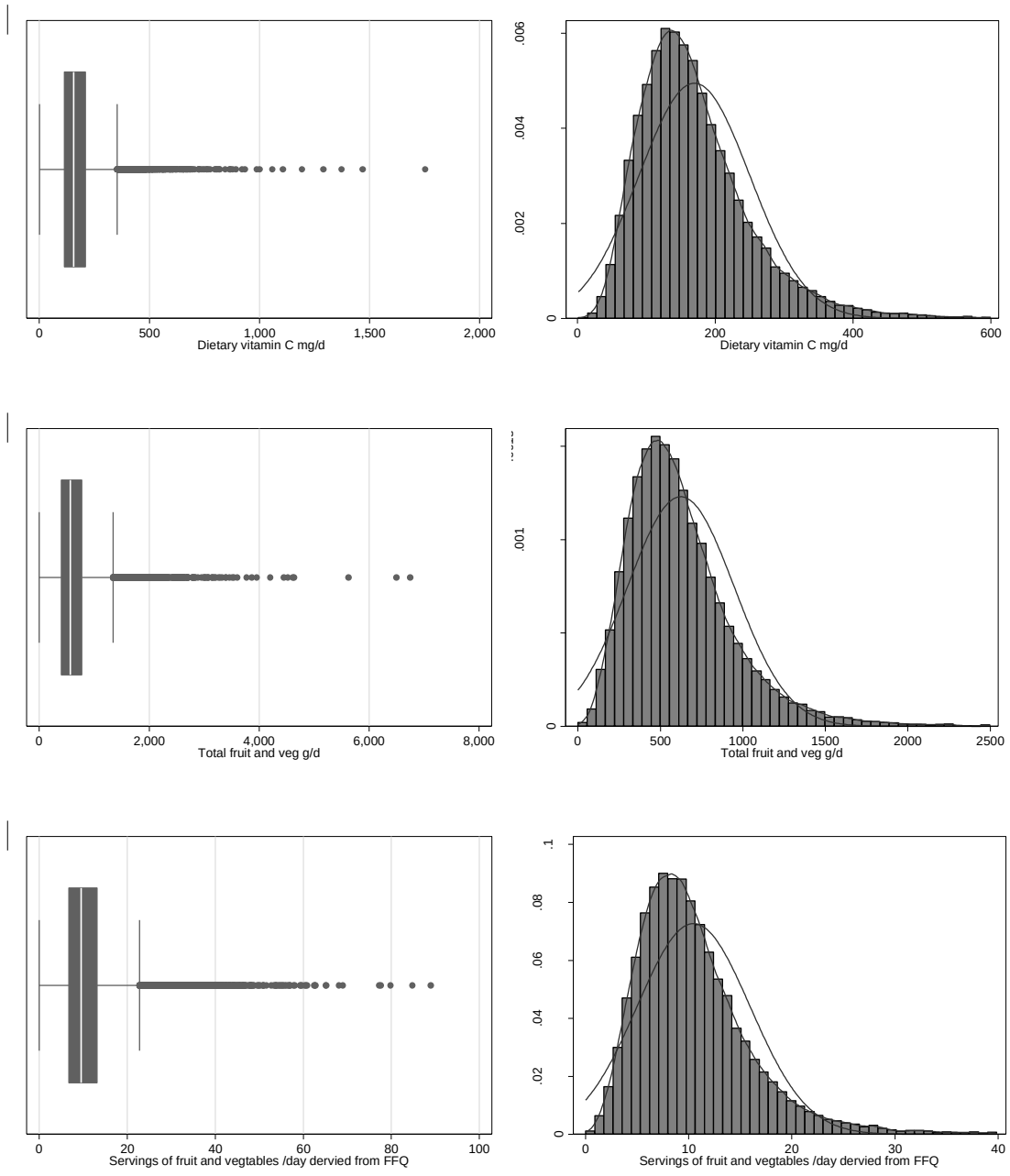
K ≥0.21 ≤0.40 fair agreement

K ≥0.41 ≤0.60 moderate agreement

K ≥0.61 ≤0.80 substantial/good agreement

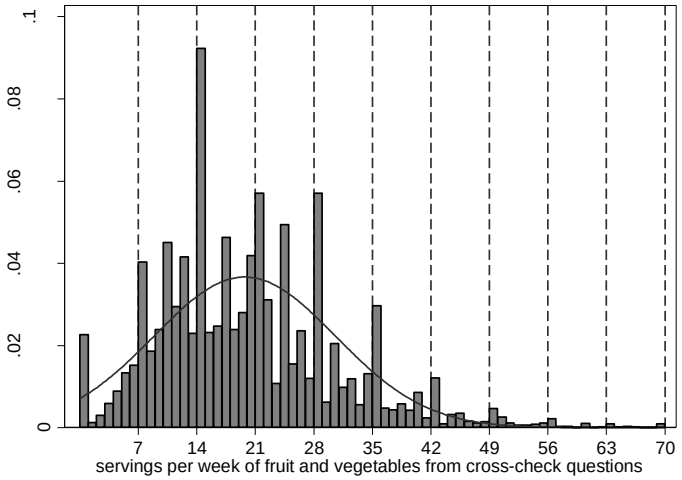
K >0.8-1.00 very good agreement

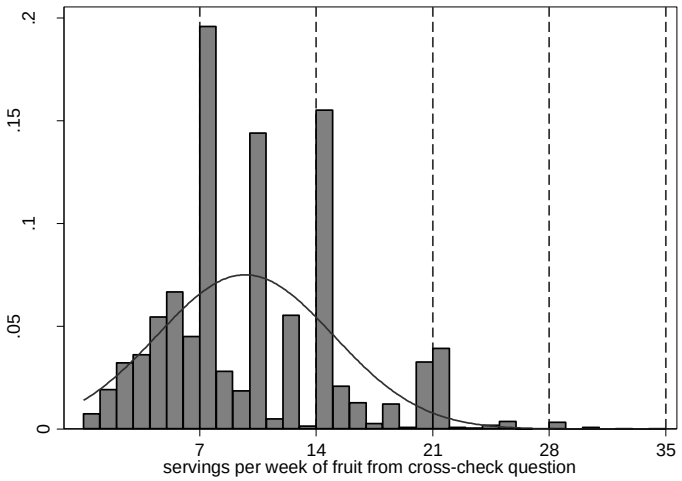
Figure 18 Box whisper plots and histograms showing distributions of FFQ derived dietary vitamin C (mg/d), total fruit and vegetable intake (g/d), and total servings per day of fruit and vegetables

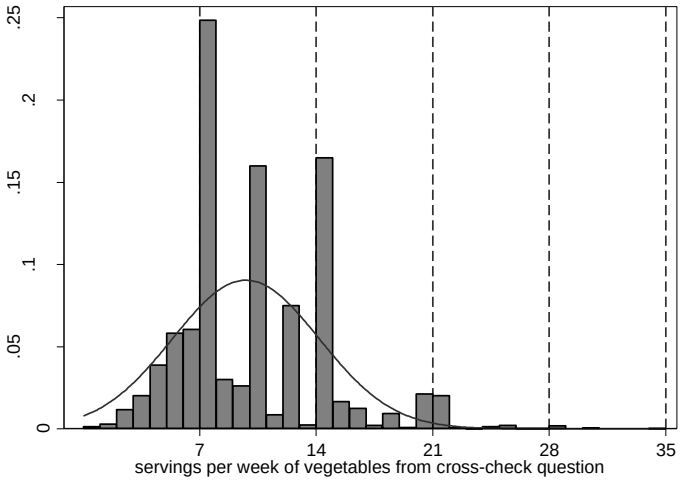


Outliers shown in box plots but not in histograms

Figure 19 Histograms showing distributions of total fruit and vegetables servings per week recorded by cross-check question, and separate distributions for fruit and for vegetables







5.3.3 Results

The median intake in grams (IQR) per day derived from the FFQ was 264g (164-399) for fruit, 281g (195-393) for vegetables, and 565g (210-400) for total fruit and vegetable including juices. The median total vitamin C derived from the FFQs was 156mg (114-210) for all women and 160mg (117-218) for vegetarians, a difference of only 3%. Mean totals were 171.2mg (sd 86.3) and 178.0mg (sd 93.9), respectively. Vegetarians were defined from the FFQ as eating meat or fish less than once per week. Dietary vitamin C intake calculated from juice, fruit, vegetables and potatoes are shown in Table 15 for all women and for vegetarians, who comprised of 19% of this dataset. The main source of vitamin C was from fruit and vegetables, making up 75% for all women and only slightly more for vegetarians at 76% (as calculated from means). Juice made up 19% of the total dietary vitamin C intake derived from the FFQ.

Table 15 The main dietary sources of vitamin C at baseline in the UKWCS for all women and for vegetarians separately

	All women			FFQ defined vegetarians		
Vitamin C from	Median (IQR) mg/d	Mean (sd) mg/d	% of total[a]	Median (IQR) mg/d	Mean (sd) mg/d	% of total[a]
Juice	24.1 (5.1-56.5)	32.4(38.5)	19%	22.1 (5.1-56.6)	33.0 (41.5)	19%
Fruit	48.9 (28.3-77.2)	61.6 (56.7)	36%	51.3 (29.7-81.3)	65.5 (64.0)	37%
Vegetable	57.7 (36.9-85.9)	66.3 (43.3)	39%	60.8 (39.7-88.8)	69.5 (44.6)	39%
Potatoes	14.0 (9.2–19.2)	15.1 (9.4)	9%	12.4 (7.51-18.3)	13.8 (10.1)	8%

[a]Percentage of totals are based on means (these add up to slightly more than 100% due to some errors in splitting vitamin C between sources)

The median (IQR) daily servings of fruit and vegetables reported on the cross-check questions were 1.4 (1-2) and 1.4 (1-2), whereas the median servings derived from the FFQ were much higher: 4.3 (2.7-6.5) and 4.7 (3.2-6.6). The medians from the cross-check questions are significantly different from the FFQ servings (P<0.001). For total fruit and vegetables from the cross-check questions and from the FFQ the mean servings were 2.8 (sd 1.8) and 10.6 (sd 6.1) respectively. These averages excluded women with missing data. Of the 33,521 women, 731 (2%) had missing data for both cross-check questions, 4122 (12%) had missing responses for fruit only and 1397 (4%) for vegetables.

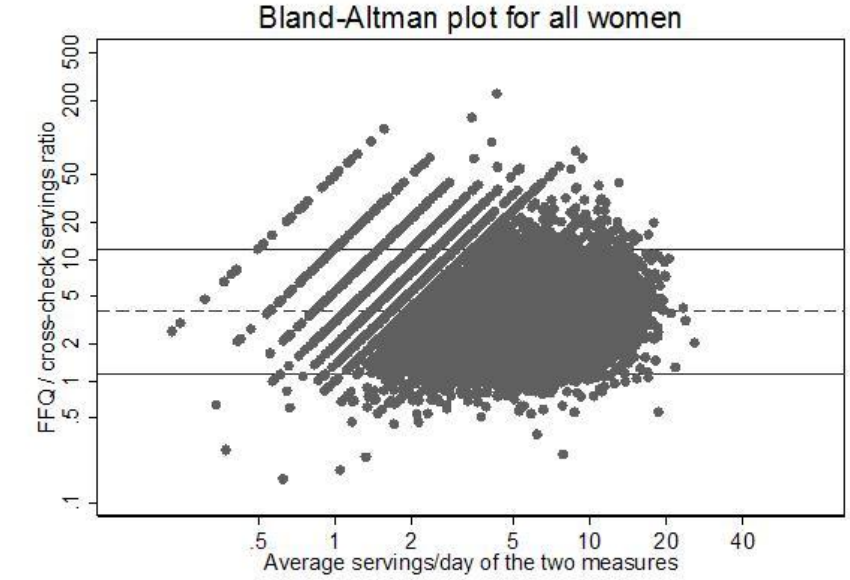
5.3.3.1 Comparisons between methods of recording fruit and vegetables

There were some anomalies relating to juice in the comparisons for fruit. Some participants may have included fruit juice in their responses to cross-check questions, others may not. Up to one portion of fruit juice per day was allowed in the calculation of number of fruit servings derived from the FFQ, which had been previously calculated.[330]Juice was not included in ‘fruit in grams’ and in ‘vitamin C from fruit’, both

derived from the FFQ. Comparisons relating to vegetables did not include potatoes, which made up 8-9% of total vitamin C intake. Participants were asked not to include potatoes in the vegetable cross-check question.

The log-transformed Bland-Altman plots in Figure 20 show that for 95% of women the fruit and vegetable servings per day derived from the FFQ exceeds the cross-check question recordings by between 1.2 and 11.9 times. The mean ratios and 95% confidence limits were similar for vegetarians (Figure 20) and non-vegetarians (not shown) and for fruit and vegetables separately (Figure 21).

Figure 20 Bland-Altman plots of ratios between FFQ and cross-check question recording total fruit and vegetable servings for all women and for vegetarians only



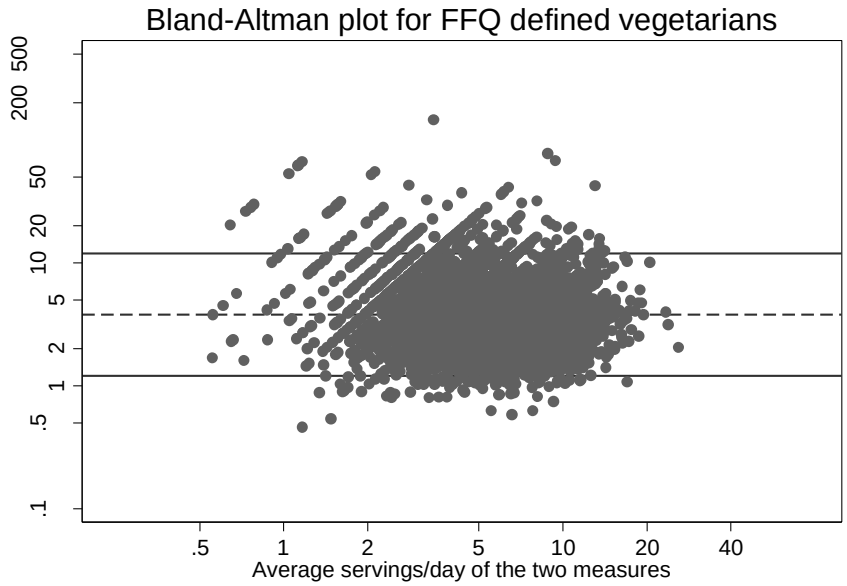
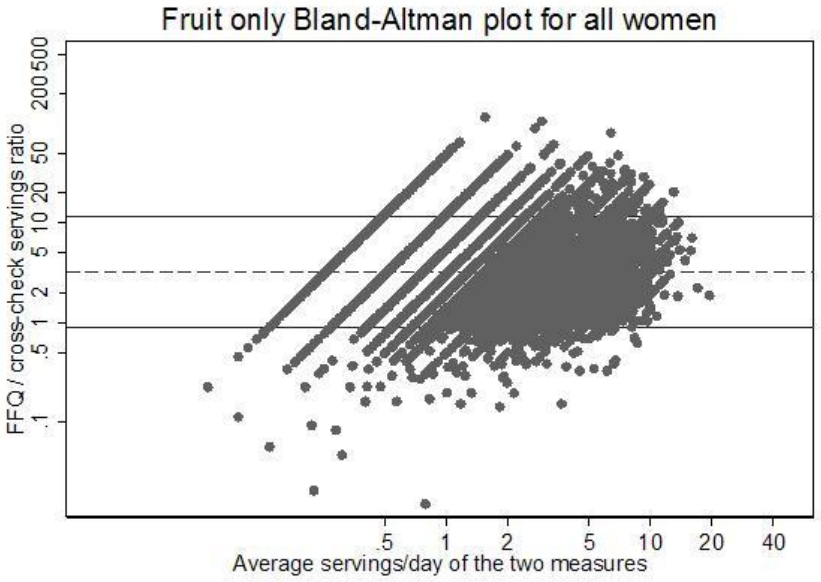
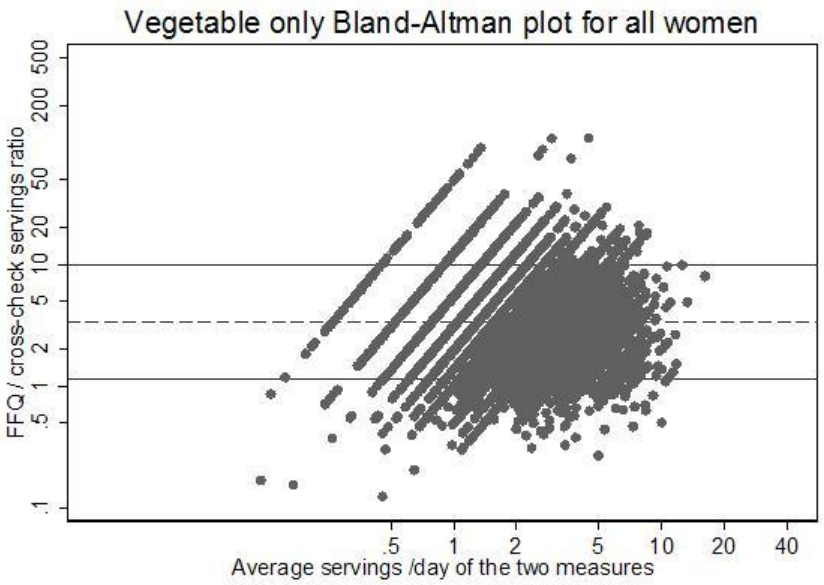


Figure 21 Separate fruit and vegetable Bland-Altman plots of ratios between servings measured by FFQ and by cross-check question





Despite the large reported discrepancies found above there was moderate correlation with Spearman's ranked correlations coefficients between the methods for recording servings for fruit (r_s=0.54) and for vegetables (r_s=0.40). There was also moderate correlation between the cross-check answers and the FFQ derived fruit or vegetable in grams or as vitamin C as seen in Table 16 and Table 17. The correlation was strong between servings (or grams) of fruit or vegetables derived from the FFQ and the vitamin C from fruit calculated from the FFQ, and similarly for vegetables (r_s=0.91 and 0.86).

Table 16 Spearman's correlations between fruit as servings and as grams, and between derived vitamin C intake

Comparison variables for fruit		Spearman's[a]	Correlation[b]
X-check fruit serving[c]	FFQ fruit servings[d]	0.54 (p<0.001)	Moderate
X-check fruit serving	FFQ fruit (g)[e]	0.61 (p<0.001)	Moderate
X-check fruit serving	FFQ vit C from fruit[f]	0.54 (p<0.001)	Moderate
FFQ fruit servings	FFQ vit C from fruit	0.91 (p<0.001)	Strong
FFQ fruit (g)	FFQ vit C from fruit	0.89 (p<0.001)	Strong

[a]Spearmans's ranked correlation coefficients for non-parametric data
[b]Given strength of correlation is based on classification of Peason's correlation coefficients
[c]Fruit servings consumed, obtained from cross-check questions
[d]Fruit servings consumed, derived from the FFQ
[e]Grams of fruit consumed, derived from the FFQ
[f]Dietary vitamin C intake from fruit, derived from the FFQ

Table 17 Spearman's correlations between vegetables as servings and as grams, and between derived vitamin C intake

Comparison variables for vegetables		Spearman's	Correlation
X-check veg serving	FFQ veg servings	0.40 (p<0.001)	Moderate
X-check veg serving	FFQ veg (g)	0.40 (p<0.001)	Moderate
X-check veg serving	FFQ vit C from veg	0.37 (p<0.001)	fair
FFQ veg servings	FFQ vit C from veg	0.86 (p<0.001)	Strong
FFQ veg (g)	FFQ vit C from veg	0.90 (p<0.001)	Strong

See notes to Table 16

Alternatively, using the Kappa method to measure the extent of the agreement, the fruit serving cross-check responses were split into five roughly equal groups and cross-tabulated against fruit servings derived from the FFQ split similarly (Table 18). Only 35% of participants were classified in the same fifths producing a kappa statistic of 0.19, of slight agreement (Table 19). However, 74% were classified in the same or adjacent fifths producing a weighted Kappa of 0.36 of fair agreement. (The kappa weighted method is explained in section 5.3.2). At the extremes, 9% were classified in groups greater than two groups difference. As seen in Table 19 the weighted Kappa

was similar for comparisons between fruit serving cross-check responses and vitamin C from fruit derived from the FFQ (K_w=0.37). As expected the latter's agreement with fruit as grams derived from the FFQ was substantial (K_w=0.71).

Table 18 Tabulation of fruit servings from cross-check answers and those derived from the FFQ excluding women with missing data

Cross-check fruit group (servings/week)	FFQ derived fruit servings per week					
	1 (0-16) 20%	2 (17-25) 20%	3 (26-34) 18%	4 (35-49) 21%	5 (50 +) 21%	Total (%) 100%
1 (0-5)	3,165	1,257	695	445	286	5,848 (20)
2 (6-7)	1,458	1,742	1,312	1,156	849	6,517 (22)
3 (8-11)	668	1,350	1,265	1,211	851	5,345 (18)
4 (12-14)	345	1,047	1,309	1,603	1,626	5,930 (20)
5 (15 & above)	145	530	882	1,674	2,526	5,757 (20)
Total	5,781	5,926	5,463	6089	6,138	29,397 (100)

Table 19 Kappa agreements between groupings of fruit consumed as servings, intake in grams and derived vitamin C intake

Comparison variables		Kappa (SE)	Agreement[321]
X-check fruit servings	FFQ fruit servings	0.19 (0.0029)	Slight
		0.36 (0.0040)[w]	Fair
X-check fruit servings	FFQ fruit (g)	0.23 (0.0029)	Fair
		0.42 (0.0040)[w]	Moderate
X-check fruit servings	FFQ vit C from fruit	0.19 (0.0029)	Slight
		0.37 (0.0040)[w]	Fair
FFQ vit C from fruit	FFQ fruit (g)	0.48 (0.0029)	Moderate
		0.71 (0.0040)[w]	Substantial

[w]Weighted to taken into account observations allocated to adjacent groups (see section 5.3.2)
SE= standard error
Women with missing fruit cross-check serving data were excluded from all the above kappa calculations

It proved difficult to split the vegetable serving cross-check responses into five roughly equal groups due to their uneven distribution. When these were cross-tabulated against vegetables servings derived from the FFQ split similarly (Table 20) only 29% of participants were classified in the same fifths producing a Kappa statistic of 0.11, of slight agreement (Table 21). However, 72% were classified in the same or adjacent fifths producing a weighted Kappa of 0.25 of fair agreement. At the extremes 9% were classified in groups greater than two groups difference. The weighted Kappa was similar for comparisons between vegetable serving cross-check responses and vitamin C from vegetables derived from the FFQ (K_w=0.24). The latter's agreement with vegetables as grams derived from the FFQ was substantial (K_w=0.72).

Table 20 Tabulation of vegetable servings from cross-check answers and those derived from the FFQ excluding women with missing data

Cross-check vegetable group (servings/week)	FFQ derived vegetable servings per week					
	1 (0-18)	2 (19-28)	3 (29-38)	4 (39-54)	5 (55+)	Total (%)
	17%	23%	22%	23%	15%	100%
1 (0-6)	2,340	1,553	1,001	621	252	5,767 (18)
2 (7)	1,530	2,000	1,642	1,473	791	7,436 (23)
3 (8-11)	982	1,830	1,703	1,529	790	6,834 (21)
4 (12-14)	574	1,411	1,771	2,154	1,628	7,538 (24)
5 (15 & above)	171	635	1,061	1,486	1,194	4,547 (14)
Total	5,597	7,429	7,178	7,263	4,655	32,122 (100)

Table 21 Kappa agreements between grouping of vegetable consumed as servings, intake in grams and derived vitamin C intake

Comparison variables		Kappa (SE)	Agreement[321]
X-check veg servings	FFQ veg servings	0.11 (0.0028)	Slight
		0.25 (0.0038)[w]	Fair
X-check veg servings	FFQ veg (g)[e]	0.11 (0.0028)	Slight
		0.25 (0.0038)[w]	Fair
X-check veg servings	FFQ vit C from veg[f]	0.11 (0.0028)	Slight
		0.24 (0.0038)[w]	Fair
FFQ vit C from veg	FFQ veg (g)	0.49 (0.0028)	Moderate
		0.72 (0.0038)[w]	Substantial

[w]Weighted to taken into account observations allocated to adjacent groups (see section 5.3.2)
SE= standard error
Women with missing vegetable cross-check serving data were excluded from all the above kappa calculations

The Kappas produced for comparisons of total fruit and vegetables were similar (not shown).

5.3.3.2 Women with high discrepancies

Fruit servings derived from the FFQ were more than double the responses for cross-check questions for 78% of women who completed the cross-check questions, similarly for 82% of women relating to vegetable servings. FFQ derived servings were more than five times larger than the cross-check answers for about a quarter of the women (23% of the all women reporting fruit and 24% reporting vegetables, and 21% and 28% respectively for FFQ defined vegetarians). The characteristics of women with these large discrepancies are shown in Table 22. On average, high discrepancy fruit and vegetable reporters had higher median FFQ servings per day compared to other women but lower median servings per day reported on the cross-check questions compared to others (Table 22). These women, including the FFQ defined vegetarians amongst them, had a median of one serving per day for fruit and for vegetables as per the cross-check question. FFQ derived servings were seven times that of the cross-check answers for over half of the high discrepancy reporters, and for about 10% of total women.

Table 22 Characteristics of women with large discrepancies between methods of measuring fruit and vegetable portions

	High discrepancy		Other women	
	Fruit reporting	Veg reporting	Fruit reporting	Veg reporting
FFQ servings, median (IQR)	6.4 (4.3-9.8)	6.7 (5.2-8.8)	4.0 (2.6-5.8)	4.1 (2.9-5.7)
X-check servings per day, median (IQR)	1.0 (0.6-1.4)	1.0 (0.7-1.3)	1.4 (1-2)	1.4 (1-2)
Age years, mean (sd)	52.4 (9.3)	51.6 (9.2)	51.6 (9.2)	52.0 (9.3)
BMI kgm^{-2}, mean (sd)	24.5 (4.3)	24.4 (4.3)	24.4 (4.3)	24.4 (4.3)
Typical food portions[a] -small	1033 (27%)	1089 (26%)	2783 (73%)	3105 (74%)
medium	5122 (23%)	5814 (24%)	17109(77%)	18492(76%)
large	659 (22%)	778 (24%)	2397 (78%)	2523 (76%)
Vegetarian - Yes	1188 (21%)	1679 (28%)	4342 (79%)	4294 (72%)
No	5695 (24%)	6075 (23%)	18174(76%)	20076(77%)
Low fat diet - Yes	1829 (24%)	2324 (28%)	5932 (76%)	6113 (72%)
No/missing	5054 (23%)	5430 (23%)	16584(77%)	18257(77%)
Slimming diet -Yes	505 (21%)	724 (27%)	1924 (79%)	1930 (73%)
No/missing	6378 (24%)	7030 (24%)	20592(76%)	22440(76%)
Socio-economic status -1	2437 (21%)	2794 (22%)	9011 (79%)	9660 (78%)
2	2951 (24%)	3294 (25%)	9374 (76%)	10124(75%)
3	1354 (26%)	1508 (26%)	3798 (74%)	4207 (74%)
Educated to degree - Yes	1481 (19%)	1736 (21%)	6150 (81%)	6476 (79%)
No	5402 (25%)	6018 (25%)	16366(75%)	17894(75%)
Supplement user - Yes	2572 (23%)	2798 (23%)	8540 (77%)	9371 (77%)
No	4223 (23%)	4855 (25%)	13788(77%)	14781(75%)

[a]Self-categorised portions

High discrepancies in vegetable reporting were more likely for women who were vegetarian, or on a low fat or slimming diet ($p<0.001$), or eating small portions ($p=0.01$) or not supplement users ($p<0.001$), despite there being no differences in BMI on average between them and other women. There was no such clear pattern between diet and high discrepancy fruit reporting, though eating small portions ($p<0.001$) and not being on a slimming diet ($p=0.001$) was significantly associated with discrepancies. Non-graduates and women from lower SES were more likely to report high discrepancies for either fruit or vegetables ($p<0.001$).

5.3.4 Discussion

The majority of the dietary vitamin C estimated from FFQ recordings at baseline in the UKWCS came from fruit and vegetables (75%) and the remaining was from juice and potatoes. This compares with an estimate of 62% vitamin C from fruit and vegetables using 24-hour recall methods for 264 women from the UKWCS.[331] In the current analysis, Spearman's correlations and weighted kappa statistics confirmed that FFQ

derived grams of fruit and vegetables had strong agreement with total derived dietary vitamin C. However the corresponding non-weighted kappa statistics showed only moderate agreement; this may reflect the fact that some women may consume some types of fruit and vegetables that are more vitamin C rich than others. Furthermore, the accuracy of vitamin C derived from diet records is limited by the accuracy of food tables that are used to convert recordings into amounts of vitamin C consumed, in addition to limitations relating to the allocation of portion weight and recording of frequency of consumption. The derivation methods used for the UKWCS have been explained in section 4.5.1 and elsewhere.[288] A review found that the inclusion of portion-size questions produced lower correlation coefficients specifically for vitamin C, than if standard portions were used.[332] Although the UKWCS FFQ included a question about portion sizes, it was not used to derive nutrient values; a standard portion size was used.

The vitamin C intake estimates for vegetarians in this UKWCS baseline dataset were only 3% more than for non-vegetarians. Women in this cohort were generally more health conscious than the general population and may be likely to consume high intakes of fruit and vegetables, whether or not they are vegetarians. Additionally, they may have given socially desirable answers they believed were related to the aims of the research.[333]

Spearman's correlation method using relative rankings showed moderate agreement between the cross-check answers and other variables; however other methods such as the Bland-Altman and the Kappa statistics showed weaker agreement. In line with the initial analysis of 6,572 women at baseline in the UKWCS,[288] the current results confirmed there were considerable inconsistencies between the two variables representing fruit and vegetable servings. The initial analysis showed 15% of participants had differences of more than five times between fruit serving derived from the FFQ and those reported on the cross-check questions. With regard to vegetables, 26% of women reported a difference of more than five times. [288] These current results showed a similar percentage for vegetables (25%) and a much higher percentage for fruit (23%).

Reasons why most FFQ derived servings were much higher than cross-check responses could be because participants may have misunderstood how to complete the FFQ; they may have ticked too many items listed on the FFQ or over-stated their frequency of consumption. For instance, from checks of questionnaires of women with very high fruit and vegetable intake it was found that one woman had ticked every fruit and vegetable listed on the FFQ as being consumed once or 2-3 times per day, yet for the cross-check response she stated vegetable servings consumed were only 14 per

week, adding 2 per day as clarification. Research has shown that in comparisons of FFQs using 20, 33 or 40 fruit and vegetable items, those with more items lead to higher reporting of fruit and vegetables, ranging from a mean intake of 23.8 to 38.8 portions per week.[334] By comparison the UKWCS FFQ had 55 fruit and vegetable items in total and a mean total of 10.6 portions per day equating to 74 portions per week, which indicates an over-estimation of intake. Some discrepancies may have occurred due to counting vegetables twice; once in mixed dishes and once in the separate listings.[335] On the other hand, many women may have misread the cross-check questions as servings per day instead of per week. Indeed servings per day calculated from cross-check answers were over seven times lower than FFQ servings for more than 10% of the women. The fact that high discrepancy fruit and vegetable reporters had both a higher median FFQ serving per day compared to other women but lower median servings per day reported on the cross-check questions compared to others, supports all explanations given above. Nevertheless the histograms in Figure 19 clearly show that a large proportion of women reported intake in multiples of seven, indicating that they correctly reported intake per week rather than per day. However, since the cross-check answers for many of these women, including the vegetarians amongst them, were on average much lower than the national average intake of fruit and vegetables at the time, [336] this indicates that many of them may have misinterpreted the cross-check question as servings per day rather than servings per week. On the other hand, the median FFQ intake of high discrepancy reporters was well above the national average calculated from a sample of 6000 households in 1998 (4.25 portions based on a total average of 340g of fruit and vegetable intake per day which included fruit juice and excluded potatoes).[336] However, this difference could be due to the women in the UKWCS being highly health conscious rather than it being an over-estimation.

Possible errors in fruit and vegetables and vitamin C intake derived from FFQs can be attenuated by applying a correcting factor for each subject using answers to summary cross-check questions as demonstrated in previous studies.[288] [325-327] For the 6,572 women in the initial UKWCS analysis, each person's intake was adjusted by dividing the response to the cross-check question on the UKWCS questionnaire by the sum of frequencies from the FFQ, for each food group, as shown below:

$$\frac{\text{Number of servings/week from cross-check question}}{\text{Number of servings/week from individual food items on the FFQ}}$$

After applying the weightings the mean vitamin C intake reduced by 44% from 151 to 85 mg/d.[288] One study found that the correction of FFQ derived vitamin C improved its Pearson's correlation coefficients with 4-d diet records.[326] Another study found that

rankings remained relatively unchanged after corrections which reduced vitamin C intake by 20%.[325]

The above weightings could be used to adjust FFQ derived vitamin C used in the analyses of associations between breast cancer risk and vitamin C intake in the UKWCS. However, it was decided not to apply this weighting, since it was unclear whether the cross-check answers reflected true intake or at least true ranking of fruit and vegetable servings any better than the FFQ in the UKWCS. Similar concerns were expressed by Amanatidis et al. (2001) who also warned against comparing corrected or uncorrected FFQ figures with national target intakes.[325]

A review of studies found that FFQ recordings of vegetable intake had weak correlations (r=0.39) with other assessment methods, whereas fruit produced somewhat higher correlations (r=0.49).[337] Since then the Norfolk-EPIC study reported their FFQ recorded fruit and vegetable intake was about double that recorded by 7-day diaries.[338] Given that the UKWCS FFQ is based on the Norfolk-EPIC FFQ but has more fruit and vegetable items, its average intake may be more than double that recorded by diaries. Comparisons with biomarkers, or weighed food records, are considered appropriate methods of evaluating dietary assessment methods, but neither was employed at baseline in the UKWCS, and therefore could not be used to evaluate fruit and vegetable intake or vitamin C intake derived from the UKWCS FFQ. However, in the next section vitamin C derived from a similar FFQ is compared with biomarkers and food diary records at phase two on a small sample of women who provided blood samples.

5.4 Comparison of vitamin C intake recorded by FFQ and by diary with biomarkers in a small sample of the UKWCS at phase 2

5.4.1 Introduction

Biomarkers are used to assess the nutrient status of individuals, and have also been used to investigate associations between nutrient status and disease risk. In relation to plasma vitamin C and breast cancer risk, no evidence of an association was fund in a nested case-control study,[268] and conflicting evidence has been produced in retrospective case-control studies.[339-341]

Biomarkers have also been used as objective measures to assess the validity of dietary measurement tools, although their usefulness in this respect is questionable for some nutrients; since the absorption and storage of vitamin C is limited, particularly above 400mg/d, [65] vitamin C plasma levels are unlikely to reflect dietary vitamin C intake well. Previously 4-d diary and 24hr records of estimated vitamin C intake have been compared with biomarkers in a small sample of women from the UKWCS.[331] Results of the regression analysis showed that plasma ascorbic acid was a good indicator of previous vitamin C intake from all foods sources but not a good proxy for specific food groups such as fruit and vegetables recorded by these dietary methods. Conversely, an EPIC-Norfolk study found plasma vitamin C correlated weakly but significantly with fruit and vegetables recorded by FFQ.[342] In a systematic review of dietary assessment methods, vitamin C intake estimated from FFQs correlated weakly with biomarkers, the mean correlation coefficient being only 0.28.[343] However, in an earlier review, Cade et al. (2004) found correlations with biomarkers in 15 studies were somewhat higher at r=0.46.[337] The large difference between reviews may be due to the small number of studies involved, the assay methods used to measure biomarkers, or the quality weighting that studies were assigned in the later review.

Diaries have been the reference method most often used to validate FFQs; these have shown moderate correlations for vitamin C of r=0.50 in two reviews,[337 343] and slightly higher in a third review (r=0.59).[332]

This analysis will compare three methods of measuring vitamin C intake in a small sub-sample of women from the UWKCS, measured in close proximity and derived from: the 217 item FFQ; the 4-d diary; and blood samples. The resulting correlation coefficients and regression analysis will be compared with previous studies.

5.4.2 Methods

5.4.2.1 Subjects

Phase 2 UKWCS participants who lived within a 30 mile radius, or one hour drive of Leeds, were contacted between 3 and 5 years after baseline and a nurse arranged to take blood samples from 273 of these women.[280] The women also completed a 4-d diary and a FFQ which were returned to the nurse when the blood samples were collected. Dietary vitamin C intake was derived from the 4-d diaries and also from the FFQ, and vitamin C supplement dose was also recorded in the FFQs.

5.4.2.2 Dietary assessment methods

Blood samples were collected into lithium heparin (8ml) after an overnight fast. They were kept cool and within 2 hours of collection were separated and prepared for storage at -70^{o}C.[280] Plasma ascorbic acid and total vitamin C (ascorbic acid and dehydroascorbic acid) was analysed by high-performance liquid chromatography,[344] in the Division of Pathological Sciences, Department of Clinical Medicine at the University of Leeds. The food listings FFQs used for this dataset were almost identical to the FFQ used at baseline. The diaries used were the ones used in the full phase 2 dataset.

5.4.2.3 Statistical analyses

Characteristics of women grouped into fifths according to self-recorded vitamin C intake were produced. The strength of the associations between plasma ascorbic acid or total vitamin C (which includes dehydroascorbic acid as well as ascorbic acid) and vitamin C intake derived from FFQs and diary records at phase 2 was calculated using Spearman's pairwise correlation coefficients, since the data was not normally distributed. The analysis was repeated for non-vitamin C supplement users; i.e. those women who did not record either frequency or dose of vitamin C supplements taken. Spearman's correlation between vitamin C intake derived from phase 2 FFQs and diary records was also calculated. Spearman's method was used as opposed to Pearson's method for parametric data because the vitamin C intake derived from phase 2 FFQs and diary records in this dataset was not normally distributed. In the pairwise correlation coefficient calculations, pairs are excluded if one of the two variables is missing. Due to vitamin C's role in the regeneration of vitamin E and in the absorption of iron and in the Fenton reaction,[76] correlations between plasma ascorbic acid or total vitamin C and plasma iron and plasma vitamin E concentration were also assessed.

Regression analysis was used to quantify the percentage increase in plasma ascorbic acid and plasma total vitamin C when vitamin C consumed from all foods were doubled, as derived from diary or from FFQ recordings. For regression estimates to be

valid, it is assumed the residuals (.i.e. the difference between actual values and the estimated line of best fit) are normally distributed and have uniform variance. These assumptions were examined using histograms of residues, and q-q plots of residues against the predictor variable. Since these did not follow normal distribution patterns, it was decided to log-transform the vitamin C variables for the regression analysis. The analysis of all women was adjusted for vitamin C supplement use and energy intake, and the sub-analysis by vitamin C supplement taking was adjusted for energy intake.

Although recommended,[337] Bland-Altman plots were not used to assess correlations between plasma concentrations and FFQs and diaries because of the different units used between assessment methods (Nevertheless, standardized units, such as standard deviations, could have been compared).

5.4.3 Results

The mean vitamin C derived from diary recordings was 128 mg compared to 191 mg from FFQs. When women were split into fifths according to dietary vitamin C intake recorded by diary and by FFQ, their mean plasma ascorbic acid and plasma total vitamin C recordings increased with increasing fifths (Table 23 & Table 24). On average women were older and more likely to self-report being a vegetarian in each fifth of increasing vitamin C intake. There were no clear patterns for BMI or vitamin C supplement use.

As observed in Table 25, the estimates of dietary vitamin C intake from diaries and from FFQs correlated weakly with plasma ascorbic acid and plasma total vitamin C with diaries showing stronger correlations (r=0.35 and 0.34 vs. 0.26). When vitamin supplement dose recorded in the FFQ was added to the dietary FFQ value then correlations with plasma total vitamin C were 0.36 and 0.35. Correlations of plasma concentrations were even weaker with fruit and vegetable portions recorded by questionnaire, being higher for fruit than vegetables (0.20 and 0.21 vs. 0.08 and 0.13). Correlations between plasma ascorbic acid or total vitamin C and plasma iron and plasma vitamin E were also poor (0.09-0.18). In general the correlations were higher when 115 women (42%) were excluded who recorded vitamin C supplement use in either the frequency or dose section of the FFQ (Table 26). Dietary vitamin C recorded by diary for all women had a substantial correlation with that recorded by FFQ (r=0.49, Table 25). For 21 women (12%) taking supplement doses of 500mg or more vitamin C, substantial correlations were also found between supplement vitamin C intake and plasma ascorbic acid or plasma total vitamin C, being 0.60 and 0.48 respectively.

Table 23 Characteristics of women by dietary vitamin C intake recorded by FFQ

	Dietary vitamin C intake recorded by FFQ split by fifths					
	lowest	2nd fifth	3rd fifth	4th fifth	highest	Total
Dietary vitamin C, mean mg (sd)	85.6 (20.7)	135.2 (11.2)	175.2 (10.8)	214.7 (14.2)	340.8 (104)	190.8 (99.5)
Plasma ascorbic acid, µg ml^{-1}(sd)	10.5 (5.4)	11.5 (3.5)	12.6(3.8)	11.8 (3.3)	14.0 (4.5)	12.1 (4.3)
Plasma vitamin C, µg ml^{-1} (sd)	12.1 (5.0)	13.4 (3.2)	14.1 (4.1)	13.7 (3.1)	15.6 (4.6)	13.8 (4.2)
Age, mean (sd)	49.8 (8.5)	48.8 (8.0)	49.9 (9.2)	50.3 (8.4)	53.8 (9.2)	50.5 (8.8)
BMI, mean (sd)	25.7 (5.6)	23.2 (3.1)	24.2 (4.0)	24.5 (3.9)	24.9 (4.3)	24.5 (4.3)
Vit C supplement use, n (%)	22(42)	22(42)	20 (37)	28 (53)	22 (41)	114 (43)
Vegetarian self-reported, n (%)	17 (34)	18 (35)	17 (33)	20 (38)	22 (42)	94 (37)

Table 24 Characteristics of women by dietary vitamin C intake recorded by diary

	Dietary vitamin C intake recorded by Diary split by fifths					
	lowest	2nd fifth	3rd fifth	4th fifth	highest	Total
Dietary vitamin C, mean mg (sd)	42.5 (13.6)	75.6 (9.2)	106.2 (10.2)	146.1 (13.4)	266.8 (122)	127.9 (95.7)
Plasma ascorbic acid µg ml^{-1} (sd)	10.3 (5.5)	11.4 (3.9)	11.7 (2.8)	13.0 (3.8)	13.9 (4.2)	12.1 (4.3)
Plasma vitamin C µg ml^{-1} (sd)	11.8 (5.0)	13.3 (3.4)	13.2 (2.9)	15.1 (3.4)	15.6 (4.1)	13.8 (4.1)
Age, mean (sd)	48.7 (8.0)	49.2 (8.5)	50.4 (8.4)	51.0 (8.2)	53.6 (10.0)	50.6 (8.7)
BMI, mean (sd)	25.1 (4.5)	24.2 (4.4)	24.8 (4.8)	24.2 (4.6)	24.3 (3.4)	24.5 (4.4)
Vit C supplement use, n (%)	27 (52)	19 (37)	20 (38)	23 (44)	25 (47)	114 (44)
Vegetarian self-reported, n (%)	13 (26)	18 (38)	15 (29)	23 (44)	23 (44)	92 (37)

Table 25 Spearman's correlation coefficients between different measures of vitamin C for all UKWCS women who provided blood samples

	Plasma		Diary	FFQ		Questionnaire		
	Ascorbic acid	Total vit C	Dietary vit C	Dietary vit C	Total vit C	Fruit portions	Veg portions	Total portions
Plasma ascorbic acid	1							
Plasma total vitamin C	**0.76**	1						
Diary dietary vitamin C	**0.35**	**0.34**	1					
FFQ dietary vitamin C	**0.26**	**0.26**	**0.49**	1				
FFQ total vitamin C	**0.36**	**0.35**	**0.33**	**0.79**	1			
Questionnaire fruit portions	**0.20**	**0.21**	**0.39**	**0.43**	**0.31**	1		
Questionnaire veg portions	0.08	**0.13**	**0.23**	**0.27**	**0.24**	**0.47**	1	
Total fruit & veg portions	**0.17**	**0.19**	**0.34**	**0.43**	**0.36**	**0.86**	**0.82**	1
Plasma iron	**0.15**	**0.18**	0.09	0.05	0.11	-0.01	0.02	0.05
Plasma vitamin E	**0.16**	0.09	-0.01	**0.14**	0.25	-0.06	0.05	0.06

Numbers in bold are statistically significant $p<0.05$
(N varies from 259 to 272)

Table 26 Spearman's correlation coefficients between different measures of vitamin C for non-vitamin C supplement users[a] in the sub-sample of UKWCS women who provided blood

	Plasma		Diary	FFQ	Questionnaire		
	Ascorbic acid	Total vit C	Dietary vit C	Dietary vit C	Fruit portions	Veg portions	Total portions
Plasma ascorbic acid	1						
Plasma total vitamin C	**0.73**	1					
Diary dietary vitamin C	**0.46**	**0.49**	1				
FFQ dietary vitamin C	**0.36**	**0.35**	**0.51**	1			
Questionnaire fruit portions	**0.33**	**0.31**	**0.41**	**0.46**	1		
Questionnaire veg portions	**0.15**	**0.16**	**0.27**	**0.31**	**0.55**	1	
Total fruit & veg portions	**0.25**	**0.22**	**0.32**	**0.46**	**0.88**	**0.86**	1
Plasma iron	0.13	**0.24**	0.04	0.06	-0.03	0.00	0.05
Plasma vitamin E	0.10	**0.01**	-0.05	**0.18**	0.09	-0.03	0.10

Numbers in bold are significant

[a]Not indicated as vitamin C supplement users on the questionnaire

(N varies from 148 to 157)

The regression analyses showed that plasma ascorbic acid concentrations increased significantly by 19% when total vitamin C from all food sources recorded by diary was doubled (Table 27). When vitamin C supplement users were excluded this rose to 23%, but for the sub-analysis for non-vitamin C users the increase was only 15%. Similar results were found for recordings by FFQ. Increases in total plasma vitamin C concentrations were not as large; an increase of 14% occurred when total vitamin C from all food sources recorded by diary were doubled. When vitamin C supplement users were excluded this rose to 19%. Again, similar results were found for recordings by FFQ. The R_2 values ranged from 0.07 for vitamin C supplement users to 0.26 for women who did not take vitamin C supplements, indicating that plasma concentrations were not explained well by FFQ and diary recordings, particularly for supplement users.

Table 27 Percentage increase in plasma concentrations of ascorbic acid and total vitamin associated with a doubling of vitamin C from all foods recorded by diary and by FFQ

	Plasma ascorbic acid % increase (95%CI)	Plasma vitamin C % increase (95%CI)
Total women (N=262)[a]		
Diary dietary vitamin C intake	18.9 (13.1, 25.0)	13.6 (9.3, 18.0)
FFQ dietary vitamin C intake	19.9 (12.0, 28.3)	14.4 (8.6, 20.4)
Excluding vitamin C supplement users (N=148)[b]		
Diary dietary vitamin C intake	23.0 (15.3, 31.3)	18.5 (12.8 24.4)
FFQ dietary vitamin C intake	26.9 (16.4, 38.4)	18.9 (11.2, 27.3)
Vitamin C supplement users (N=114) [b]		
Diary dietary vitamin C intake	14.5 (5.7, 24.0)	8.8 (2.3, 15.6)
FFQ dietary vitamin C intake	12.5 (1.1, 25.2)	9.1 (0.8, 18.2)

[a]Adjusted for energy intake and supplement taking
[b]Adjusted for energy intake

5.4.4 Discussion

The FFQ vitamin C correlations with plasma ascorbic acid and also with diary derived dietary vitamin C for the UKWCS were weak (ranging from 0.26 to 0.51) but in line with the mean coefficients between these measurement methods calculated in two reviews.[337 343] However, the UKWCS results are different from many of the energy adjusted correlations between plasma ascorbic acid and vitamin C intake for women derived from EPIC FFQs from a variety of European countries; these correlations ranged from 0.04 in Greece, to 0.29 in Italy, to 0.65 in Spain.[345] As observed in a previous EPIC-Norfolk study,[346] UKWCS diary derived vitamin C intakes correlated better with plasma ascorbic acid than FFQ data when they were recorded in close

temporal proximity. Both plasma concentrations and diaries reflect relatively short-term intake compared to FFQs which usually assess the previous 12 months' intake. However, in the previous study, correlations with biomarker levels re-measured several years later were found to be similar for diaries and FFQs. [346 347] In the current study, although significant, correlations between plasma ascorbic acid and total fruit and vegetables portions were even weaker than those with FFQ derived vitamin C intake; reflecting the fact that fruit and vegetables, excluding juice and potatoes, make up only about two thirds of total vitamin C consumptions, as observed in section 5.3.3. Similarly, the weak correlations between ascorbic acid and plasma iron and plasma vitamin E were expected to be greater due to ascorbic acids' role in the regeneration of vitamin E, in the absorption of iron and in the Fenton reaction.[76]

The most recent review found no difference in correlations for vitamin C between FFQs and diaries which recorded seven or more days' intake and those recording less than seven days.[343] However another review observed lower correlations for diaries recording less than seven days and these were more in line with the 4-d UKWCS diary correlations than the longer diaries (0.55 vs. 0.63).[332] Additionally, the most recent review found no large differences in correlations between studies that included vitamin supplements and those that did not;[343] however, this result has been questioned due to inconsistencies found in classifying studies as those including supplements.[348] Contrary to this, correlations for the UKWCS were substantially stronger when women taking supplements were excluded. Indeed dietary only intake is likely to correlate more strongly with plasma levels when the latter does not contain vitamin C from supplements. Surprisingly though, correlations in the UKWCS were the strongest between supplement vitamin C intake and plasma ascorbic acid for women taking high doses (>=500mg/d); such doses are unlikely to be absorbed or stored by the body and plasma levels are likely to fall within hours due to excretion. This may be a spurious result due to low numbers analysed. In contrast, the previous UKWCS analysis reported that plasma levels of ascorbic acid reached a plateau with dietary intakes around 60mg/d,[331] which is much lower than the average dietary vitamin C intake of the UKWCS, and lower than the 400mg/d plateau reported by Levine et al. (2001).[65]

In one review correlation coefficients between FFQs and references measures were higher in general if participants were allowed to describe their own portion sizes (r=0.54).[337] However another review found that portion-size questions produced lower correlation coefficients specifically for vitamin C (0.60 vs. 0.68).[332] Although a general question about portion sizes (small, medium or large) was asked in the UKWCS, it was not used to calculate the weight of fruit and vegetables consumed and derived vitamin C intake. The review also observed lower vitamin C correlations for FFQs developed

from the EPIC FFQ (as was the case for the UKWCS FFQ) than ones developed from the Willet or Block FFQs (0.53 vs. 0.67 and 0.63).[326 332 345 349] The EPIC based FFQ on average contained substantially more food items than the other two FFQs (154 vs. 113 and 100), but in general FFQs containing 200 items (as was the case for the UKWCS FFQ) correlated slightly better for vitamin C with non-biomarker reference measures than those containing 150 or 100 (r=0.64 vs. 0.60 vs. 0.56).[332]

The results of the regression analysis, which measures the extent of agreement between methods, were inline with those from the earlier study of the UKWCS after excluding women who were vitamin C supplement users.[331] For vitamin C supplement users in the current study, the increase in plasma ascorbic acid from a doubling of dietary intake recorded by diary or FFQ was about half that of the non-users, since their plasma levels were likely to be generally higher due to intake from supplements.

As mentioned in section 2.2.2, there are a number of physiological processes that determine plasma ascorbic acid concentrations meaning they do not simply reflect intake. Plasma ascorbic levels reflect the effects of digestion, absorption, uptake, utilisation, metabolism, excretion, homeostatic mechanisms, intake of other nutrients as well as the influence of health-related behaviours and the presence of chronic illness. Therefore, plasma records naturally will not correlate well with FFQ, diary or 24-hr recordings of intake. Since the human body has limited capacity to absorb and store water-soluble vitamin C,[65] excess is excreted within hours of consumption. Given this, plasma vitamin C biomarkers are likely to reflect short-term intake. In general, FFQs, including the UKWCS FFQ, estimate the previous 12 months' intake; therefore they are unlikely to correlate well with short-term measures of vitamin C intake. Furthermore, repeatability, an aspect of validity, is poor for biomarkers of vitamin C as reported in an earlier UKWCS assessment where second samples were taken after 18 months.[331]

Derived values of vitamin C consumed may be inaccurate, however the relative ranking of individuals, important in the risk analyses and observed from the correlations, were acceptable compared to previous studies, although weak. None of the measurement methods of recording vitamin C intake in the UKWCS are ideal and all have strengths and weaknesses; biomarker and diary recordings tend to reflect short-term intake and although FFQs cover longer-term intake and are easier to administer at lower costs, they are limited by their retrospective nature and by problems reporting portion sizes and fruit and vegetable consumption. Nevertheless, the general difficulties in accurately measuring vitamin C consumed and correctly ranking the cohort individuals by intake, needs to be taken into account when interpreting the risk analysis results in the following chapters. Measurement error of intake can lead to substantial reduction of calculated disease-exposure relative risks.[350]

5.5 Conclusion from evaluations

The comparisons between FFQs, diary and plasma concentrations in all three sections in this chapter showed some weaknesses in methods used in the UKWCS for recording food and supplements containing vitamin C. However, on the whole the results were in line with those from other studies and were considered suitable for further analyses. Nevertheless, the weaknesses found should be taken into account when interpreting results of associations between vitamin C intake and breast cancer risks in the UKWCS.

In the following chapters, breast cancer risk will be assessed in relation to

- Any supplement use (chapter 7)
- Vitamin C contained in supplements at phase 2 of the UKWCS (chapter 8)
- Dietary vitamin C intake from FFQs (split by supplement users and non-users) at baseline in the UKWCS (chapter 9)
- Total vitamin C intake (from diet and supplements) from dairy records in the UK Dietary Cohort Consortium (chapter 10)

First, in the next chapter, the characteristics of women which predict vitamin C supplement use at phase 2 in the UKWCS will be determine and also whether women who have a history of breast cancer are more likely to use high dose vitamin C supplements.

CHAPTER 6

6 Is vitamin C supplement use associated with a personal or family history of cancer and what characteristics predict vitamin C supplement use in UK women? A phase 2 analysis of the UKWCS

6.1 Summary

Objectives: To determine whether frequent vitamin C supplement use is associated with a personal or family history of cancer and to determine what characteristics predict vitamin C supplement use in UK women.[1]

Methods: This cross-sectional analysis of 12,453 women aged between 37-79 years from phase 2 of the UKWCS examines the odds of taking supplements containing vitamin C as recorded in 4-day food diaries, based on lifestyle characteristics and cancer history self-reported by questionnaire.

Results: Women frequently taking supplements containing vitamin C, compared to those who did not, had healthier behaviours, including higher consumption of fruit and vegetables. Frequent high dose vitamin C takers (>=1000mg) had a higher socioeconomic status, visited alternative practitioners more often than family or private doctors, and were more likely to be ex-smokers, and to drink little or no alcohol than women who did not take high doses. Women who self-reported having had cancer (OR=1.33; 95% CI: 1.00, 1.76) or specifically breast cancer (OR=1.70; 95% CI: 1.14, 2.55), or reported a family history of cancer (OR=1.16 (95% CI: 0.95, 1.41)) or breast cancer (OR=1.26; 95% CI: 1.01, 1.58) had increased odds of being frequent high dose users after adjusting for socio-demographic and health behaviours.

Conclusion: High dose vitamin C use by UK women was associated with healthier behaviours and a history of breast cancer.

6.2 Introduction

Despite lack of evidence of benefits,[7 351] vitamin supplement use reported by UK women increased from 17% in 1986/1987 to 41% reported in 2008/9.[24 25] A recent UK survey showed users are most likely to be women above 55 years and of higher socioeconomic status.[26] An analysis of the UK Women's Cohort Study (UKWCS) found that users were significantly more likely to lead healthier lifestyles: to be more physically active; have a lower alcohol intake; a lower body mass index (BMI) and eat diets which met recommended dietary intakes.[27] Therefore they were less likely to need supplements than non-users.[27] Further support for this 'inverse supplement hypothesis' has been found in the UK,[25 28 29] and elsewhere.[30-35]

Moreover, those classifying themselves as high strength supplement users in the 2008 UK Random Location Omnibus Survey were particularly health conscious.[26] They were more likely to consider the health implications of what they ate, to actively look for information about how to stay healthy and to believe that they needed vitamin and mineral supplements to feel and stay healthy.[26] Thirty eight percent of the 801 people surveyed reported taking high strength supplements,[26] and only 6% of these were likely to report they were in excellent health compared to 25% of non-users.[26]

Vitamin C is one of the most commonly used supplements in the UK,[26 352] and is often taken at high doses, however little is known about the characteristics of women who frequently take them. Although many users may take vitamin C supplements to boost their immune systems, suggestions that vitamin C is able to reduce the incidence of colds, have been unsubstantiated in randomised controlled trials.[45] It is unknown whether users take vitamin C supplements for other health reasons, such as reducing cancer risk or cancer reoccurrence. Despite some clear evidence of an association between plasma vitamin C levels and reduced mortality from all causes in men and women, and also reduced cancer mortality in men,[67] there is limited evidence to suggest that supplementation with vitamin C is associated with reduced risk of mortality or cancer.[7 351] The Vitamins and Lifestyle (VITAL) study in the US found no overall association between vitamin C supplement-taking (>=150mg) and prevalent cancer in a cross-sectional analysis,[353] despite general supplement use being widespread in cancer survivors in the US, particularly in breast cancer survivors.[354] However, a US study of women physicians showed those with pre-existing breast cancer were more likely to take vitamin C supplements than women who were free from breast cancer.[31]

An EPIC study reported that UK health-conscious women were more likely to be supplement users if they had prevalent cancer than if they were cancer free.[352] However, no study has examined the relationship between vitamin C supplement use in

UK women and a personal or family history of cancer. The main aim of this analysis was to determine whether women in the UKWCS who reported having had cancer, or reported a relative who had cancer, were more likely to use vitamin C supplements than those who did not report these histories. Breast cancer was the principle focus of the analyses. A further aim, which has not been examined in UK women, was to determine which characteristics and health behaviours predicted vitamin C supplement use, particularly at high doses of 1000mg/d or more.

6.3 Methods

UKWCS baseline data was gathered between1995-1998 from 35,367 women who completed a 217-item Food Frequency Questionnaire (FFQ).[279] Further details are found in section 4.1.1.1. At baseline 62% of participants took some type of dietary supplement.

All the initial participants were re-contacted at phase 2 between 1999 and 2004, on average 4 years after recruitment; 12,453 (35%) completed a follow-up health and lifestyle questionnaire and a 4-day food diary. For each day, the diaries requested supplement brand, name, amount taken and dosage of any supplement taken. This information was matched against a database of supplement descriptions and ingredient composition as explained in section 4.5.3.1 of the methods chapter. The average daily vitamin C intake contained in all supplement types was calculated for the total number of diary days vitamin C was taken.

Univariate logistic regression was applied to the phase 2 data to determine which participant characteristics predicted frequent supplement use in two different classifications of users: those taking any dose of vitamin C (yes/no); and those taking high doses of vitamin C (>=1000mg, yes/no). These were compared to women not frequently taking ‘any’ or ‘high’ doses respectively. Intakes above 1000mg/d have been linked to adverse effects, particularly gastrointestinal disturbance.[52 355] This level is more than 15 times the recommended daily allowance (EU RDA = 60mg/day[53]) normally found in multivitamins. Frequent use of supplements containing vitamin C in this study was defined as taking on at least three out of the four diary days. As described in section 5.2, this agreed well with daily use of vitamin C and multivitamin supplements reported on the phase 2 questionnaire, which was completed concurrently with the diary. Socio-demographic and health related lifestyle variables that were significantly associated with either any dose or high intake were all included in a logistic regression model for mutual adjustment. Variables that remain significant should be predicative of vitamin C use over and above the effects of the other variables in these multivariable models.

Social class and marital status variables were derived from answers to the questionnaire used at baseline since this information was not provided at phase 2. All other variables were taken from responses to the phase 2 questionnaire: BMI (kg/m^2); smoking status; level of physical activity; parity; drinking alcohol less than once a week, red meat servings; total fruit and vegetable servings; frequency of visits to doctors and alternative practitioners. Vigorous activity was defined as activity causing shortness of breath, rapid heart rate and sweating. Attendance at routine health checks was not significantly associated with vitamin C supplement use, and therefore was excluded from the models.

These variables, excluding visits to doctors and alternative practitioners, were used in logistic regression analyses to adjust the odds of women with a family or personal history of cancers taking any or high doses (>=1000mg) of vitamin C. Additional analyses were performed at doses above or equal to 250mg; 500mg; and 2000mg. Given that vitamin C supplements are more likely to be taken in winter because well-publicised research had linked it to reduced duration of the seasonal common cold,[45] sensitivity analyses were performed to assess the robustness of results to weighting the analyses by the inverse of the probability of being sampled in each season.

All information relating to family or personal history of cancers was reported by the participant at phase 2. They were asked whether or not family members (blood relatives only) ever had the following cancers: breast, skin, lung, colon and rectum, ovary, stomach, cervix, ovary, pancreas, or prostate. The cancer history of first and second degree relatives was used to identify women potentially at raised or high risk of hereditary breast cancer (further information on determining this is given in section 4.7.6.3.2 of the methods chapter). It was unknown whether affected relatives were on the same side of the family, therefore this could only approximate to the guidelines provided by the UK's National Institute for Health and Clinical Excellence (NICE).[319] Participants were also asked to report their own history of disease, including whether they had previously been told they had a diagnosis of one of the cancers listed above.

6.4 Results

Thirty-four percent (4242) of women frequently took supplements containing any dose of vitamin C, on at least three of the four diary days, and 5% (579) frequently took high doses of 1000mg or more. Twenty-seven percent (1165) of those frequently taking any dose and 52% (299) taking high doses of vitamin C took four or more types of supplements. Furthermore, 82% of users taking any dose and 86% of the high dose users took some type of supplement at baseline, on average four years earlier.

After mutual adjustment, significant lifestyle predictors of frequently taking supplements containing either high dose or any dose of vitamin C were eating more than five servings of fruit and vegetables per day; eating less servings of red meat; and visiting an alternative practitioner more often than women not frequently taking these supplements (Table 28). Odds of visiting an alternative practitioner four or more times in the last 12 months were substantially greater for high dose takers compared to any dose takers (OR=2.84; 95% CI: 2.20, 3.66 vs. OR=1.75; 95% CI: 1.51, 2.03). Additionally, the odds of taking supplements containing any dose of vitamin C were significantly higher in women who exercised vigorously more than three times a week (OR=1.52; 95% CI: 1.23, 1.8); or were aged 45 years or more; of intermediate social class; divorced; childless; frequent visitors to their GP; or leaner. Significant predictors of frequent high dose taking were being an ex-smoker when compared with never smokers (OR=1.25; 95% CI: 1.02, 1.53), drinking alcohol less than once a week (OR=1.37; 95% CI: 1.12, 1.67) and being of high socio-economic status compared to low status (OR=1.45; 95% CI: 1.06, 2.00).

Table 28 Characteristics associated with taking supplements containing any dose of vitamin C and taking supplements containing high doses of vitamin C (1000mg or above)

Characteristics	Any dose OR (95% CI)[a]	P value	>=1000mg OR (95% CI)[a]	p value
Age (years)[b]				
<45	1	0.07	1	0.3
45-54	1.20 (1.03, 1.41)		1.11 (0.81, 1.54)	
55-64	1.26 (1.07, 1.48)		0.85 (0.60, 1.20)	
65 and above	1.23 (1.03, 1.47)		0.91 (0.62, 1.34)	
Social class[c]				
High	1	0.1	1	0.04
Intermediate	1.10 (1.01, 1.21)		0.96 (0.78, 1.17)	
Low	1.07 (0.94, 1.22)		0.69 (0.50, 0.94)	
Marital status[c]				
Married/living together	1	0.4	1	0.9
Divorced/separated	1.31 (1.14, 1.51)		1.25 (0.94, 1.66)	
Widowed	0.95 (0.78, 1.16)		1.14 (0.72, 1.80)	
Single	0.86 (0.72, 1.03)		0.88 (0.61, 1.28)	
Had children				
Yes	1	0.001	1	0.09
No	1.24 (1.11, 1.39)		1.23 (0.97, 1.56)	
Body mass index (BMI kg/m^2)[b]				
Underweight (<18)	1.03 (0.72, 1.46)		1.07 (0.53, 2.15)	
Normal (18-24.99)	1	0.08	1	0.6
Overweight (25-30)	0.90 (0.82, 0.99)		0.87 (0.69, 1.09)	
Obese (>30)	0.93 (0.80, 1.07)		1.11 (0.81, 1.54)	
Smoking status[c]				
Never smoked	1	0.4	1	0.02
Ex-smoker	1.07 (0.98, 1.17)		1.25 (1.02, 1.53)	
Current smoker	0.91 (0.75, 1.00)		1.19 (0.79, 1.81)	
Drinks alcohol more than once a week				
Yes	1	0.1	1	0.001
No	1.07 (0.98, 1.17)		1.37 (1.12, 1.67)	
Physical activity[c]				
No weekly activity	1	<0.001	1	0.008
Light moderate	1.16 (0.95, 1.41)		0.94 (0.60, 1.48)	
Vigorous 1-2/week	1.18 (0.96, 1.46)		0.98 (0.61, 1.57)	
Vigorous >=3/week	1.52 (1.23, 1.89)		1.36 (0.85, 2.19)	
Servings of red meat eaten per week[c]				
None	1	<0.001	1	<0.001
1-3	0.79 (0.72, 0.87)		0.68 (0.55, 0.85)	
4 or more	0.61 (0.54, 0.68)		0.48 (0.35, 0.65)	
Servings of fruit and veg eaten per day[c]				
<=2	1	<0.001	1	0.01
3-5	1.21 (1.08, 1.37)		1.11 (0.84, 1.48)	
More than 5	1.45 (1.26, 1.67)		1.40 (1.02, 1.92)	

Table continued: Characteristics associated with taking supplements containing any dose of vitamin C and taking supplements containing high doses of vitamin C (1000mg or above)

Characteristics	Any dose OR (95% CI)[a]	P value	>=1000mg OR (95% CI)[a]	p value
Number of visit to doctors in last 12mths[c]				
None	1	<0.001	1	0.9
1-4	1.27 (1.12, 1.42)		0.98 (0.76, 1.25)	
>4	1.45 (1.26, 1.67)		0.98 (0.72, 1.33)	
Number of visit to alternative practitioner in last 12mths[c]				
None	1	<0.001	1	<0.001
1-4	1.41 (1.23, 1.61)		1.77 (1.35, 2.31)	
>4	1.75 (1.51, 2.03)		2.84 (2.20, 3.66)	
Number of participants in the models	10161		10161	

[a]Mutually adjusted for the other variables listed above, OR =Odds ratio

[b]p for trend over the continuous variable

[c]p for trend over categories

Table 29 shows that, after adjustment, frequent high dose use of vitamin C remained significantly associated with a personal history of any cancer (OR=1.33; 95% CI: 1.00, 1.76) and any hormone related cancer (OR=1.68; 95% CI: 1.16, 2.43); specifically breast cancer (OR= 1.70; 95% CI: 1.14, 2.55). Additionally, frequent high dose use was significantly greater for women with a family history of breast cancer (OR=1.26; 95% CI: 1.01, 1.58) and appeared more likely, though non-significant, in women with a family history of any cancer (OR=1.16; 95% CI: 0.95, 1.41), any hormone related cancer (OR=1.19; 95% CI: 0.98, 1.46), and pancreatic cancer (OR=1.44; 95% CI: 0.94, 2.21). Taking any dose of vitamin C was significantly associated with a family history of cancer of the uterus (OR=1.38; 95% CI: 1.10, 1.74). These results were very similar when the analysis was weighted to take into account differential sampling in each season. For instance, the seasonally weighted odds of being a high dose user with a personal history of breast cancer was OR = 1.61 (95% CI: 1.07, 2.41), or with a family history of breast cancer was OR = 1.29 (95% CI: 1.03, 1.62) or with a family history of pancreatic cancer was OR = 1.53 (95% CI: 0.99, 2.36). However, the result for a personal history of total cancers was not significant (OR = 1.30; 95% CI: 0.97, 1.73) after seasonally weighting.

It may be observed in Table 30 that the odds of taking vitamin C increased with increasing doses above 500mg for women who had any family member with a history of breast cancer or who had a personal history of breast cancer. For the latter, ORs were 1.09 (95% CI: 0.78, 1.52) at >=500mg, 1.70 (95% CI: 1.14, 2.55) at >=1000mg and 2.36 (95% CI: 1.00, 5.56) at intakes of 2000mg or above. A similar pattern occurs for those with a personal history of cancer of the uterus or cervix, and was also seen in the total analyses of any cancer or any hormone related cancer. The small numbers of women in some of the categories, however, may have produced spurious results. Although the odds of having a mother or sister with breast cancer or potentially being at raised risk of this cancer increased at higher intakes, these were not statistically significant.

Table 29 Odds ratios of taking supplements containing vitamin C: any dose; or 1000mg or more for UKWCS women who self-reported a personal or a family history of cancer

		Any Dose: N=4242 (34%)		>=1000mg: N =579 (5%)	
Type of cancer	*N*[b]	Unadjusted OR(95% CI)	Adjusted[a] OR(95% CI)	Unadjusted OR(95% CI)	Adjusted[a] OR(95% CI)
Personal history of					
Any cancer	1268	1.14 (1.01, 1.29)	1.12 (0.97, 1.28)	1.31 (1.02, 1.68)	1.33 (1.00,1.76)
Any hormone	642	1.11 (0.94, 1.31)	1.08 (0.89, 1.31)	1.50 (1.09, 2.08)	1.68 (1.16, 2.43)
Breast	523	1.13 (0.94,1.36)	1.10 (0.89, 1.35)	1.53 (1.08, 2.18)	1.70 (1.14, 2.55)
Uterus	75	0.85 (0.52, 1.39)	0.77 (0.44, 1.34)	1.78 (0.77, 4.12)	1.97 (0.77, 5.02)
Ovarian	60	1.29 (0.77, 2.17)	1.28 (0.71, 2.33)	1.35 (0.60, 3.07)	0.84 (0.20, 3.51)
Any non-hormone cancer	584	1.16 (0.98, 1.40)	1.11 (0.91, 1.34)	1.16 (0.80, 1.70)	1.05 (0.68, 1.60)
Skin	324	1.14 (0.91, 1.43)	1.04 (0.81, 1.34)	0.85 (0.48, 1.49)	0.71 (0.36, 1.39)
Cervix	190	1.26 (0.94, 1.69)	1.20 (0.86, 1.66)	2.03 (1.22, 3.36)	1.70 (0.94, 3.05)
Colon Rectum	63	1.19 (0.71, 1.98)	1.30 (0.73, 2.30)	1.07 (0.34, 3.44)	0.98 (0.24, 4.10)
Family history of					
Any cancer	7,259	1.08 (1.00, 1.16)	1.04 (0.96, 1.13)	1.15 (0.97, 1.36)	1.16 (0.95, 1.41)
Any hormone cancer	3,629	1.09 (1.01, 1.18)	1.09 (0.99, 1.19)	1.16 (0.97, 1.38)	1.19 (0.98, 1.46)
Breast	2,370	1.06 (0.97, 1.17)	1.04 (0.94, 1.16)	1.23 (1.00, 1.51)	1.26 (1.01, 1.58)
Prostate	958	1.09 (0.95, 1.25)	1.13 (0.97, 1.32)	1.04 (0.76, 1.41)	1.09 (0.77, 1.51)
Ovarian	423	1.10 (0.90, 1.35)	1.12 (0.90, 1.41)	1.07 (0.69, 1.70)	1.09 (0.66, 1.79)
Uterus	380	1.41 (1.14, 1.73)	1.38 (1.10, 1.74)	1.08 (0.68, 1.73)	1.11 (0.66, 1.87)

Table continued: Odds ratios of taking supplements containing vitamin C: any dose; or 1000mg or more or for UKWCS women who self-reported a personal or a family history of cancer

		Any Dose: N=4242 (34%)		>=1000mg: N =579 (5%)	
Type of cancer	*N*[b]	Unadjusted OR(95% CI)	Adjusted[a] OR(95% CI)	Unadjusted OR(95% CI)	Adjusted[a] OR(95% CI)
Family history of					
Any non-hormone	5,227	1.07 (0.99, 1.16)	1.03 (0.95,1.12)	1.03 (0.87, 1.22)	1.04 (0.86, 1.25)
Lung	2,066	1.06 (0.96, 1.17)	1.00 (0.89, 1.11)	1.07 (0.86, 1.33)	1.00 (0.78, 1.29)
Colon/Rectum	1,608	0.96 (0.86, 1.08)	0.98 (0.86, 1.11)	0.97 (0.76, 1.25)	1.08 (0.82, 1.43)
Stomach	1,300	1.02 (0.90, 1.15)	1.01 (0.88, 1.16)	0.91 (0.69, 1.21)	0.97 (0.71, 1.33)
Skin	957	1.01 (0.88, 1.16)	0.97 (0.83, 1.13)	0.88 (0.64, 1.23)	0.86 (0.60, 1.24)
Pancreas	455	1.13 (0.93, 1.37)	1.11 (0.90, 1.38)	1.41 (0.96, 2.08)	1.44 (0.94, 2.21)
Cervix	311	1.03 (0.81, 1.31)	1.04 (0.79, 1.36)	0.68 (0.36, 1.28)	0.74 (0.38, 1.46)

[a]Adjusted for BMI, age, social class, marital status, children, smoking status, level of physical activity, low alcohol consumption, red meat servings, total fruit and vegetable servings.

[b]Total numbers with history of cancer

Table 30 Odds ratios of taking supplements containing vitamin C for a range of doses for UKWCS women who self-reported a personal history of cancer or a family history of breast cancer

	Frequent use of supplements containing vitamin C doses greater or equal to							
		250mg		500mg		1000mg		2000mg
	N[b]	OR(95% CI)[a] N=1,448 (12%)	*N*[b]	OR(95% CI)[a] N=1,195(10%)	*N*[b]	OR(95% CI)[a] N=579 (5%)	*N*[b]	OR(95% CI)[a] N==92(1%)
Personal history								
Any cancer	159	1.06 (0.87, 1.30)	131	1.03 (0.83, 1.29)	74	1.33 (1.00,1.76)	19	2.86 (1.64, 4.98)
Any hormone cancer	81	1.04 (0.79, 1.39)	69	1.08 (0.80, 1.46)	43	1.68 (1.16, 2.43)	12	3.50 (1.75, 7.01)
Breast cancer	68	1.10 (0.81, 1.49)	56	1.09 (0.78, 1.52)	36	1.70 (1.14, 2.55)	8	2.36 (1.00, 5.56)
Uterus	8	0.99 (0.45, 2.22)	8	1.25 (0.56, 2.78)	6	1.97 (0.77, 5.02)	3	8.64 (2.52, 29.6)
Ovarian	7	0.50 (0.15, 1.62)	7	0.64 (0.20, 2.06)	3	0.84 (0.20, 3.51)	1	2.75 (0.37, 20.8)
Any non-hormone cancer	69	0.98 (0.73, 1.30)	56	0.97 (0.71, 1.33)	31	1.05 (0.68, 1.60)	8	2.52 (1.19, 5.32)
Skin	34	0.79 (0.53, 1.20)	26	0.74 (0.47, 1.19)	13	0.71 (0.36, 1.39)	2	1.08 (0.26, 4.49)
Cervix	32	1.43 (0.93, 2.21)	29	1.60 (1.03, 2.52)	17	1.70 (0.94, 3.05)	4	3.14 (1.10, 8.94)
Colon Rectum	5	0.69 (0.24, 1.94)	3	0.41 (0.10, 1.72)	3	0.98 (0.24, 4.10)	2	7.20 (1.62, 32.1)
Family history of breast cancer								
Any family member	299	1.15 (0.99, 1.34)	244	1.10 (0.93, 1.30)	129	1.26 (1.01, 1.58)	27	1.69 (1.01, 2.83)
Mother or sister	163	1.13 (0.93, 1.37)	129	1.04 (0.84, 1.29)	67	1.16 (0.87, 1.55)	15	1.55 (0.81, 2.96)
Respondent at raised risk	32	1.11 (0.73, 1.68)	25	1.04 (0.66, 1.65)	15	1.31 (0.73, 2.32)	4	2.03 (0.62, 6.56)
Respondent at high risk[c]	9	0.67 (0.30, 1.47)	8	0.71 (0.31, 1.65)	4	0.69 (0.22, 2.23)		

[a]Adjusted for BMI, age, social class, marital status, children, smoking status, level of physical activity, low alcohol consumption, red meat servings, total fruit and vegetable servings. Comparison group = all respondents not taking stated dose.

[b]Total numbers with a history of cancer listed taking doses specified.

[c]Insufficient numbers at higher doses.

6.5 Discussion

The frequent use of supplements containing any dose or high doses of vitamin C in the UKWCS was associated with healthier lifestyle behaviours, and therefore supports the inverse supplement hypothesis, as seen in analyses of any supplement-taking in the UK or elsewhere.[25 28-35] Women taking either high (>=1000mg per day) or any dose of vitamin C were more likely to consume over five servings of fruit and vegetables, the main dietary source of vitamin C.[67] This is consistent with evidence from studies of any supplement-taking,[27 28 33] and US studies of vitamin C supplement-taking,[30 33] and suggests that many high dose vitamin C takers are less likely to need them. Furthermore, in-line with US findings, UKWCS vitamin C takers were likely to eat less meat.[31] They also exercised vigorously more often, supporting previous research linking activity to supplement use.[27-29 32-35] Distinguishing characteristics of high dose vitamin C takers in the UKWCS were being an ex-smoker, drinking alcohol less than once a week and being of high socio-economic status. These characteristics were not significant predictors of using supplements containing any dose of vitamin C in the UKWCS, however they have been positively associated with taking any type of supplement in other studies.[26 29] Additionally, the current results indicated high dose vitamin C users relied more on alternative practitioners rather than family or private doctors. Healthy behaviours associated with vitamin C supplement use are likely to reduce health risks, therefore these behaviours identified should be considered for adjustment in longitudinal studies of risks.[33]

Despite controversy surrounding evidence of benefits of high dose vitamin C supplementation for prolonged cancer survival,[87-89] our results showed prior to seasonal weighting women with any type of cancer were more likely to be high dose vitamin C supplement-takers than women with no history of cancer. Since antioxidants can potentially reduce the effectiveness of anti-cancer drugs,[77 356] patients should be encouraged to discuss their supplement use with their doctors in order to avoid contraindications. For some cancer patients supplement use may be a coping behaviour and a way of taking control.[37 357] Similar health related behaviours may also occur in women with concerns about risk of developing cancer: for instance women who attended mammography have also been positively associated with supplement use in the US.[33] Likewise, women attending UK breast screening clinics had similar characteristics to supplement users in the UKWCS and wanted diet and exercise advice to be provided at these clinics.[16] Doses below 1000mg/d and any dose of vitamin C, however, were not significantly associated with total cancer in the UKWCS; this has been also observed in some US studies.[353 358]

To the best of our knowledge this is the first UK study to analyse associations between vitamin C supplement use and specific prevalent cancers, and therefore the first to report significant associations of frequent high dose vitamin C taking (=>1000mg/day) in UK women with a personal or family history of breast cancer. This supports findings that US women physicians with breast cancer were more likely to take vitamin C.[31] Furthermore, our results show the odds of having a history of cancer increased at higher doses (>=2000mg). However, whilst US research found that women at high risk of breast cancer and with inconclusive genetic test results were significantly more likely to take any supplements, the increased odds of taking high doses of vitamin C in the UKWCS for women with increased risk of hereditary breast cancer or those having mothers or sisters with breast cancer were not significant.[359] Our results may be due to low numbers and lack of power. In general, a history of non-hormone related cancer did not appear to be associated with vitamin C supplement-taking in the UKWCS, nevertheless associations with a personal history of cervical cancer remained significant at some doses after adjustments, including adjustment of socio-economic status which is known to be linked with this cancer.[360]

In relation to prevention of cancer and other chronic diseases, the 5 A Day fruit and vegetable initiative based on the World Health Organisation (WHO) recommendations could have influenced antioxidant supplement sales at the time of the phase 2 UKWCS follow-up.[9] Proactive marketing by supplement companies would have also increased sales. The 1997 WCRF report, nevertheless, stated that supplements were probably unnecessary and unhelpful for reducing cancer risk.[8] Whilst the recent 2007 WCRF report found no probable or convincing evidence that vitamin C supplementation affects cancer risk there was evidence of an increased or decreased risk with some other supplement types (Appendix B), though this was usually from studies of high-risk groups.[7] In summary the 2007 report states that it is unwise to recommend widespread supplement use for cancer prevention since effects cannot be confidently predicted in the general population.[7] Indeed high doses of some supplements, including vitamin C may promote cancer,[77] although doses above 400mg of this water soluble vitamin are likely to be excreted in healthy women.[65] Its antioxidant properties may reduce DNA damage by reactive oxygen species during the initial stage of cancer particularly in individuals with high levels of ROS.[77] This antioxidant property, however, may decrease beneficial apoptosis, the ROS induced programmed death of damaged cells,[81] and thereby lead to the progression of cancer particularly in individuals with low levels of ROS.[77] Vitamin C may also act as a pro-oxidant creating highly reactive and damaging hydroxyl radicals via the Fenton reaction in the presence of iron.[76] However this hypothesis is controversial since free iron is normally unavailable in vivo.[76] Apart from a family history of breast cancer and a moderate but non-significant association

with a family history of pancreatic cancer our results indicate that UK women were probably not taking high dose vitamin C supplements as a preventive measure due to a family history of cancer in general. Since cancer of the pancreas has a poor prognosis, some women with this family history may have been more motivated to take high doses of vitamin C supplements.

It is unknown why vitamin C supplements were taken by women in the UKWCS. Given that only a relative small proportion of the UK population are advised by the medical profession to take supplements for health reasons,[26] some health conscious UK women may be self-treating with vitamin C. Alternatively, women with or without cancer may take supplements under the belief supplements can make them feel better generally or increase immune function.[361] Additionally, due to the cross-sectional nature of the study the direction of cause and effect cannot be determined; it is unknown whether vitamin C has been taken to prevent or manage cancer symptoms or whether vitamin C has caused cancer.

Lack of information to determine whether cancer developed before or after frequent vitamin C supplement use started is a limitation of this study. Although cancer registry data was available for the cohort we suggest it is women's self-reported perceived health, whether accurate or not, that influences their supplement-taking behaviour. Self-reporting of supplement descriptions, for only four days by diary were limitations of the study. Whilst 4-day diaries are capable of capturing daily, and near daily intake, in reality some three to four day diary recordings may represent spasmodic rather than frequent intake. Nevertheless, our results in section 5.2 of the evaluation chapter show that substantial agreement was found between these frequent diary recordings and daily use recorded concurrently by questionnaire. Although the number of years of supplementation was not collected in either the diary or questionnaire, and no further follow-up was conducted, the majority of vitamin C users (82%) were taking a supplement of some type on average four years earlier at baseline. An additional problem was the wide variety of formulations of supplements used which made coding difficult. Whilst high dose vitamin C supplements were unlikely to contain other micronutrients,[35] our results show that consistent with other research,[26] women taking high doses were highly likely to take other supplements. Therefore vitamin C use may be a marker for the intake of other supplements.

Our study capitalises on the large sample size of the UKWCS, substantial numbers of women frequently taking vitamin C (34%) and as well as the wide variety of characteristics and cancers recorded. However, the small numbers of women in some of the categories, particularly those shown in Table 30 and especially those taking

2000mg/day or more, may have produced spurious results. Another limitation of our study is that UKWCS participants were more health conscious than the general population and therefore not representative of the whole UK population. Differences in characteristics between frequent takers and non-frequent takers in the UKWCS may not be as pronounced as that found in the general population.

Our research may help to identify high-dose users, such as ex-smokers, low alcohol drinkers and women with a history of breast cancer who could be made aware of inconsistencies in evidence relating to suggested benefits, and of warnings relating to high strength supplements.[362] Furthermore, patients should be encouraged to discuss their supplement use with their doctors to avoid contraindications.[81] [356] Finally, additional research is needed to establish the effects of both supplement and dietary vitamin C intake on cancer initiation and development. Breast cancer risks for UK women in relation to vitamin C intake are reported in chapter 8, 9 and 10. First, breast cancer risk in relation to general supplement use is explored in the next chapter.

CHAPTER 7

7 Is general supplement use associated with breast cancer risk in the UKWCS?

7.1 Summary

Objectives: To determine whether general supplement use is associated with breast cancer risk at baseline in the UK Women's Cohort Study (UKWCS) and whether use modifies risks in different groups of women. In addition breast cancer risks for women who were users at both baseline and phase 2 will be determined.

Methods: Hazard ratios were calculated for 33,138 middle-aged women in the UKWCS in relation to any supplement use recorded by questionnaire at baseline. The median follow-up period was 11.2 years with 982 registered breast cancer incidences. Risks for 12,917 of these women, who also completed the phase 2 questionnaires on average 4.4 years later, were analysed in relation to consistent supplement use. Median follow-up for these women was 11.6 years from baseline during which 414 breast cancers occurred. Adjustments were made for potential confounders measured at baseline. Interactions between baseline supplement use and other characteristics of the women were tested.

Results: There was no evidence of associations with breast cancer risk for baseline supplement users (HR=1.00; 95% CI: 0.87, 1.16) compared to non-users, or for women who were still taking supplements at phase 2 (HR=0.96; 95% CI: 0.74, 1.26) compared to never-users. There were also no significant risks for pre- (HR=1.14; 95% CI: 0.91, 1.43) or post-menopausal women (HR=0.89; 95% CI: 0.73, 1.08) for baseline users or similarly for consistent users. Significant interactions were found between baseline supplement use and menopausal status (p=0.04), socio-economic status (p=0.01) and vigorous exercise (p=0.05). Increasing healthier behaviours were apparent from never users, to inconsistent users, to consistent users.

Conclusion: There were no overall associations in this UK cohort between breast cancer risk and general supplement use, including women who were users on average 4.4 years later. However, the results indicate supplement users may have different risks from non-users depending on their menopausal status, their socio-economic status or level of physical activity.

7.2 Introduction

Due to the unregulated increased use of supplements by UK women, which has risen from 17% in 1986/1987 to 41% reported in 2008/9,[24 25] it is important to establish whether or not general supplement use is associated with detrimental effects, such as an increased risk of breast cancer. Indeed the 2007 WCRF review clearly states that supplements are not recommended for cancer prevention, furthermore it reports no convincing evidence that supplements protect against breast cancer.[7] Most studies evaluating the relationship between supplement use and breast cancer risk have focused on individual supplement types or ingredients. Only a few studies mentioned in the 2007 WCRF review examined general supplement use in relation to breast cancer and these were case-control studies which may be prone to selection bias and recall bias.[257-259] No associations were found in the Danish and US studies reported in the 2007 review,[257 259] whereas a significant protective effect of supplements on breast cancer was found in a Taiwanese study (OR=0.40, 95% CI: 0.3, 0.7). Furthermore, there is a lack of evidence on whether general supplement use modifies factors linked to breast cancer risk; supplement use could be beneficial for some groups of women but detrimental for others.

Additionally, no study has assessed whether consistent use of supplements in general is associated with breast cancer risk. Only one study has analysed the consistency of general supplement use in relation to cancer, though this was for total cancer mortality and no significant associations were found for women who were consistent users at three survey points over 11 years.[363] Nevertheless, in this German cohort, consistent users had a healthier diet compared to inconsistent users as well as non-users.[364] As reported in the previous chapter, 6, supplement users are more likely to lead healthier lifestyles, for instance they are more likely to be physically active, have a lower BMI and eat diets which meet recommended dietary intakes. However, there is no research on whether consistent users are more health conscious than inconsistent users in the UK.

The current study prospectively examines the relationship between breast cancer incidence and use of any type and amount of supplements at baseline in the UKWCS, and also compares the risk for women who are still using any supplements at phase 2 with those who are inconsistent users and those who are never-users. It also describes characteristics of consistent and inconsistent users. Furthermore, it capitalises on the large sample size of the UKWCS at baseline by testing for interactions between supplement use at baseline and women's characteristics and their associations with breast cancer risk.

7.3 Method

7.3.1 Study population

General information about the recruitment of the UKWCS baseline population used in this analysis is provided in section 4.1.1.1 of the methods chapter. Of the 35,372 women in the UKWCS who completed the baseline questionnaire, 34,958 (99%) provided information about whether or not they took supplements. Women with any prevalent malignant cancers recorded in the cancer registries before baseline questionnaire date and women diagnosed with breast cancer within 6 months after the questionnaire date were excluded. To be consistent with other baseline analyses that will be presented in this thesis, women with extremely low or high total energy intake (more than 6000kcal and less than 500kcal) were also excluded. This left 33,138 women for the time-to-event analysis. Over the median follow-up period of 11.2 years there were 982 incident breast cancers which were registered by the censor date 01/01/08.

Risks for 12,917 of these women who also completed supplement questions on the UKWCS phase 2 questionnaires on average 4.4 years after baseline, were analysed in relation to consistent supplement use. Consistent users are defined in this analysis as women who were taking any type of supplement at both baseline and phase 2. The same types of exclusions were made as above. Over a median follow-up of 11.6 years from baseline there were 414 incident breast cancers registered to censor date 01/07/08.

7.3.2 Exposure measurement

General supplement use at baseline was determined by questionnaire using yes/no answers to:

Q29 *Do you take any vitamins, minerals, fish oils or other food supplements?*

Supplement use at phase 2 was determined by questionnaire using yes/no answers to:

Q15 *Do you presently use any dietary supplements?*

Supplements had previously been defined on the phase 2 questionnaire as vitamins, minerals, fibre, fish oils or other food supplements.

Additionally, if participants did not answer yes to the above questions but provided details of any type of supplements taken, regardless of amount taken, then these women were designated as being general supplement users. As a result an extra 3083 women were coded as general supplement users at baseline, and also an extra 846

women at phase 2 were coded as users who detailed they took supplements weekly or more frequently on the phase 2 questionnaire (see sections 4.5.1 and 4.5.2). These numbers are before exclusions mentioned in 7.3.1.

7.3.3 Statistical analyses

Characteristics of users and non-users at baseline were described using means and percentages; significant differences between means were established using t-tests and significant differences between categories were established using chi squared tests. Women who had also completed the phase 2 questionnaire were split into three categories: Never users at baseline and phase 2; inconsistent users (use at only one collection point); Consistent users (use at both baseline and phase 2). Characteristics of these women were described using means and percentages. Significant trends for means across groups from never users to inconsistent users to consistent users were calculated using linear regression followed by tests for linear hypotheses.

Cox proportional hazard ratios were used to estimate breast cancer risk relating to supplement use of women in the UKWCS. The proportional hazard assumption was checked using graphs of log-log curves. Covariates used for adjustment were based on those previously identified as risk factors for breast cancer which had also previously been associated with supplement use, as illustrated in the DAG in Figure 14 in section 4.7.5.2. These were age; BMI (underweight, normal, overweight, obese); parity (none, 1-2, 3 or more); estimated cumulative breast feeding (wks); age at menarche; minutes sweating exercise per week; contraceptive pill use (never, past, current); HRT use (never, past, current); alcohol intake (g/day); smoking status (never, past, current); food group (meat, oily fish, fish, vegetarians); total energy intake; and also where appropriate supplement use (yes, no); menopausal status (postmenopausal; premenopausal) and socio-economic status (high: professional and managerial; intermediate; low: routine and manual). Menopausal status was determined at baseline based on the answers to this questionnaire (see section 4.7.4 including Figure 12). Covariates were derived from the health and lifestyle part of the baseline questionnaire apart from total energy intake, total alcohol intake and food groups which in turn had been derived from the FFQ part of the baseline questionnaire.

To investigate whether supplement use at baseline modified the effects of women's characteristics on breast cancer risks, adjusted likelihood ratio tests for overall interactions were performed between supplement use status and each of the following characteristics: menopausal status; HRT use; socio-economic status (SES); educational level; BMI; level of exercise; and allocated food group. These are factors that have been associated with breast cancer risk, many of which are discussed in the

literature review chapter, 3, and/or in section 4.7.5.2 of the methods chapter. The hazard ratios shown in Table 35 include interaction terms for joint effects of supplement use status and each characteristic, which were adjusted for the same covariates in the other time-to-event analyses. Linear combinations of coefficients were used to calculate the hazard ratios for the users after running the main Cox proportional hazard command (using the Stata lincom command).[365]

7.4 Results

Of the 33,138 total women, 62% percent were supplement users at baseline and 54% of the 12,917 women followed-up at phase 2 had taken supplements at both recording points, and were classed as consistent users. Twenty-four percent of women at phase 2 were inconsistent users (had taken supplements at either recording point but not at both) and 21% were never users. Of the 8,896 (69%) women who were currently taking supplements at phase 2 as many as 6,992 (79%) were classed as consistent users.

There were significant differences between general supplement users and non-users at baseline for the majority of characteristics listed in Table 31 except for age at menarche, smoking status, socio-economic status and family history of breast cancer. In particular users were more likely to have a higher fruit and vegetable intake (mean 652 vs. 591 g/d), a lower meat intake (mean 59.8 vs. 72.9 g/d), more likely to exercise vigorously (15.9 mins/d vs. 14.1 mins/d), but less likely to engage in breast feeding (22.6 vs. 25.1 cumulative weeks) than non-users. Users were slightly less likely to have a degree than non-users. There were similar differences in the prospective comparison in Table 33 which showed increasingly healthier behaviours from never users at baseline and phase 2, inconsistent users, to consistent users at phase 2 (e.g. fruit and vegetable intake respectively was 603 vs. 627 vs. 676 g/d).

There was no difference in breast cancer incidence between supplement users and non-users for total women either in the unadjusted or adjusted analyses (HR=1.00; 95% CI: 0.87, 1.16) For post-menopausal women hazard ratios for users were lower and for pre-menopausal hazard ratios were raised, compared to non-users (HR=0.89; 95% CI: 0.73, 1.08 and HR=1.14; 95% CI: 0.91, 1.43 respectively), but they were not significant (Table 32). For women who reported taking any supplement at both baseline and phase 2 similar patterns of non-significant risks were found; these also were not significant (Table 34).

Table 31 Characteristics of supplement users and non-users at baseline in the UKWCS

	Non-users N=12678 (38%)	Users N=20460 (62%)	p
Age (years) mean (sd)	51.5 (9)	52.3 (9)	<0.001
BMI (kg/m^2) mean (sd)	24.8 (4)	24.2 (4)	<0.001
Age at menarche (years)	12.8 (2)	12.8 (2)	0.8
Parity mean (sd)	1.9 (1)	1.8 (1)	<0.001
Cumulative breast feeding (weeks) mean (sd)	25.1 (31)	22.6 (34)	<0.001
Vigorous exercise (mins/d) mean (sd)	14.1 (28)	15.9 (30)	<0.001
Total energy intake (cal/d) mean (sd)	2323 (710)	2360 (717)	<0.001
Alcohol intake (g/day) mean (sd)	9.0 (11)	8.5 (10)	<0.001
Total meat intake (g/day) mean (sd)	72.9 (63)	59.8 (60)	<0.001
Total fruit & veg (g/day) mean (sd)	591 (329)	652 (354)	<0.001
Never smoked (%)	58.2	57.5	0.2
Never used HRT (%)	70.0	66.5	<0.001
Never used pill (%)	31.1	32.6	0.008
Socio-economic status (%)			
Higher	39.2	39.0	0.8
Middle	42.3	42.7	
Lower	18.5	18.3	
Education level, (%)			
Degree	26.9	25.7	0.05
Family history of breast cancer (%)	7.5	7.7	0.4

Table 32 Breast cancer risks according to any supplement use at baseline in the UKWCS

Any supplements use at baseline	Cases/ Non-cases	Unadjusted		Adjusted[a]	
		HR	(95% CI)	HR	(95% CI)
Total women					
Non-users	364/12314	1		1	
Users	618/19842	1.04	(0.92, 1.18)	1.00	(0.87, 1.16)
Post-menopausal					
Non-users	218/6061	1		1	
Users	353/10514	0.91	(0.76, 1.08)	0.89	(0.73, 1.08)
Pre-menopausal					
Non-users	146/6253	1		1	
Users	265/9328	1.21	(0.99, 1.48)	1.14	(0.91, 1.43)

[a]Adjusted for baseline covariates: age, BMI (grouped), socio-economic status, parity, estimated cumulative breast feeding, age at menarche, hrs exercise sweating per week, contraceptive pill use, HRT use, alcohol intake, smoking status, food group, total calories

Table 33 Baseline characteristics of women in the UKWCS according to supplement use at baseline and phase 2: never users; inconsistent users; and consistent users

	Never[a] users N=2756 (21%)	Inconsistent[b] users N=3169 (24%)	Consistent[c] users N=6992 (54%)	p_{trend}
Age (years) mean (sd)	52.1 (9)	51.2 (9)	52.6 (9)	<0.001
BMI (kg/m^2) mean (sd)	24.4 (4)	24.4 (5)	23.9 (4)	<0.001
Age at menarche (years) mean (sd)	12.8 (2)	12.8 (2)	12.8 (2)	0.2
Parity mean (sd)	1.4 (1)	1.4 (1)	1.3 (1)	0.002
Cumulative breast feeding (weeks) mean (sd)	28.5 (39)	26.1 (37)	24.4 (35)	<0.001
Vigorous exercise (mins/d) mean (sd)	13.4 (26)	13.9 (23)	16.6 (31)	<0.001
Total energy intake (cal/d) mean (sd)	2341 (674)	2366 (709)	2364 (694)	<0.001
Alcohol intake (g/day) mean (sd)	8.9 (13)	8.6 (10)	8.0 (10)	<0.001
Total meat intake (g/day) mean (sd)	67.0 (62)	60.5 (59)	53.0 (56)	<0.001
Total fruit & veg (g/day) mean (sd)	603 (312)	627 (337)	676 (347)	<0.001
Never smoked (%)	63.4	61.0	61.3	0.2
Never used HRT (%)	72.0	69.6	65.3	0.007
Never used pill (%)	34.2	28.4	33.0	0.03
Socio-economic status (%)				0.8
Higher	41.9	41.0	41.0	
Middle	41.7	41.7	42.7	
Lower	16.4	16.7	16.3	
Education level, (%)				
Degree	31.0	30.0	27.2	0.001
Family history of breast cancer (%)	8.0	7.7	8.2	0.7

[a]Never users: women who reported no supplement use at baseline and at phase 2

[b]Inconsistent users: women who reported supplement use on only one questionnaire, either baseline and at phase 2

[c]Consistent users: women who reported supplement use at both baseline and at phase 2

Table 34 Breast cancer risks according to any supplement use for never users, inconsistent users and consistent users according to use at baseline and phase 2 of the UKWCS

Any supplements use at baseline or phase 2	Cases/ Non-cases	Unadjusted		Adjusted[a]	
		HR	(95% CI)	HR	(95% CI)
Total women					
Never users at BL & P2	85/2671	1		1	
Inconsistent users	99/3070	1.00	(0.75, 1.34)	0.92	(0.67, 1.26)
Consistent users	230/6762	1.05	(0.82, 1.35)	0.96	(0.74, 1.26)
Post-menopausal					
Never users at BL & P2	54/1389	1		1	
Inconsistent users	54/1438	0.95	(0.65, 1.39)	0.87	(0.57, 1.33)
Consistent users	130/3667	0.89	(0.65, 1.23)	0.80	(0.56, 1.15)
Pre-menopausal					
Never users at BL & P2	31/1282	1		1	
Inconsistent users	45/1632	1.13	(0.71, 1.79)	1.01	(0.63, 1.63)
Consistent users	100/3095	1.32	(0.88, 1.98)	1.16	(0.77, 1.76)

[a]Adjusted for baseline covariates: age, BMI (grouped), socio-economic status, parity, estimated cumulative breast feeding, age at menarche, hrs exercise sweating per week, contraceptive pill use, HRT use, alcohol intake, smoking status, food group, total calories

In the test for interactions, supplement use was found to modify the effect of socio-economic status (SES), vigorous exercise, as well as menopausal status on breast cancer incidence after adjustment for potential confounders and inclusion of interaction terms for joint effects of supplement use status and each characteristic. Table 35 shows that pre-menopausal users tend to have a slightly higher breast cancer risk than non-users whereas post-menopausal users have a slightly lower risk than non-users, though individual risk estimates are not significant. Table 35 also shows that supplement use has little effect on breast cancer risk for women of high SES, but risks are different for middle and low SES groups depending on whether or not they used supplements. Non-users in the lowest SES group had a higher breast cancer risk (HR=1.52; 95% CI: 1.11, 2.07) than users in the lowest SES group (HR=0.97; 95% CI: 0.72, 1.31), when compared to women of high SES who did not take supplements. However, supplement users in the middle SES group had higher risks (HR=1.23; 95% CI: 0.98, 1.54) than non-users in the middle SES group, though these estimates were not statically significant. The unadjusted increased risk for middle SES group supplement users compared to other SES groups of supplement users is illustrated in the Kaplan Meier curve in Figure 22. Additionally, women who exercise vigorously had a higher risk of breast cancer if they took supplements than if they were not users (HR=1.16; 95% CI: 0.94 1.42). However women who did not exercise vigorously were at greater risk (HR=1.27; 95% CI: 0.99 1.62) if they were non-users than if they took supplement (HR=1.10; 95% CI: 0.87 1.39). However individual risk estimates did not reach statistical significance. Effects of HRT use, BMI, educational level and food group were not significantly modified by supplement use.

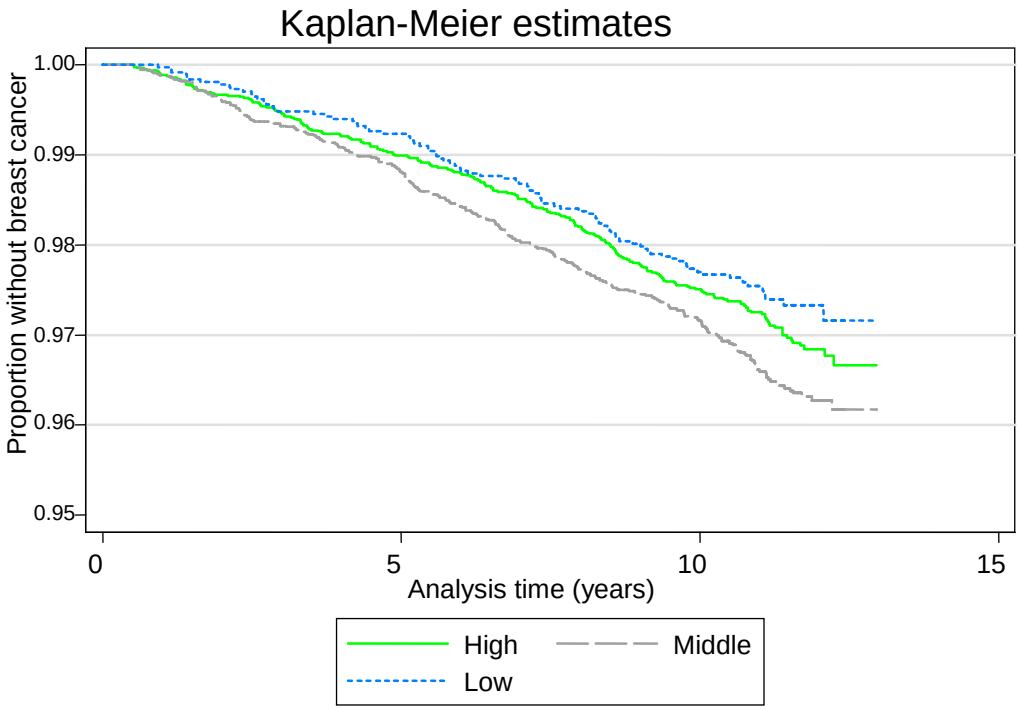
Table 35 Assessment of modifying effects of supplement use at baseline on breast cancer risk using interaction terms for joint effects of supplement use and other characteristics

	Cases/ Non-cases		HR (95% CI)[a]		p^b
	Non-users	Users	Non-users	Users	
Menopausal status					
Post	174/4713	271/8213	1	0.88 (0.73, 1.07)	
Pre	124/5293	219/7803	0.98 (0.72, 1.33)	1.17 (0.89, 1.55)	0.04
HRT use					
Never	182/6987	302/10661	1	1.07 (0.89, 1.29)	
Prior	27/775	56/1603	1.23 (0.82, 1.85)	1.23 (0.90, 1.67)	
Current	89/2244	132/3751	1.43 (1.14, 1.97)	1.32 (1.03, 1.68)	0.5
Socio-economic status					
High	109/4018	189/6351	1	1.09 (0.86, 1.38)	
Medium	123/4250	230/6826	1.08 (0.84, 1.40)	1.23 (0.98, 1.54)	
Low	66/1738	71/2838	1.52 (1.11, 2.07)	0.97 (0.72, 1.31)	0.01
Education level					
None	50/1330	75/2260	1	0.87 (0.61, 1.25)	
O'level	90/2894	145/4741	0.93 (0.65, 1.32)	0.91 (0.64, 1.24)	
A'level	69/2340	120/3731	0.88 (0.60, 1.29)	0.95 (0.67, 1.33)	
Degree	72/2710	110/4082	0.83 (0.56, 1.22)	0.83 (0.57, 1.19)	0.8
BMI					
Low	2/97	5/232	0.99 (0.19, 5.14)	0.80 (0.33, 1.95)	
Normal	166/6071	311/10610	1	1.04 (0.86, 1.26)	
Overweight	92/2699	133/3810	0.94 (0.68, 1.31)	1.19 (0.94, 1.50)	
Obese	38/1139	41/1363	0.85 (0.53, 1.38)	1.08 (0.76, 1.52)	0.9
Exercise vigorously					
Yes	138/5515	293/9812	1	1.16 (0.94 1.42)	
No	158/4455	193/6136	1.27 (0.99 1.62)	1.10 (0.87, 1.39)	0.05
Food Group					
Meat	231/7444	345/10667	1	1.00 (0.85, 1.19)	
Oily fish	4/163	12/488	0.80 (0.39, 2.15)	0.79 (0.44, 1.42)	
Fish	17/743	49/1670	0.75 (0.46, 1.22)	0.93 (0.68, 1.27)	
Vegetarian	46/1656	84/3190	0.94 (0.68, 1.30)	0.88 (0.68, 1.13)	0.9

[a]Adjusted for age, BMI (grouped), socio-economic status, parity, estimated cumulative breast feeding, age at menarche, hrs exercise sweating per week, contraceptive pill use, HRT use, low alcohol, smoking status, food group, total calories.

[b]Test for interaction

Figure 22 Breast cancer time-to-event curve for supplement users split by socio-economic status



7.5 Discussion

In line with previous research, descriptive results from these UKWCS baseline and phase 2 analyses support the inverse supplement hypothesis that supplement users lead a healthier lifestyle than non-users, as found in the UK[25 28 29] and elsewhere.[30-35] In particular, supplement users had on average a higher intake of fruit and vegetables, a lower intake of meat and spent more time exercising vigorously. Whilst UKWCS supplement users were generally more health conscious, they had spent less time on average breast feeding than non-users or never-users. Additionally, UKWCS users were less likely to be educated to degree level. However a previous analysis of an initial 13,822 respondents from the baseline UKWCS found no significant association for education, though this was after mutual adjustment for other variables,[27] and other studies have reported higher qualifications for supplement users. [32,353]

Furthermore, the descriptive results showed that consistent users (users at both baseline and phase 2) have healthier behaviours than inconsistent users, as well as never users. Only one other cohort has analysed the characteristics of consistent supplement users compared to non-users and inconsistent users;[364] women in this EPIC-Heidelberg cohort who were consistent users had the highest intake of dairy products, fish, fruit and vegetables and wine but the lowest intake of meat compared to inconsistent users as well as non-users. In this German cohort only inconsistent use was associated with self-reported cancers. In the UKWCS inconsistent or consistent users were not more likely to have a family history of breast cancer. Further analysis of the UKWCS using a multivariate logistic regression model mutually adjusting for variables is required to assess significant predictors of consistent supplement use in the UKWCS cohort, which remain significant over and above associations with other factors. In comparison to the German study which reported a low percentage of consistent women users (23%), a large percentage (54%) of women at phase 2 of the UKWCS were consistent users from baseline. Reasons for the differences may be as follows: baseline supplement use in the German study was much lower than in the UKWCS (39% vs. 62%); supplement recordings occurred over three follow-up time points in the German study compared to two in the UKWCS; and the German cohort may not have been as health conscious as the women in UKWCS which included a high percentage of vegetarians.

In the current analysis there was no evidence of significant associations between breast cancer risk and supplement use at baseline for total women and by menopausal status. This supports the results of both the Danish and US study reported[257 259] in the 2007 WCRF review which found no convincing evidence for beneficial effects of

supplements on breast cancer risk.[7] However the current results are in contrast to the Taiwanese study where general supplement use was associated with a reduced risk.[258] Taiwanese women may generally have lower intake levels of nutrients than western women and therefore may require supplementation to reduce their risk. In contrast, there was a weak increased risk for pre-menopausal supplement use at baseline in the UKWCS which was close to significance, but only in the unadjusted analysis. Given that users generally have a healthier lifestyle than non-users, lower breast cancer risks for users before adjustment for lifestyle factors had been expected.

Additionally, there was no evidence of significant associations between breast cancer risk and consistent supplement use between baseline and phase 2 for total women and by menopausal status. This is the first study to assess breast cancer risk and long-term supplement use. Only one previous study has analysed the consistency of general supplement use in relation to cancer, though this was for total cancer mortality.[363] This EPIC-Heidelberg analysis reported a weak increased cancer mortality risk for consistent users which was non-significant, but reported evidence of a significant moderate increased risk in new users which became non-significant after excluding women diagnosed between the baseline and follow-up surveys.[363] This supports research mentioned in the previous chapter 6 which reported cancer patients tend to take supplements after cancer diagnosis. A limitation in both studies is that it is unknown whether participants were taking the same types of supplements at the follow-up as at baseline: for how long, how frequently, how many and at what doses they were taking them is also unknown. Some breast cancer cases (129 of the total 414) occurred between baseline and phase 2 in the UKWCS, consequently the shorter duration of supplement use for these women was likely to have had less influence on cancer development than incidences occurring after phase 2. Additionally, it is unknown how many years supplements were used before baseline.

Additionally, the current analysis found that supplement use modified the effects of menopausal status, socio-economic status and vigorous exercise on breast cancer risk in UK health conscious women. For supplement users, differences in breast cancer risks between menopausal statuses could arise because pre-menopausal women may tend to take different supplements from post-menopausal women and these may have different effects on breast cancer risk. For instance, a small 2008 UK national survey reported cod liver oil was used by half of those aged 55 plus (who were likely to be post-menopausal) compared with a quarter (26%) of those aged 16-54, whereas multivitamins and vitamin C were more likely to be used by 16-54 year old supplement users than those aged 65 plus.[26] Another explanation for higher risk in pre-menopausal women is that there may be a large proportion of pre-menopausal women at high risk

of genetically inherited breast cancer in this cohort, who in the hope of reducing their cancer risk may have been more likely to take supplements. Sensitivity analyses could be undertaken excluding women who had a family history of breast cancer, though these women were not associated with supplement use and the recording of this at baseline appeared incomplete, as mentioned in section 4.7.6.3.1. One reason why non-supplement users of lower SES were found to have significantly higher risks of breast cancer than low SES supplement users or non-supplement of other SES is perhaps they were more likely to be poorly nourished. In relation to exercise, one study reported vitamin C supplementation was detrimental to the beneficial effects of physical exercise on insulin resistance[366] which in turn may affect breast cancer risk, as mentioned in section 3.5.6 of chapter 3. Further sub-group analysis was not undertaken since it has been acknowledged that this should be discouraged because it may lead to spurious findings from multiple testing, especially when no overall significance was found prior to sub-grouping.[367] Conversely Hemila and Kaprio (2008) argue that given the investment of time and resources into the establishment of large databases it is morally right to sub-analyse data, especially when biological and social affects are complex and not well established.[368]

Although general supplement use in poorly nourished populations may be required for the body to function adequately against the development of cancer, any effects of general supplement use per se on breast cancer risk in well-nourished populations may be more difficult to explain biologically. The women in the UKWCS appear to be well-nourished, and high intakes of different nutrients from supplements are likely to have different effects on cancer risk, some may produce opposing effects. What is more, as seen in the previous chapter, 6, a large proportion of women take many different types of supplements. Alternatively differences in risk could arise due to lifestyles or characteristic differences between users and non-users that have not been adequately adjusted for. Another limitation is that UKWCS participants were more health conscious than the general population and therefore not representative of the UK population as a whole. Results were not weighted to account for the large proportion of vegetarians in the UKWCS; nevertheless the aim was to examine the effects on health conscious women who may be prepared to alter their behaviour in light of new evidence.

To conclude, there was no evidence of significant associations between general supplement use and breast cancer risk in this UK cohort which comprised of mainly health conscious women. However, the results suggest that UK women who take supplements may have different breast cancer risks from non-users depending on their menopausal status, their socio-economic status or level of physical activity.

CHAPTER 8

8 Do women who take supplements containing vitamin C in the UK have an increased risk of breast cancer? Phase 2 analyses of the UKWCS.

8.1 Summary

Objective: To determine whether breast cancer incidence in the UKWCS is associated with the use of supplements containing vitamin C.

Method: 11,184 middle-aged women from phase 2 of the UK Women's Cohort Study (UKWCS) were followed up for a median of 7.4 years. Associations between 239 registered incident breast cancers and vitamin C contained in supplements recorded by 4-day diaries were analysed by Cox's regression models using four intake categories: no frequent use of supplements containing vitamin C; frequent intake up to and including EU recommended allowances (1-60mg/d); between 60mg and 500mg/d; and high intake (≥500mg/d). Additionally, the relationship between breast cancer risk and phase 2 questionnaire reports of daily multivitamin use, a common source of low dose vitamin C, was examined for 12,642 women, including 269 cases. Adjustments were made for relevant covariates.

Results: Compared to women who did not use supplements containing vitamin C, there was no evidence of significant associations between breast cancer incidence and frequent vitamin C supplementation in any intake category, or in post-menopausal sub-analyses. Additionally, no dose-response associations were found (HR=0.98 per 60mg/d; 95% CI: 0.94, 1.02; P_{trend}=0.3). However pre-menopausal women in the lowest intake category (1-60mg/d) had statistically significantly increased risks (HR=2.56; 95% CI: 1.41, 4.66; p=0.002) compared to non-users of vitamin C. There was also a moderate increased risk for pre-menopausal daily multivitamin users, however, this was not significant (HR=1.51; 95% CI: 0.90, 2.54).

Conclusion: There was little evidence that supplementation with vitamin C per se was associated with breast cancer incidence in UK women, even at high doses. The increased breast cancer risk for pre-menopausal women consuming supplements containing vitamin C less than or equal to EU recommendations may be due to the effects of other ingredients in the supplements, though the multivitamin use results did not entirely support this. Alternatively the positive associations may be spurious.

8.2 Introduction

As mentioned in chapter 2, vitamin C is one of the most commonly used dietary supplements in the UK.[26 352] In a small 2008 nationally representative UK survey 24% of people reported its use and it was one of the top three reported supplements used along with multivitamins (36%) and cod liver oil (35%).[26] In the UKWCS 17.5% of women recorded they took vitamin C on the phase 2 questionnaire; other popular supplements were multivitamins, fish oils and evening primrose oil. In total, phase 2 questionnaire recordings showed that 35.5% of UKWCS women used vitamin C, multivitamins, or antioxidants; all likely to contain vitamin C (Figure 2, chapter 2). This was comparable to diary recordings at phase 2 which, as seen in chapter 6, showed that 34% of women frequently took supplements containing any dose of vitamin C. Additionally, diary recordings showed that 10% frequently took high doses of 500mg or more and 5% frequently took doses of 1000mg or above (chapter 6).[1]

In addition to associations with healthier lifestyle behaviours, the frequent use of supplements containing vitamin C by UKWCS participants was also associated with a family history of breast cancer, as reported in chapter 6.[1] It is unknown, however, whether these women took vitamin C supplements for cancer prevention, or took them to increase immune function or for general health and well-being, the latter being a popular reason for taking supplements in general in the 2008 UK survey.[26] The 2007 WCRF/AICR report states that it is unwise to recommend widespread supplement use for cancer prevention since effects cannot be confidently predicted in the general population.[7] Indeed there are mechanisms whereby vitamin C could potentially increase or decrease cancer, as discussed in chapter 2.[77 81] Whilst supplement use by UK women continues to increase,[24 25] evidence is lacking regarding the benefits of vitamin C, in relation to mortality,[351] cancer and breast cancer specifically.[7 351]

As seen in section 3.7.4 of the literature review chapter, results from prospective studies examining associations between vitamin C from supplements and breast cancer risk have been inconsistent. Only six cohort studies have assessed the relationship between breast cancer incidence and vitamin C supplement intake by dose, without the inclusion of dietary intake.[242 245 247 267 270 273] These are summarised together in Table 36, along with one RCT.[14] Each study has assessed different categories of intake, but no pattern of risk emerges.

Table 36 Studies analysing breast cancer risk by vitamin C supplement dose

Author, year	Study	Design	Supplement information requested	Follow up and cancer incidence	Menopause status	Vitamin C supplement associations, compared to non-users: OR, RR, HR
Rohan et al. 1993[267]	Canadian Breast screening Study	Prospective Nested case-control Age 50-59	Specific vitamins Multivitamins	1982-1987 519 cases 1184 controls	Total of pre & post	<250mg/d: 1.04 (0.78-1.39) >250mg/d: 1.46 (1.05-2.01)
Kushi et al. 1996[242]	Iowa Women's Health Study, US	Prospective Cohort N=34,387 Age 55-69	A,C,E, Multivitamin brand name frequency	Av 7yrs: 1986-1992 879 cases	Post	<200mg/d: 0.91 (0.77-1.08) 200-500: 0.94 (0.73-1.21) 500-1000: 0.86 (0.65-1.14) >1000: 0.77 (0.50-1.17) p_{trend} = 0.20
Zhang et al. 1999[247]	Nurses Health Study US	Prospective Cohort N=77,925 Age 33-60	Brand, doses, duration of specific vitamins & multivitamins Ingredients obtained	14yrs: 1980-1994 2523 cases Pre-meno ~27%	Total of pre & post	<400mg/d: 0.96 (0.73-1.25) 400-700: 1.08 (0.93-1.27) 750-1250: 1.02 (0.85-1.23) >1300: 1.04 (0.77-1.42)
Nissen et al. 2003[245]	Danish Diet & Cancer Cohort	Prospective Nested case-control Age 50-64	Brand, doses, frequency in last year Ingredients obtained	Mean 4.7yrs: 1993-1997 228 cases 246 controls	Post	Per additional 100mg/d 1.06 (1.01-1.13)
Cui et al. 2008[270]	Women's Health Initiative US	Prospective Cohort N=84,805 Age 50-79	Multiple and single vitamins A,C, E estimated	Av 7.6yrs 2879 cases	Post	>0<61mg/d: 1.03 (0.91-1.16) 61<347: 0.99 (0.88-1.11) 347-711: 1.04 (0.92-1.16) >711: 1.16 (1.04-1.30) p_{trend} = 0.029
Roswall et al 2010[273]	Diet, Cancer & Health Cohort Denmark	Prospective Cohort N=26,224 1993-1997 Age 50-64	Vitamin C content by brand obtained	Median 10.6yrs 1072 cases	Post	>0≤40mg/d: 0.95 (0.74-1.21) >40 ≤64: 1.03 (0.80-1.33) >64 : 0.96 (0.77-1.21) p_{trend} = 0.41
Lin et al. 2009[14]	Women's Antioxidant Cardio-vascular Study, US	RCT Factorial design N=7,627 Age >=40	500mg/d vitamin C 600 IU/d vitamin E 50/2d beta carotene	Av 9 yrs 135 cases	Post	Compared to placebo group 500mg/d: 1.11 (0.87–1.41)

Table 37 Methods and factors controlled for in studies of breast cancer risk and vitamin C supplement use

Author, year	Exclusions	Hormonal factors controlled for	Dietary factors controlled for	Other factors controlled for
Rohan et al. 1993[267]	History of breast cancer Had mammograms	Age at menarche Age at first live birth Surgical menopause	Energy intake	Age Years of education Family history of breast cancer History of benign breast disease
Kushi et al. 1996[242]	Post menopausal Full or partial breast removal Cancer (other than skin) Missing >30 items on FFQ. Extreme total energy intake	Age at menarche Age at first live birth Parity Age at menopause	Alcohol intake (Energy intake in sensitivity analysis)	Age Educational attainment BMI @ BL & 18yrs Waist/hip ratio Breast cancer family history in first degree relatives History of benign breast disease
Zhang et al. 1999[247]	Previous cancer diagnosis Implausible total energy intake <500>3500kcal/d Missing >10 FFQ blank	Age at menarche Age at first live birth Parity Age at menopause HRT use Menopausal status	Alcohol intake Energy intake	Length of follow up Age Height BMI & BMI @18 yr Weight change from 18 Breast cancer in mother or sister
Nissen et al. 2003[245]	Previous cancer diagnosis Pre-menopausal Missing items on FFQ Missing items on lifestyle questionnaire regarded as potential confounders	Age at first birth Parity HRT duration	Intake of vitamin A & E Alcohol intake (Energy intake in sensitivity analysis)	School education History of benign breast disease BMI (Matched on age at baseline)
Cui et al. 2008[270]	History of breast cancer Implausible total energy intake <600>5000kcal/d	Age at menarche Age at first live birth Parity Age at menopause Contraceptive use HRT use Hysterectomy Bilateral oophorectomy	Energy intake Alcohol intake Dietary folate intake Dietary vitamin C	Age Ethnicity Educational level Smoking Family history of breast cancer History of benign breast disease

Table continued - Methods and factors controlled for in studies of breast cancer risk and vitamin C supplement use

Roswall et al 2010[273]	Cancer diagnosis No lifetime menstruation Missing covariates and micronutrient intake	HRT Parity Age at first birth	Alcohol intake Total intake of vitamin E and β-carotene Dietary or supplement vitamin C intake mutually adjusted	Age (used as the underlying time scale for hazard ratio rather than adjusted for it as a covariate) BMI Education
Lin et al. 2009[14]	Self-reported cancer diagnosis (other than skin) Unwilling to avoid vitamin A,C or E use	Randomised	Randomised	Randomised

Half of the cohorts in Table 36 show no evidence of associations compared to women with no intake. In the Nurses' Health Study, Zhang et al. (1999) reported no evidence of associations for intake categories of <400mg/d, 400-700mg/d, 750-1250, and >=1300mg/d supplement vitamin C for pre-menopausal, post-menopausal or total women.[247] Kushi et al. (1996) also found no significant associations with risk for intake categories of <200mg/d, 200-500mg/d, 500-1000 or >1000 or per 100mg/d increase intake for post-menopausal women in the Iowa Women's Health Study.[242] The recent analyses of the full Danish cohort by Roswall et al. (2010) using much lower categories of intake (<40, 40-64, >64mg/d) also reported no associations.[273] Similarly, in comparisons between total vitamin C users and non-vitamin C users, no associations with breast cancer risk were found in the Netherland Cohort Study or in the Nurses Health Study.[246 247] Duration of use was also not associated with risk.[247] Only one RCT has used vitamin C supplements to assess breast cancer risk, where in a factorial design 500mg/d was taken singly or with vitamin E or beta carotene by North American women at high risk for cardiovascular events. There was no evidence of a statistically significant effect on breast cancer risk for vitamin C (OR=1.11; 95% CI: 0.87, 1.41).[14]

Conversely, in the Danish nested case-control study of post-menopausal women, Nissen et al. (2003) provided weak evidence of an increased risk per 100mg/d increase intake (OR=1.06; 95% CI: 1.01, 1.13), and results were similar after excluding users who consumed above 300mg/d.[245] In another nested case-control study Rohan et al. (1993) reported evidence of a larger increase in breast cancer risk in the Canadian Breast Screening Study for women consuming more than 250mg/d (OR=1.45; 95% CI: 1.05, 2.01) compared to women with no vitamin C intake from supplements.[267] Results split by post-menopausal or other women were not statistically significantly different.[267] In addition, a modest increase in breast cancer incidence was reported by Cui et al. (2008) for post-menopausal women in the Women's Health Initiative Observational Study for intakes of 711mg/d and over (HR=1.16 95% CI: 1.0, 1.30).[270]

The current analysis is the first UK study to examine the relationship between breast cancer risk and vitamin C intake from supplements. The study is important since a variety of supplements containing vitamin C are commonly consumed in the UK, as single or multi-ingredient types, and the consequences of taking them are unclear at population level. In contrast to previous studies, the aim for the current study was to determine whether the frequent use of supplements containing vitamin C recorded by diary, and categorised in relation to the EU recommended daily allowance (60mg/d) and high dose intake, was associated with breast cancer risk in UK women. Evidence of a dose-response relationship was also assessed. The initial study hypothesis, based on antioxidant and pro-oxidant mechanisms discussed in chapter 2, was that women

taking supplements containing low to medium amounts of vitamin C (up to 60mg/d or between 60-500mg/d) may be at lower risk than non-users and also high users (over 500mg/d); i.e. there may be a U-shaped relationship with breast cancer incidence. Additionally, the relationship between breast cancer risk and questionnaire reports of daily multivitamin use, a common source of low dose vitamin C, was examined.

8.3 Methods

8.3.1 Subjects

UKWCS baseline data was gathered between 1995-1998 from 35,367 women.[279] Further details are found in section 4.1.1.1. At baseline 62% of participants took some type of dietary supplement.

All the initial participants were re-contacted at phase 2 between 1999 and 2004, on average 4 years after recruitment; 12,453 (35%) completed a follow-up health and lifestyle questionnaire and a 4-day food diary. Of the 12,453 women who had completed both the phase 2 diary and questionnaire, 1011 women were excluded who had any registered malignant cancer prevalent at diary date (except for skin cancer). An extra 252 women who self-reported cancer and a further six were excluded because they developed breast cancer within six months of the diary date. This left 11,184 women for the vitamin C analyses, including 239 cases.

8.3.2 Determining supplement use

8.3.2.1 Vitamin C use from diary recordings

The diaries requested supplement brand, name, amount taken and dosage of any supplement taken for each of the four diary days as shown in section 4.5.3.1 of the methods chapter. As explained in section 4.5.3.2, the supplement data provided by the women were matched against a database of supplement descriptions and ingredient composition. The mean daily vitamin C intake contained in all supplement types was calculated across the total number of diary days vitamin C was taken; this meant the women were more likely to be categorised by doses actually taken than if intake had been averaged across the four diary days. (A focus towards doses actually taken was decided because averaging across the four diary days could not provide a meaningful mean vitamin C amount absorbed by the body since vitamin C is not stored in the body in large amounts. Excretion from the body in urine starts at intakes of about 100mg/day and tissue saturation is reached between 200-400 mg/day.[65] Doses above 400mg are likely to be excreted in healthy women.[65]) Frequent use of supplements containing vitamin C in this study was defined as taking these on at least three out of the four

diary days. Because excess vitamin C is excreted, the nutrient is most likely to have an effect on the body if taken daily, or almost daily. Women taking supplements containing vitamin C on only one or two diary days were categorised in the reference category as non-frequent users.

The study population was split into four intake categories: women not frequently taking supplements containing vitamin C; those frequently taking up to and including 60mg/d; frequently taking between 60mg and less than 500mg/d; frequently taking 500mg/d or more vitamin C. Substantial agreement with frequencies given in the phase 2 questionnaire, completed concurrently with the diary, was observed and reported in section 5.2 of chapter 5. Category cut-off points in the current analysis were chosen with the aim of determining whether intakes in relation to the EU recommended daily allowance (60mg/d) were beneficial or detrimental in relation to breast cancer risk. As described in section 4.5.3.2 of the methods chapter, multivitamins consumed in the UK often contain the EU recommended daily allowance (EU RDA 60mg/d),[53] and 'antioxidant' supplements usually include more than the EU RDA but less than 500mg/d. The highest intake category of 500mg/d or more was chosen because supplements containing 500mg/d and above are usually classed as high dose and these usually do not contain other micronutrients. Women's characteristics by these categories of consumption were described using means and percentages by intake category. Significant differences in characteristics between vitamin C intake categories were established using chi squared tests for trend over categorical characteristics or tests for linear trend over the continuous variables.

The breast cancer risk of users taking high doses of 1000mg/d was assessed separately. It was observed in chapter 6 that phase 2 women who had a family history of breast cancer were significantly more likely to frequently take supplements containing 1000mg/d or more of vitamin C. In line with this analysis and in contrast to the main analysis, these high dose users were compared to women who did not take 1000mg/d or more vitamin C, instead of being compared to women who did not take any vitamin C from supplements. These 502 users, however, comprised only 4.5% of the study population, providing considerably less numbers to power this secondary analysis compared to the main analysis where there were 9.5% of women in the highest intake category (≥500mg/d). This high dose of 1000mg/day has been recommended as the safe upper limit; intakes at this level and above have been linked to adverse effects, particularly gastrointestinal disturbance.[52] This level is more than 15 times the recommended daily allowance (EU RDA = 60mg/day[53]) normally found in multivitamins.

Note that dietary vitamin C could not be included in any analyses since this had not been captured electronically from the food diary details; however total fruit and vegetable intake, the main source of vitamin C, had been electronically captured from diary data, and thus was available for adjustment.

8.3.2.2 Multivitamin use from questionnaire recordings

Since multivitamins are a common source of low-dose vitamin C (as seen in section 4.5.3.2) additional analyses were produced to explore whether multivitamin use as recorded on the phase 2 questionnaire was associated with breast cancer risk. The frequency use of multivitamins with minerals, and multivitamins without minerals had been recorded as: more than daily; daily; weekly; monthly; less than monthly (as seen in section 4.5.2 in the methods chapter). The study population was split into: women currently taking multivitamins or multivitamins with minerals either daily or more than daily; and those women not taking these supplements daily. In relation to frequent use, the reporting of daily multivitamin use on the questionnaires may be more representative than 4-d diary recordings. Of the 14172 women who had completed the phase 2 questionnaire, 78 were excluded because of a missing questionnaire date, 1154 women were excluded who had any registered malignant cancer prevalent at diary date (except for skin cancer). An extra 292 women who self-reported cancer and a further 6 were excluded because they developed breast cancer within six months of the diary date. This left 12,642 women for the analysis including 269 cases.

8.3.3 Statistical analyses

Cox proportional hazard modelling was used to estimate breast cancer risks relating to vitamin C intake from supplements. Hazard ratios were adjusted for age, BMI (underweight; normal; overweight; obese), parity, estimated cumulative breast feeding (wks), age at menarche, estimated units of alcohol per week (based on a glass of wine = 2.5 unit; half pint of beer = 1 unit; glass of sherry =1 unit; spirit measure= 1.5 units), physical activity (none; light/moderate physical activity most weeks; vigorous activity for at least 20 minutes once or twice a week; vigorous activity at least 20 minutes three or more times a week), smoking status (never; past; current), highest education level (none; CSE/O' level; A' level/City & Guilds; HNC; Degree; other), current HRT use, ever used a contraceptive pill, menopausal status at phase 2 (post-menopausal; pre-menopausal) and family history of breast cancer. Vigorous activity was defined as activity causing shortness of breath, rapid heart rate and sweating. Unlike the majority of prior studies, further adjustment was made for total fruit and vegetable intake (gms/d) and intake of vitamin E from supplements (mg/d), both recorded by diary, as explained in the discussion. All variables were taken from responses to the phase 2

questionnaire except for education level which was derived from answers to the questionnaire used at baseline since this information was not provided at phase 2. Reasons for selecting confounders are discussed in section 4.7.5 of this thesis. Menopausal status at phase 2 was determined as explained in chapter 4. Red meat was adjusted for in an additional sensitivity analysis since intake was found to be significantly and inversely associated with vitamin C supplementation in chapter 6,[1] and was positively associated with breast cancer incidence in this dataset in unadjusted analysis. A further sensitivity analysis was performed to adjust for fish oil supplementation which had been inversely associated with breast cancer risk in a US cohort,[369] though not significantly associated in this cohort.

The length of follow-up was calculated for each individual in the study as the number of days since completion of the phase 2 diaries for the vitamin C analyses (or questionnaire date for the multivitamin analyses) until the date of breast cancer diagnosis or to 1st July 2008, whichever is the sooner. The median follow-up period was 7.4 years. The assumption of proportional hazards over follow-up time was met; the log-log curves for intake categories were more or less parallel for pre-menopausal, post-menopausal and total women. An increment of 60mg/day was used to test for linear trends over continuous intake.

Likelihood ratio tests were used to determine whether breast cancer risk differed by menopausal status and sub-group time-to-event analyses were produced by menopausal status. Likelihood ratio tests were also performed for socio-economic status (high: professional and managerial; intermediate; low: routine and manual) and exercise behaviour since these variables were found to have significant effect modifications on breast cancer risk with supplement use/none use in chapter 7 of this thesis.

8.3.4 Excluding family history of breast cancer

Exclusions were made in two separate sensitivity analyses for women with a family history of breast cancer, and for women estimated to be at raised genetic risk of breast cancer. A family history of breast cancer was defined as having a blood relative diagnosed with breast cancer as reported by participants at phase 2 in the questionnaire; they were questioned about cancers in mothers, fathers, sisters, brothers, aunts, and uncles. This information was also used to identify a small percentage (2%) of phase 2 UKWCS women who may have been at raised risk of developing breast cancer based on guidelines from the NICE report on familiar breast cancer.[319] As explained in detail in section 4.7.6.3.2 and 0 of this thesis, being at raised risk depended on the age of diagnosis of their relatives and whether they were

1st or 2nd degree relatives. Unfortunately these NICE guidelines[319] could not be followed in some areas for this analysis; the UKWCS had no information on daughters or sons with breast cancer. NICE guidelines also stated that women with a first degree relative diagnosed with bilateral breast cancer before the age of 50 would also be at raised risk; however cases of bilateral breast cancer in the UKWCS could not be identified.

8.4 Results

In this dataset 34% of women frequently took supplements containing vitamin C, on at least three of the four diary days; 13% (1493) took doses less than or equal to 60mg/d; 11% (1235) took between 60mg and 500mg/d; 9.5% (1063) took 500mg/d or more (Table 38) and 4.5% (503) frequently took high doses of 1000mg or more.

There was a tendency for women who took supplements containing higher doses of vitamin C (≥500mg/d) to have a lower BMI, or to consume more fruit and vegetables or less red meat (Table 38). They also were more likely to take many types of other supplements, visit alternative practitioners, have a family history of cancer, or have fewer children. Women in the low or mid-range categories (1-60mg/d and >60<500mg/d) were less likely to be educated to A' level compared to women taking higher doses or not taking any. Although 49% of women not taking any supplements containing vitamin C at phase 2 did take some other types of supplements at baseline, the percentage taking other supplements at baseline was much higher in the other vitamin C intake categories (78-86%).

Table 38 Characteristics of women at phase 2 in the UKWCS by vitamin C supplement intake group

	Vitamin C supplement intake on 3-4 diary days					
	None N=7393	1-60mg/d N=1493	>60<500mg/d N=1235	≥500mg/d N=1063	Total N=11184	P value[a]
Age (years), mean (sd)	56.5 (9.1)	55.9 (8.7)	57.5 (8.8)	56.0 (8.7)	56.5 (9.0)	0.9
BMI (kg/m^2), mean (sd)	24.9 (4.5)	24.4 (4.2)	24.6 (4.2)	24.2 (4.1)	24.7 (4.4)	<0.001
Waist, mean (sd)	81.7 (11.4)	80.7 (10.8)	81.0 (11.0)	79.9 (10.7)	81.3 (11.3)	<0.001
Age at menarche, mean (sd)	12.8 (1.6)	12.8 (1.6)	12.7 (1.6)	12.8 (1.5)	12.8 (1.6)	0.2
Age at first birth, mean (sd)	26.1 (4.6)	25.7 (4.4)	25.6 (4.7)	26.0 (4.9)	26.0 (4.6)	0.006
Number of children, mean (sd)	1.9 (1.3)	1.8 (1.3)	1.8 (1.3)	1.7 (1.3)	1.8 (1.3)	<0.001
Est. cumulative breastfeeding weeks (sd)	27 (37)	26 (36)	23 (33)	24 (36)	26 (37)	<0.001
Portions red meat /week median (IQR)	2 (0-4)	1 (0-3)	1 (0-3)	0 (0-2)	1 (0-3)	<0.001
Portions white meat /week median (IQR)	2 (0-3)	1 (0-3)	1 (0-3)	1 (0-3)	2 (0-3)	<0.001
Portions fish meat /week median (IQR)	1 (1-2)	1 (0-2)	1 (0-2)	1 (0-2)	2 (0-3)	0.02
Portions fruit /week median (IQR)	12 (7-18)	14 (8-20)	14 (7-20)	14 (8-20)	14 (7-20)	<0.001
Portions vegetables /week median (IQR)	12 (7-15)	12 (7-17)	13 (7-18)	14 (8-18)	12 (7-16)	<0.001
Diary fruit intake (grams) mean (sd)	858 (628)	943 (658)	947 (647)	1026 (702)	895 (644)	<0.001
Diary veg. intake (grams) mean (sd)	827 (452)	865 (462)	922 (506)	960 (536)	855 (536)	<0.001
Vitamin E sup. intake (mg), mean (sd)	8 (39)	25 (69)	49 (82)	75 (130)	21 (67)	<0.001
Number of supplement types taken	1.2 (1.6)	2.7 (1.7)	3.7 (2.1)	4.5 (2.4)	2.0 (2.1)	<0.001
Professional or managerial n(%)	2981 (41)	592 (40)	510 (42)	455 (43)	4538 (41)	0.03
Educated to 'A'-level or above n(%)	4307 (60)	807 (55)	691 (57)	641 (61)	6446 (59)	0.7
No children n(%)	1492 (20)	307 (21)	290 (23)	300 (28)	2389 (21)	<0.001
Ever used contraceptive pill n(%)	4860 (68)	1030 (70)	832 (69)	752 (72)	7474 (68)	0.002
Current HRT use n(%)	1481(20)	343 (23)	300 (24)	254 (24)	2378 (21)	<0.001
Post-menopausal at phase 2	4952 (67)	973 (65)	894 (72)	705 (66)	7524 (67)	0.2
Ex-smoker n(%)	2151 (29)	438 (30)	398 (32)	377 (36)	3364 (30)	<0.001
Drinks alcohol less than once a week	2450 (33)	512 (34)	423 (34)	371 (35)	3756 (34)	0.2
Vigorous activity >=3/week n(%)	1241 (18)	294 (21)	275 (24)	236 (24)	2046 (19)	<0.001
Bowel movements more than once/day	1226 (17)	279 (19)	258 (22)	237 (23)	2000 (18)	<0.001
Takes any supplements at baseline n(%)	3312 (49)	1054 (78)	924 (82)	842 (86)	6132 (60)	<0.001
Takes more than 4-10 supplement types	560 (8)	324 (22)	479 (39)	476 (45)	1839 (16)	<0.001
Visits alternative practitioner >=5 /year	463 (6)	119 (8)	132 (11)	159 (15)	873 (8)	<0.001
Family history of breast cancer n(%)	1362 (18)	261 (17)	242 (20)	211 (20)	2076 (19)	0.2
Family history of any cancer n(%)[b]	4232 (57)	851 (57)	764 (62)	638 (60)	6485 (55)	0.006

[a]p= test for trend over continuous variable, sd =standard deviation

8.4.1 Breast cancer risk and use of supplements containing vitamin C

Compared to women who did not take supplements containing vitamin C there was no evidence of an association between breast cancer risk and regular intake of supplements containing vitamin C for total women for any of the intake categories in the unadjusted and adjusted analyses (Table 39). Neither was there evidence of a dose-response association across these supplement users (HR=0.98 per 60mg/d; 95% CI: 0.94, 1.02; p_{trend}=0.3) or when non-users of these supplements were included. Excluding women with a family history of breast cancer or women at raised risk of breast cancer made little difference to the results (Table 40 and Table 41). In the tests for effect modification, menopausal status was found to significantly modify the effect of vitamin C supplement intake groups on breast cancer incidence (adjusted p=0.02). Socio-economic status was also found to modify the associations of risk with vitamin C intake (adjusted p=0.03) but exercise behaviour did not (adjusted p=0.7). Unfortunately the number of cases was considered too low to power sub-analyses by socio-economic status.

In the subgroup analysis by post-menopausal status no significant associations were found relating to dose-response relationships (HR=1.00 per 60mg/d; 95% CI: 0.96, 1.05; p_{trend}=1.0) or intake category.

Dose-response relations for pre-menopausal women were also non-significant. However pre-menopausal women who frequently took supplements containing less than or equal to 60mg/d of vitamin C were over twice as likely to develop breast cancer (HR=2.37; 95% CI: 1.32, 4.27; p=0.004) compared to women not taking any supplements containing vitamin C, in the multivariate model after adjustment of the majority of potentially confounding factors. The odds increased further after adjustment by fruit and vegetable intake and vitamin E supplement intake recorded by diary (HR=2.56; 95% CI: 1.41, 4.66; p=0.002). Additional adjustments for red meat intake strengthened the risks slightly e.g. for all pre-menopausal women in the low dose group (HR=2.78; 95% CI: 1.52, 5.09; p=0.001), and adjusting for daily fish oil supplementation made little difference to hazard ratios (both not tabled). As observed in Table 40 and Table 41, a significant positive association for pre-menopausal women who frequently took supplements containing less than or equal to 60mg/d of vitamin C was also found in sensitivity analyses excluding women with a family history of breast cancer (HR=3.02; 95% CI: 1.53, 5.96; p=0.002) and when only women at raised risk of breast cancer (HR=2.78; 95% CI: 1.52, 5.09; p=0.001) were excluded.

Table 39 Breast cancer risks of women supplementing with vitamin C recorded by diaries at phase 2 in the UKWCS

Supplement vitamin C intake[a]	Cases/ Non-cases	Unadjusted	Multivariate[b]	Fully adjusted[c]
		HR (95% CI)	HR (95% CI)	HR (95% CI)
Total women				
No vitamin C	156/7237	1	1	1
1-60mg/d	35/1458	1.10 (0.76, 1.58)	1.16 (0.77, 1.75)	1.15 (0.76,1.74)
>60<500mg/d	29/1206	1.10(0.74, 1.64)	1.17 (0.75, 1.82)	1.15 (0.73,1.80)
≥500mg/d	19/1044	0.83 (0.51, 1.33)	0.77 (0.43, 1.37)	0.75 (0.41,1.35)
Per 60mg/d (users)	83/3708	1.00 (0.97, 1.02)	0.98 (0.94, 1.02)	0.98 (0.94, 1.02)
p_{trend}		0.8	0.3	0.3
Per 60mg/d (all)	239/10945	1.00 (0.99, 1.04)	0.99 (0.96, 1.02)	0.99 (0.95, 1.02)
p_{trend}		0.5	0.4	0.4
Post-menopausal				
No vitamin C	112/4840	1	1	1
1-60mg/d	15/958	0.67 (0.39, 1.14)	0.67 (0.36, 1.26)	0.65 (0.34, 1.23)
>60<500mg/d	25/869	1.22 (0.79, 1.89)	1.25 (0.77, 2.05)	1.21 (0.74, 2.00)
≥500mg/d	14/691	0.86 (0.49, 1.50)	0.77 (0.38, 1.53)	0.70 (0.34, 1.43)
Per 60mg/d (users)	54/2518	1.02 (0.99, 1.05)	1.00 (0.96, 1.05)	1.00 (0.96, 1.05)
p_{trend}		0.2	0.8	1.0
Per 60mg/d (all)	166/7358	1.02 (0.99, 1.04)	1.00 (0.96, 1.03)	1.00 (0.96, 1.03)
p_{trend}		0.2	0.9	0.8
Pre-menopausal				
No vitamin C	44/2397	1	1	1
1-60mg/d	20/500	2.14 (1.26, 3.63)	2.37 (1.32, 4.27)	2.56 (1.41, 4.66)
>60<500mg/d	4/337	0.64 (0.23, 1.79)	0.83 (0.29, 2.34)	1.01 (0.34, 2.99)
≥500mg/d	5/353	0.76 (0.30, 1.91)	0.77 (0.27, 2.20)	0.91 (0.31, 2.65)
Per 60mg/d (users)	29/1190	0.93 (0.86, 1.01)	0.91 (0.81, 1.01)	0.92 (0.82, 1.02)
p_{trend}		0.08	0.07	0.1
Per 60mg/d (all)	73/3587	0.98 (0.93, 1.03)	0.96 (0.89, 1.03)	0.97 (0.90, 1.04)
p_{trend}		0.4	0.2	0.3

Table 40 Breast cancer risks of women supplementing with vitamin C recorded by diaries in the UKWCS, excluding women with a family history of breast cancer

Supplement vitamin C intake	Cases/ Non-cases	Unadjusted		Fully adjusted[a]	
		HR	(95% CI)	HR	(95% CI)
Total women					
No vitamin C taken 3-4 days	115/5916	1		1	
1-60mg/d	30/1202	1.26	(0.85, 1.89)	1.22	(0.77, 1.94)
>60<500mg/d	24/969	1.26	(0.81, 1.96)	1.20	(0.72, 2.00)
≥500mg/d	15/837	0.90	(0.53, 1.55)	0.75	(0.38, 1.48)
Per 60mg/d (users)	69/3008	0.98	(0.94, 1.02)	0.98	(0.94, 1.03)
p_{trend}			0.4		0.4
Per 60mg/d (all)	184/8924	1.01	(0.98, 1.03)	0.99	(0.96, 1.03)
p_{trend}			0.6		0.6
Post-menopausal					
No vitamin C taken 3-4 days	85/3925	1		1	
1-60mg/d	13/787	0.75	(0.42, 1.35)	0.67	(0.33, 1.36)
>60<500mg/d	20/694	1.32	(0.81, 2.14)	1.20	(0.68, 2.14)
≥500mg/d	11/553	0.90	(0.48, 1.69)	0.68	(0.30, 1.54)
Per 60mg/d (users)	44/2034	1.01	(0.97, 1.04)	1.01	(0.96, 1.06)
p_{trend}			0.7		0.7
Per 60mg/d (all)	129/5959	1.02	(0.99, 1.05)	1.00	(0.96, 1.04)
p_{trend}			0.2		0.9
Pre-menopausal					
No vitamin C taken 3-4 days	30/1991	1		1	
1-60mg/d	17/415	2.67	(1.47, 4.84)	3.02	(1.53, 5.96)
>60<500mg/d	4/275	0.95	(0.34, 2.70)	1.32	(0.43, 3.99)
≥500mg/d	4/284	0.91	(0.32, 2.59)	1.01	(0.29, 3.47)
Per 60mg/d (users)	25/974	0.91	(0.82, 1.01)	0.87	(0.75, 1.01)
p_{trend}			0.07		0.07
Per 60mg/d (all)	55/2965	0.97	(0.90, 1.03)	0.96	(0.88, 1.05)
p_{trend}			0.3		0.4

[a]Adjusted for age, BMI (grouped), age at menarche, parity, estimated alcohol units consumed per week, activity intensity and frequency, smoking status (never, ex, current), education level, current HRT use, ever used a contraceptive pill, estimated cumulative breast feeding, total fruit and vegetable intake recorded by diary (gms/d) and vitamin E supplements use (mg/d) recorded by diary

Table 41 Breast cancer risks of women supplementing with vitamin C recorded by diaries in the UKWCS, excluding women at raised risk of breast cancer

Supplement vitamin C intake	Cases/ Non-cases	Unadjusted		Fully adjusted[a]	
		HR	(95% CI)	HR	(95% CI)
Total women					
No vitamin C taken 3-4 days	147/7103	1		1	
1-60mg/d	35/1425	1.17	(0.81, 1.69)	1.24	(0.81, 1.88)
>60<500mg/d	28/1177	1.14	(0.76, 1.70)	1.19	(0.75, 1.90)
≥500mg/d	18/1023	0.83	(0.51, 1.36)	0.81	(0.45, 1.47)
Per 60mg/d (users)	81/3625	0.98	(0.95, 1.01)	0.98	(0.94, 1.02)
p_{trend}			0.3		0.3
Per 60mg/d (all)	228/10728	1.00	(0.98, 1.02)	0.98	(0.96, 1.02)
p_{trend}			0.9		0.5
Post-menopausal					
No vitamin C taken 3-4 days	106/4739	1		1	
1-60mg/d	15/938	0.70	(0.41, 1.21)	0.70	(0.37, 1.33)
>60<500mg/d	24/851	1.24	(0.80, 1.93)	1.26	(0.76, 2.11)
≥500mg/d	13/680	0.84	(0.47, 1.49)	0.77	(0.37, 1.57)
Per 60mg/d (users)	52/2469	1.00	(0.96, 1.04)	1.00	(0.96, 1.05)
p_{trend}			1.0		0.9
Per 60mg/d (all)	158/7208	1.01	(0.98, 1.04)	1.00	(0.96, 1.04)
p_{trend}			0.5		0.9
Pre-menopausal					
No vitamin C taken 3-4 days	41/2364	1		1	
1-60mg/d	20/487	2.32	(1.36, 3.96)	2.78	(1.52, 5.09)
>60<500mg/d	4/326	0.70	(0.25, 1.96)	1.08	(0.36, 3.19)
≥500mg/d	5/343	0.82	(0.33, 2.08)	1.00	(0.34, 2.92)
Per 60mg/d (users)	29/1156	0.93	(0.86, 1.01)	0.92	(0.82, 1.02)
p_{trend}			0.08		0.1
Per 60mg/d (all)	70/3520	0.98	(0.93, 1.03)	0.97	(0.91, 1.04)
p_{trend}			0.5		0.4

[a]Adjusted for age, BMI (grouped), age at menarche, parity, estimated alcohol units consumed per week, activity intensity and frequency, smoking status (never, ex, current), education level, current HRT use, ever used a contraceptive pill, estimated cumulative breast feeding, family history of breast cancer, total fruit and vegetable intake recorded by diary (gms/d), and vitamin E supplements use (mg/d) recorded by diary

No associations were found between breast cancer risk and frequent use of supplements containing 1000mg or more vitamin C compared with women not taking these amounts. Adjusted hazard ratios for pre-menopausal women were HR=0.37 (95% CI: 0.05, 2.70) and for post-menopausal women were HR=1.08 (95% CI: 0.47, 2.52); none were close to significance (Table 42). The number of cases in these analyses was extremely low.

Table 42 Breast cancer risks of women supplementing with 1000mg/d or more vitamin C (N=502) compared to women not taking these doses at phase 2 in the UKWCS

Supplement vitamin C intake	Cases /Non-cases	Unadjusted HR (95% CI)	Multivariate[a] HR (95% CI)	Fully adjusted[b] HR (95% CI)
Total women				
<1000mg/d[c]		1	1	1
1000mg or more (y/n)	10/492	0.92 (0.49, 1.73)	0.88 (0.41, 1.87)	0.85 (0.39, 1.84)
Post-menopausal				
<1000mg/d[c]		1	1	1
1000mg or more (y/n)	8/310	1.14 (0.56, 2.32)	1.17 (0.51, 2.66)	1.08 (0.47, 2.52)
Pre-menopausal				
<1000mg/d[c]		1	1	1
1000mg or more (y/n)	2/182	0.52 (0.13, 2.14)	0.32 (0.04, 2.34)	0.37 (0.05, 2.70)

There were no substantial differences in any of the above results if self-reported prevalent cancers were not excluded.

8.4.2 Breast cancer risk and multivitamin use

From the questionnaire data on supplement use it was observed that compared to women who did not take multivitamin supplements every day there was no evidence of significant associations between breast cancer risk and daily use of multivitamin supplements for total women in the unadjusted and adjusted analyses (Table 43) or for post-menopausal women. However both the unadjusted and adjusted results for pre-menopausal women showed a moderate positive association and the unadjusted result was close to significance (HR=1.55; 95% CI: 0.98, 2.45 and HR=1.51; 95% CI: 0.90, 2.54 respectively). Results did not change substantially when women with a raised risk of breast cancer were excluded (Table 45). When women with a family history of breast cancer were excluded the risk for pre-menopausal multivitamin users was strengthened, the unadjusted results reached significance and the adjusted result was close to significance (unadjusted HR=1.81; 95% CI: 1.08, 3.05 and adjusted HR=1.79; 95% CI: 0.99, 3.22, Table 44).

Results did not alter substantially when women who recorded daily vitamin C supplement use on the phase 2 questionnaires were excluded (results not shown).

Table 43 Breast cancer risks of women using multivitamins recorded by questionnaire at phase 2 in the UKWCS

Daily multivitamin intake	Cases/	Unadjusted		Adjusted[a]	
	Non-cases	HR	(95% CI)	HR	(95% CI)
Total women					
Not taking daily multivitamins	198/9362	1		1	
Taking daily multivitamins	71/3011	1.09	(0.81, 1.43)	1.12	(0.81, 1.54)
Post-menopausal					
Not taking daily multivitamins	149/6396	1		1	
Taking daily multivitamins	42/1901	0.93	(0.66, 1.31)	0.95	(0.63, 1.42)
Pre-menopausal					
Not taking daily multivitamins	49/2966	1		1	
Taking daily multivitamins	29/1110	1.55	(0.98, 2.45)	1.51	(0.90, 2.54)

[a]Adjusted for age, BMI (grouped), age at menarche, parity, estimated alcohol units consumed per week, activity intensity and frequency, smoking status (never, ex, current), education level, current HRT use, ever used a contraceptive pill, estimated cumulative breast feeding, family history of breast cancer and total fruit and vegetable intake recorded by diary (gms/d).

Table 44 Breast cancer risks of women using multivitamins recorded by questionnaire at phase 2 in the UKWCS, excluding women with a family history of breast cancer

Daily multivitamin intake	Cases/	Unadjusted		Adjusted[a]	
	Non-cases	HR	(95% CI)	HR	(95% CI)
Total women					
Not taking daily multivitamins	151/7668	1		1	
Taking daily multivitamins	59/2447	1.20	(0.89, 1.62)	1.25	(0.88, 1.78)
Post-menopausal					
Not taking daily multivitamins	116/5204	1		1	
Taking daily multivitamins	35/1537	1.00	(0.69, 1.46)	1.05	(0.67, 1.65)
Pre-menopausal					
Not taking daily multivitamins	35/2464	1		1	
Taking daily multivitamins	24/910	1.81	(1.08, 3.05)	1.79	(0.99, 3.22)

[a]Adjusted for age, BMI (grouped), age at menarche, parity, estimated alcohol units consumed per week, activity intensity and frequency, smoking status (never, ex, current), education level, current HRT use, ever used a contraceptive pill, estimated cumulative breast feeding and total fruit and vegetable intake recorded by diary (gms/d).

Table 45 Breast cancer risks of women using multivitamins recorded by questionnaire at phase 2 in the UKWCS, excluding women at raised risk of breast cancer

Daily multivitamin intake	Cases/	Unadjusted		Adjusted[a]	
	Non-cases	HR	(95% CI)	HR	(95% CI)
Total women					
Not taking daily multivitamins	190/9193	1		1	
Taking daily multivitamins	68/2938	1.09	(0.83, 1.44)	1.16	(0.84, 1.61)
Post-menopausal					
Not taking daily multivitamins	143/6271	1		1	
Taking daily multivitamins	40/1860	0.92	(0.65, 1.31)	0.97	(0.64, 1.48)
Pre-menopausal					
Not taking daily multivitamins	47/2922	1		1	
Taking daily multivitamins	28/1078	1.58	(0.99, 2.52)	1.60	(0.94, 2.70)

[a]Adjusted for age, BMI (grouped), age at menarche, parity, estimated alcohol units consumed per week, activity intensity and frequency, smoking status (never, ex, current), education level, current HRT use, ever used a contraceptive pill, estimated cumulative breast feeding and total fruit and vegetable intake recorded by diary (gms/d) and family history of breast cancer.

8.5 Discussion

This analysis of phase 2 of the UKWCS found little evidence of associations between breast cancer incidence and vitamin C supplementation per se, when compared to women not consuming supplements containing vitamin C, as recorded by food diaries. Specifically, there was no evidence of a dose-response relationship for total, pre- or post-menopausal women. Additionally there was no evidence of a U-shaped relationship that was initially hypothesised to occur between vitamin C intake and breast cancer risk. Conversely, the Danish nested-case control study found evidence of a slight dose-response relationship: a significant increase in risk per additional 100mg/d of vitamin C intake from supplements for postmenopausal women (HR=1.06; 95% CI: 1.01, 1.13) (Table 36).[245]

No significant associations were found for total women or for post-menopausal women in any of the intake categories. This is consistent with US results from the Nurses Health Study, the Iowa Women's Health Study and the RCT of the Women's Antioxidant Cardiovascular Study,[14 242 247] and also the most recent findings from a Danish cohort.[273] However, it is in contrast with another US study, the Women's Health Initiative, and also with the Canadian and a nested case-control analysis of the Danish study which did find increased risks for post-menopausal women taking vitamin C supplements.[245 267 270] The latter two studies used case-control methodology which unlike full cohort studies could have been affected by biased selection of controls.[245 267] The Women's Health Initiative had the highest average age of all the cohorts (64 years), involving women as old as 79,[270] and their positive associations (shown in Table 36) could indicate that high doses of vitamin C may promote the progression of cancer in the later stages of the disease. Differences in results cannot be explained by the number of cases involved to power the analyses since the two largest studies, the Nurses Health Study and Women's Health Initiative, produced conflicting results.[247 270] The length of follow-up for all but one of the studies, including ours, was on average between four and nine years; the average follow-up for the Nurses Health Study which found no association was considerably longer: 14 years.[247] Longer follow-up studies may be less likely to find associations if supplement usage did not remain relatively constant. Another explanation for the null results of studies for total and post-menopausal women, is that vitamin C may not affect hormonal mechanisms of cancer initiation or development; as discussed in Chapter 3, levels of endogenous sex hormones are one of the main risk factors for post-menopausal breast cancer.[147]

In the current study, however, for pre-menopausal women an increased risk of breast cancer was associated with the lowest category of intake: below or equal to 60mg/d.

Similar to most studies, the classification of pre-menopausal women was at recording date rather than diagnosis or censor date, meaning that pre-menopausal women taking supplements in this category appear to have an increased risk of later developing breast cancer; whether this was when they were pre- or post-menopausal. Although vitamin C intake has been associated with increased breast density, an increased risk factor for breast cancer, this result for low dose vitamin C is difficult to explain biologically. The result may be due to other characteristics of these pre-menopausal women in the UKWCS. Indeed, in a previous UKWCS analysis of pre-menopausal women another result was difficult to account for; vegetarians were found to have a significantly higher breast cancer risk than low meat consumers.[82] The authors suggested a family history of breast cancer may have increased a tendency for vegetarianism as well as an increased risk of breast cancer. [82] However, the significant association in the current analysis is unlikely to be attributed to women genetically at high risk since the results were adjusted for women with a family history of breast cancer. Furthermore, excluding these women in sensitivity analyses increased the risk further. The level of significance was high therefore this result may not have occurred by chance despite the multiple testing through sub-analyses, although the number of cases was low and could have produced spurious results. Previously, the Canadian study reported a significant moderate increase in risk evident for intakes above 250mg/d compared to women with no intake for a subgroup combining pre- and peri-menopausal women.[267] No previous study has analysed pre-menopausal women consuming intakes as low as 60mg/d vitamin C. In the UKWCS analysis there was no evidence of significant associations for pre-menopausal women supplementing with vitamin C above the EU recommended daily intake of 60mg/d. However, since there were less than 10 cases in these higher intake groups, the analysis is likely to have been underpowered to find an effect for pre-menopausal women. In chapter 7 of this thesis a non-significant increased risk of breast cancer was found for pre-menopausal women who took any type of supplement at baseline, compared to those who did not; the use of supplements containing low levels of vitamin C may have contributed to this result. The reason the results did not reach significance, may have been due to dilution by use of other supplement types which did not have the same effect.

Although the use of supplements containing 1000mg/d of vitamin C was associated with a family history of breast cancer in the UKWCS in chapter 6 of this thesis,[1] there was no evidence in the current analysis that women taking these high doses had a significantly different risk of breast cancer compared to women not taking them, even when adjusting for family history of breast cancer. Due to the very small number of women who developed breast cancer and who had taken doses more than or equal to 1000mg/d, the analysis is likely to have been underpowered to find an effect. Only one

previous study assessed intakes specifically above 1000mg/d, the Iowa Women's Health Study,[247] which showed a non-significant inverse association for post-menopausal women with wide confidence intervals (HR=0.77; 95% CI: 0.50, 1.17).

Women with prevalent self-reported cancers were excluded in the UKWCS analyses, which meant that some women who had non-malignant cancers may have been inadvertently excluded from the analyses. However sensitivity analyses which included prevalent self-reported cancers did not produce substantially different results. From the reports of previous studies it was not clear whether their exclusions for prevalent cancers were self-reported or not.

It is unlikely that differences in results between this and previous studies were due to variations in adjustments for potential confounding factors; the current analyses controlled for the majority of those used in previous studies (as shown in Table 37). However, unlike previous studies, no adjustment was made for energy intake in the UKWCS since this data was not electronically captured for all women. Nevertheless two studies reported that adjustments for energy intake in sensitivity analyses did not alter their results.[242 245] Contrary to previous studies no adjustment was made for age at first live birth in the UKWCS analyses due to large numbers of missing data; instead cumulative time of breast feeding and parity was used which have been found to be a good predictor of breast cancer risk.[167] Of prior studies that reported an increase in risk for post-menopausal women, the Women's Health Initiative adjusted for dietary vitamin C intake,[270] and the Danish study adjusted for vitamin A and E supplement intake;[245] this was in contrast to the other studies detailed in Table 37. Although dietary vitamin C and supplement vitamin A was not available in the UKWCS, further adjustments were made for vitamin E supplement intake and for dietary fruit and vegetable intake, which has been found to correlate well with plasma vitamin C;[67] these, however, did not substantially alter the results. Adjustment were made for these variables since vitamin C supplement use has been significantly associated with fruit and vegetable consumption[1] and due to vitamin C's role in the regeneration of vitamin E[76] the latter could potentially modulate vitamin C's activity relating to cancer development. The majority of supplements containing vitamin C at medium to low doses such as those classed as multivitamins and antioxidants also contain vitamin E. However, as discussed in 4.7.6.2, vitamin E may be a mediator between vitamin C and breast cancer incident so adjusting for it could create bias, therefore this was only undertaken in the sensitivity analyses.

Fish oil supplements, which are taken by 26.5 % of UKWCS women (figure 2, chapter 2), have been inversely associated with breast cancer risk;[369] and in a recent UK survey half of women over 55 years of age reported taking cod-liver oil compared to a

quarter of younger women.[26] However, additional adjustment for daily use of fish oil supplements did not change the results for the breast cancer and vitamin C analysis in the UKWCS. Furthermore, although there was evidence of a reduced breast cancer risk from daily use of fish oil supplements in the UKWCS, it was not significant. Previous studies in Table 36 did not adjust for fish oil supplements.

Since the body has limited capacity to absorb and store this water soluble vitamin; [65] daily vitamin C supplement use is plausibly more likely to have an effect on the body than infrequent intake. Therefore only women recording at least 3 out of 4 days intake of supplements containing vitamin C were classed as frequent users in this analysis. Regardless of this, the diary recordings may represent short-term episodic intake, particularly because vitamin C is promoted for reducing seasonal common colds and no seasonal adjustments to the results have been made. Nevertheless, the majority of vitamin C users (82%) in the UKWCS reported taking a supplement on average four years earlier, however the type taken is unknown and respective seasons were not assessed.[1] Therefore due to its short-term nature, diary data may not be more superior than questionnaire data for elucidating the relationship between breast cancer risk and long-term use of supplements, unless repeated measures are taken.[370] Unfortunately baseline details of supplements used had not been electronically captured.

Vitamin C contained in a wide variety of supplement types recorded by diary was determined using a database of brand specific vitamin C content and extensive cleaning was done (as described in the methods chapter). Therefore intake assessed in relation to breast cancer risk in the UKWCS may be more accurate than other approaches used in some of the previous studies. Only the Nurses Health Study, which found no associations, and the two Danish studies, which found conflicting associations, appeared to have used a database of supplement ingredients.[245 247 273] In contrast some of the other studies appear have estimated or not accounted for the vitamin C content of many supplements used, and this may have reduced their ability to find a dose-response affect if one existed. Nevertheless, comparisons between UKWCS diary data and questionnaire data in section 5.2 of chapter 5 indicated that taking account of multivitamins and antioxidants, as well single supplement vitamin C, may pick up a large proportion of supplements containing vitamin C.

As seen in section 5.2.3 of chapter 5, the vitamin C responses on the phase 2 questionnaire had reasonable agreement with the recordings of supplements containing 500mg/d or more vitamin C from diaries. Therefore, it is possible that high dose users may be defined more accurately by combining recordings from both the phase 2 questionnaire and diaries. However, since some reporting was not consistent,

this would result in fewer high dose users in the analysis and therefore would lower the power to detect significant associations.

Previous chapters have provided evidence that women in the low intake category (less than or equal to 60mg/d of vitamin C) were likely to be taking multivitamins which contained a wide range of other micronutrients (as reported in section 4.5.3.2 and section 5.2.3.1, though the Kappa agreement was only weak to moderate Κ=0.41(0.40-42)). Conversely, women in the reference group did not take supplements containing any vitamin C and therefore were unlikely to be frequently taking multivitamins, most of which contain vitamin C. Furthermore, it was also observed from the questionnaire responses that 22% of women in the low intake category took four to ten types of supplements (though women in the highest vitamin C intake category were twice as likely to do this). Therefore, one or more, or an interaction of these micronutrients in multivitamins or from the variety of supplements taken, may have had an effect on breast cancer risk for premenopausal women rather than the low dose of vitamin C per se. For instance iron and vitamin C together may exert a pro-oxidant effect via the Fenton reaction,[76] or ingredients other than vitamin C may have an effect. A clearer way to assess the effects of vitamin C intake per se without confounding by other supplement ingredients would be by analysing the dietary vitamin C intake of non-supplement users (as reported in chapter 9) or by implementing an RCT using a vitamin C supplement verse a placebo where the consumption of other supplements would be randomised between intervention groups.

A higher proportion of pre-menopausal compared to post-menopausal women took multivitamin supplements in the UKWCS (as seen in Table 43). Similarly, the 2008 UK survey found multivitamins were more likely to be used by 16-54 year old supplement users than those aged 65 plus.[26] The UKWCS adjusted analysis of multivitamin use recorded by questionnaire at phase 2 showed a moderate non-significant increased risk of breast cancer for pre-menopausal women. This strengthened and became close to significance when women with a family history of breast cancer were excluded. One explanation for the association with multivitamins being non-significant and weaker than the low dose vitamin C results may be because the former analysis did not exclude higher doses of vitamin C, whereas the latter did. This may indicate that taking multivitamins without also taking medium to high doses of vitamin C may increase breast cancer risk. A possible biological explanation is, perhaps, at low concentrations in the presence of certain nutrients vitamin C is a pro-oxidant but at high concentrations it is an antioxidant. However, the multivitamin use results did not alter substantially when women who recorded daily vitamin C supplement use on the phase 2 questionnaires were excluded, who were likely to be consuming high doses. Further

analyses could be undertaken to explore the risks for women specifically reporting multivitamin use in the diaries.

Results of a recent Swedish study show that multivitamin use was significantly associated with a 22% increased risk of breast cancer for all women using them for over 3 years.[261] The authors suggest that folic acid in the multivitamins may have promoted cancer, since there is no mandatory folic acid fortification of food in Sweden. Conversely, the US does fortify food with folic acid and several US studies, including the Nurses Health Study, have not found an increased risk of breast cancer with multivitamin use.[247] [371] A recent meta-analysis found no evidence of a significant association between multivitamin use and breast cancer risk.[260] Since fortification is not mandatory in the UK, folic acid in multivitamins could explain the increased risk for UKWCS pre-menopausal women who took supplements containing low vitamin C doses, though the multivitamin results do not strongly support this. Despite many multivitamins containing folic acid, very few women reported on the UKWCS questionnaire that they took folic acid supplements; therefore the questionnaire data would be underpowered to find an association with breast cancer incidence. However, an analysis of associations between breast cancer risk and folic acid use by dose ascertained from the phase 2 diary supplement database may be able to illuminate this hypothesis further.

To conclude, this UK study found little evidence that supplementation with vitamin C per se was beneficial or detrimental in relation to breast cancer incidence in UK women, even at high doses. However the results indicate that frequent use of supplements containing less than or equal to 60mg/d vitamin C, or the frequent use of multivitamins, may increase breast cancer risk in pre-menopausal women. Although these results may be spurious, it would be prudent to conduct further research on supplement use in the UK, particularly relating to multivitamins, to identify whether some micronutrients or combination of micronutrients might produce an increase in breast cancer risk in pre-menopausal users.

CHAPTER 9

9 Is breast cancer incidence associated with dietary vitamin C or fruit and vegetable intake? Does supplement use modify these relationships? A baseline analysis of the UKWCS

9.1 Summary

Objectives: To determine whether dietary vitamin C or fruit and vegetable intake at baseline in the UKWCS is associated with breast cancer risk. Since the use of supplements may have obscured associations in previous studies, sub-analyses by general supplement use were also undertaken.

Method: This time-to-event analysis of 33,520 middle-aged women at baseline in the UK Women's Cohort Study (UKWCS) calculated Hazard Ratios (HRs) over a median follow-up period of 11.2 years for 989 registered incident breast cancers in relation to fruit and vegetable and dietary vitamin C intake recorded by Food Frequency Questionnaire (FFQ) and cross-check questions. Sub-analyses by supplement use and menopausal status were produced. Additionally, non-linear relationships between intakes of 60mg-350mg/d vitamin C were modelled using restricted cubic splines.

Results: All hazard ratios were non-significant relating to intake and breast cancer risk. Furthermore, in tests for interactions, relationships were not significantly different between women who took any type of supplement and non-users for total fruit and vegetable servings (p =0.6) and for dietary vitamin C intake (p=0.2). Additionally, no significant interactions were found relating to menopausal status or family history of breast cancer. Although breast cancer risks appeared to increase with increasing dietary vitamin C intake for non-supplement users, and specifically post-menopausal women (HR per 60mg/d vitamin C HR=1.05; 95% CI: 0.94, 1.19), risks were non-significant. In the tabled analysis by fifths of intake, a U-shaped relationship was apparent for these women when vitamin C was expressed as nutrient density, but this was less clear on the graphed models.

Conclusion: There was no evidence of significant associations between breast cancer incidence and intake of fruit and vegetables or dietary vitamin C derived from FFQ in

the UKWCS. There was also no statistically significant evidence that supplement use modified the relationship between breast cancer risk and intake.

9.2 Introduction

Fruit and vegetables are the main dietary sources of vitamin C,[67] whose antioxidant properties are believed to protect against DNA damage and cancer development by decreasing reactive oxygen species (ROS) that may cause DNA damage.[74] Additionally, many other bioactive compounds such as phytochemicals in fruit and vegetables are thought to protect against cancer.[7] Although it is widely believed that high intakes of fruit and vegetables reduce cancer risk, there is no conclusive evidence for this or for breast cancer specifically.[7 11 12] The 2007 WCRF report stated there was probable rather than convincing evidence that they decreased the risk of many cancers such as mouth, oesophagus, stomach and colorectal cancer, but not breast cancer.[7] Furthermore, a recent EPIC analysis found only a very small inverse association between intake of total fruits and vegetables and total cancer risk.[212] Initial findings from retrospective case-control studies reported a reduction in breast cancer risk with increasing intake by as much as 25%.[8 213] However, results of a subsequent pooled analysis of cohort studies showed that fruit and vegetable consumption during adulthood was not significantly associated with breast cancer risk.[12]

Similarly, as stated in section 3.6.4.1 of the literature review, the meta-analyses in the WCRF 2007 report of 14 case-control studies of dietary vitamin C intake provides evidence of a statistically significant protective association for breast cancer (RR=0.88, 95% CI: 0.84-0.92, per 100mg/day); analyses comparing high versus low intake categories, plus pre-menopausal and post-menopausal analyses support this.[7] However, overall cohort results were inconclusive.[239 240 242 243 245-247 264-267] In the 2007 WCRF report no significant associations were produced for dietary vitamin C in the post-menopausal women meta-analyses of three cohort studies (HR=1.15 per 100mg/d, 95% CI: 0.92-1.43), [7 245 246 265] or in the high versus low intake analysis of two additional studies.[242 247] Differences in findings between study types may be due to selection or recall bias in the retrospective case-control studies. Most recently no significant evidence was found between dietary intake of vitamin C and breast cancer risk for either pre- or post-menopausal women in the EPIC study, involving 7,502 breast cancer cases,[244] or for post-menopausal women in a Danish cohort analysed by Roswall et al. (2010).[273] A recent analysis of the Women's Health Initiative Observational Study found that dietary vitamin C was not associated with breast cancer risk, although weak positive associations were found for breast cancer for total vitamin C and supplemental vitamin C intake in the highest versus lowest intake analyses.[270]

In contrast to the overall WCRF meta-analysis results, Nissen et al. (2003) in an earlier nested case-control analysis of the Danish 'Diet, Cancer and Health' cohort (418 case and 394 controls) did find a significant doubling in risk per 100mg/d increase in intake for post-menopausal women (RR= 2.06, 95% CI: 1.45-2.91).[245] Unlike other studies mentioned, this dietary analysis was restricted to women who consumed vitamin C through both diet and supplements; women who did not consume vitamin C supplements were excluded.[245] This nested case-control study followed Danish, postmenopausal women for 4.7 years; a shorter period than the other studies. Unlike the other studies in the WCRF meta-analysis, adjustment was made for intake of the other antioxidant vitamins A and E.

As seen in previous chapters supplement users in general have healthier behaviours than non-users,[27] therefore excluding all types of supplement users from analyses will reduce potential confounding and provide a clearer picture of associations between dietary vitamin C intake and risk. There is a lack of evidence relating to non-supplement users, other than non-significant findings reported above for the recent EPIC study, which did exclude all supplement users in their dietary vitamin C analysis.[244] When vitamin C supplement users were excluded in the Danish nested case-control study reported by Nissen et al. (2003), this reduced the association substantially from a doubling in risk to RR=1.54 (95% CI: 0.80-2.96 per 100mg/day), which also became non-significant.[245] No analysis, however, was produced excluding all supplement users. The more recent analysis of the Danish study by Roswall et al. (2010) also did not exclude supplement users.[273] An analysis of the Iowa Women's Health Study excluded women who took antioxidant supplements A,C and E and this increased the point estimate in the high verse low calculation from an non-significant decrease (HR=0.88 95% CI: 0.70-1.11) to a non-significant increase in risk (HR=1.06 95% CI: 0.77-1.47).

Furthermore, it is possible that the effect of dietary intake on cancer risk may be different for women genetically predisposed to developing breast cancer. In a test for interaction Zhang et al. (1999)[247] found that family history of breast cancer significantly modified the relationship between dietary vitamin C and breast cancer risk ($p<0.05$). Moreover pre-menopausal women with a family history of breast cancer whose dietary vitamin C intake was in the highest fifth were found to have a significantly reduced risk of breast cancer compared to those in the lowest fifth; RR=0.37 (95% CI: 0.17, 0.80). However, no association was apparent when vitamin C from supplements was included; RR=0.97 (95% CI: 0.52, 1.79).

The current analyses examined the relationship between breast cancer incidence and fruit and vegetable intake, and dietary vitamin C intake at baseline in the UKWCS, which incorporates a wide range of intakes. Based on the hypothesis that a U-shaped relationship may exist, non-linear relationships between intake and breast cancer risk were explored, in addition to linear. Although the amount of vitamin C in supplements taken by users was unknown at baseline, women who reported supplement use of any type were excluded in sub-analyses to provide a clearer picture of the relationship between dietary vitamin C and breast cancer risk. The effects of family history and menopausal status were also examined.

9.3 Method

9.3.1 Study population

General information about the recruitment of the 35,372 women who completed the baseline questionnaire is provided in section 4.1.1.1 of this thesis. All analyses excluded 1735 women with any prevalent malignant cancers recorded in the cancer registry before the baseline questionnaire date and excluded a further 33 women who were diagnosed with breast cancer within 6 months after the questionnaire date. The analyses also excluded an additional 83 women with extremely low or high total energy intake (more than 6000kcal and less than 500kcal). During the median follow up period of 11.2 years, 989 women developed incident breast cancers; these were registered with cancer registries by the censor date 01/01/08.

Of the 33,520 women included in the analyses of dietary vitamin C and fruit and vegetable intake, 33,137 (99%) provided information on whether or not they took dietary supplements. Sixty-two percent (20,460 women) in the cohort took supplements. Ninety-four percent (31,559 women) also provided information on whether or not they had a family history of cancer, 8% (2,406) of whom had a family history of breast cancer and 39% (12,350) had a family history of any type of cancer.

9.3.2 Exposure measurement

9.3.2.1 Fruit and vegetable intake

Fruit and vegetable intake had been measured in grams and also by two methods of calculating total servings: from answers to the 217 item FFQ; also from answers to cross-check questions 7 and 11 of the baseline questionnaire. Fruit and vegetable intake in grams was derived from total servings from the FFQ.

The cross-check questions were:

Q7 How many servings of vegetables or vegetable containing dishes (excluding potatoes) do you usually eat each week?

Q11 How many servings of fruit or fruit containing dishes do you usually eat each week?

Five roughly equal groups were created from the cross-check question data for servings per day. Total servings derived from the answers to the FFQ, and also total grams of fruit and vegetable consumed per day derived from the FFQ, were divided into fifths. The range of fruit and vegetable servings in each group and the mean servings for each group are shown in Table 48. As reported in section 5.3 of the evaluation chapter, the agreement between the two methods of calculating servings was not good, and reporting misjudgements by participants are likely to have occurred with both methods. However, because there were substantial correlations between vitamin C derived from FFQ and fruit and vegetable servings or grams calculated from FFQ, analyses using FFQ derived fruit and vegetable servings and grams were not undertaken apart from for comparisons in the initial analysis in Table 48.

9.3.2.2 Vitamin C intake

Dietary vitamin C consumed per day was derived from FFQs. As explained in section 4.5.1 in the methods chapter, the FFQ data had been entered into the DANTE program which used micronutrient content of foods as listed in The Royal Society of Chemistry Food tables, version 5 and supplements to estimate vitamin C consumed.[287]

Vitamin C nutrient density per mega joule was calculated for each participant as vitamin C/ MJ. The reason for undertaking additional analyses with nutrient densities is discussed in section 4.7.5.3 of the methods chapter.

Five equal groups were created from the dietary vitamin C as absolute values and also from vitamin C expressed as nutrient densities. The mean intake for each fifth of dietary vitamin C intake is shown in Table 48.

9.3.2.3 Supplement use

Supplement users at baseline had been determined from yes/no answers to the question:

Q29 *Do you take any vitamins, minerals, fish oils or other food supplements?*

Women who did not answer this question but recorded information about the type of supplements taken on the questionnaire were also classed as supplement users.

9.3.3 Statistical analyses

Women's characteristics by category of consumption were described using means and percentages, and displayed in separate tables for absolute vitamin C intake and for fruit and vegetables intake recorded by the cross-check questions. Significant differences in characteristics in relation to intake were established from p-values using regression tests for trend for continuous variables and chi squared tests for trend for categorical variables.

Cox proportional hazard ratios were used to model the associations between breast cancer incidence and fifths of vitamin C and also fifths of fruit and vegetable intake. To test for linear trends, continuous intake variables were used per increment of one serving for fruit and vegetable intake and approximately one standard deviation of mean vitamin C intake (e.g. 60mg/d for absolute vitamin C intake and 8mg/MJ/day for vitamin C intake density). Tables of hazard ratios were adjusted for age, BMI (under; normal; overweight), parity, estimated cumulative breast feeding (weeks), age at menarche, hours exercise sweating per week, contraceptive pill use (never, past, current), HRT use (ever, past, current), low alcohol use, socio-economic class (professional and managerial; intermediate; routine and manual), smoking status (never, past current), total energy intake, and also supplement use (yes, no), and menopausal status (postmenopausal; premenopausal) where appropriate. Reasons for selecting confounders are discussed in section 4.7.5 of this thesis. In sensitivity analyses further adjustment was made for family history of cancer in first degree relatives (any, breast). As detailed in section 4.7.6.3 women were asked whether their mother, father, brothers or sisters ever suffered from cancer, and were asked to provide details. Family history may be a confounder since women in the UKWCS who were aware that they may be at increased risk of breast cancer may have consumed more fruit and vegetables and supplements in an effort to reduce the risk.

Both unadjusted and adjusted likelihood ratio test for overall interactions were used to test whether breast cancer risk differed between supplement groups, menopausal status or family history of breast cancer. Sub-group time-to-event analyses were produced by supplement use and for menopausal status.

Non-linear relationships between intake and breast cancer risk were modelled using restricted cubic splines consisting of four cubic polynomial segments ($\alpha + \beta_1 x + \beta_2 x^2 + \beta_3 x^3$) separated by five knots. This produced a smooth curve for each model: the models show straight lines before the first and after the last knot and are continuous at knot boundaries.[365] Harrell (2001) recommended using five knots which are placed at

the following percentiles of the exposure variable: 5, 27.5, 50, 72.5, 95.[372] These models were adjusted using the means of the continuous covariates: age, BMI, total calories parity, estimated cumulative breast feeding (weeks), age at menarche, hours exercise sweating per week. This produced estimated breast cancer risks over a range of vitamin C intakes for a woman with these average UKWCS characteristics. No adjustments were made for categorical variables, since each of the categories would need to be adjusted for in separate analyses, thereby reducing the power of the analyses. In the model of total women (minus exclusions and outliers) knots occurred at 76.7, 120.3, 155.2, 197.7 and 288.5mg/d vitamin C. Separate knots and covariate means were calculated for menopausal and supplement status sub-groups.

Women with a family history of breast cancer were excluded from the restricted cubic spline models. The upper threshold for excluding outliers, i.e. 350mg/d of vitamin C, was determined as 1.5 times the inter-quartile range and comprised 3.6% of the total women.

25% percentile = 113.6mg/d vitamin C
75% percentile = 209.7mg/d
Inter-quartile range = 96.1mg/d
1.5 * range = 144.2mg/d
Upper threshold = 209.7 + 144.2 = 353.9mg/d

Women with intakes below the RDA of 60mg/d were also excluded since they formed less than 3% of the total and appeared to disproportionately influence the extremities of the graphs. Seventy-one other data points were excluded using Mahalanobis distance calculations between vitamin C intake and the total fruit and vegetable intake from the cross-check question. This multivariate method of determining outliers was developed by Hadi and applied in Stata v10[316,317] (see section 4.7.6.1 of the methods chapter). The predicted hazard ratios shown for the graphs are relative to women taking 60mg/d vitamin C.

9.4 Results

The mean (sd) values for the lowest intake groups were 0.9 (0.4) and 4.4 (1.2) servings of fruit and vegetables per day recorded by cross-check question and by FFQ respectively, and 19mg per day of dietary vitamin C. Mean values for the highest intake groups were 5.1 (1.3) and 19.7 (6.6) servings per day for the cross-check question and FFQ respectively and 301mg (86) per day dietary vitamin C.

From the tests for trends across intakes, as observed in Table 46 and Table 47, there were significant relationships between the majority of the characteristics listed and increasing intakes of fruit and vegetables (as per the cross-check question) and also increasing intakes of dietary vitamin C. For instance, with increasing intake, there were obvious trends in relation to longer breast feeding, exercising vigorously for longer, higher energy intake, and also higher dietary vitamin A and E intake. Similarly, there were obvious trends towards having no children, taking supplements, and being a current smoker in the higher intake groups. Paradoxically, although lower meat consumption occurred with increased fruit and vegetables intake in Table 46, whereas, an increase in meat consumption occurred with increased vitamin C consumption in Table 47. Although there were no significant trends for total alcohol (ethanol) intake, significant trends were seen in relation to drinking less than once a week; women who had low fruit and vegetable or vitamin C intake were also more likely to have a very low alcohol intake.

In the breast cancer time-to-event analyses in Table 48 there was no significant evidence of differences between the lowest intake group and other intake groups for any of the methods of recording intake before or after adjustment of potentially confounding covariates, or after further adjustment for family history of cancer. Furthermore, no linear trends in risks were apparent in this table, which was prior to testing for modification effects of supplements. Instead, the highest but weak and non-significant risks for breast cancer were found in the middle intake group of fruit and vegetables and also for absolute vitamin C values (HR=1.16; 95% CI: 0.93, 1.46; mean intake 156mg/d vitamin C). The lowest risks were found for the highest fruit and vegetable intake groups derived from the FFQ analysed by servings (HR=0.85; 95% CI: 0.66, 1.09) and by grams consumed (HR=0.89; 95% CI: 0.69, 1.15), though these were also non-significant. The restricted cubic spline model (Figure 23) also shows no clear association between breast cancer risk and dietary vitamin C, with little evidence of any significant non-linear relationships, as seen from the wide confidence intervals in the bottom graph.

Table 46 Characteristics of women at baseline in the UKWCS by category of fruit and vegetable servings consumed (as per Q7 & Q11)

	Total fruit and vegetable consumption (as per Q7 & Q11) grouped						
	1	2	3	4	5	Total	P value[a]
Mean (sd) servings/day (sd)	0.9 (0.4) N=6638 (20%)	1.9 (0.2) N=6235 (19%)	2.5 (0.2) N=6298 (19%)	3.3 (0.3) N=6675 (20%)	5.1 (1.3) N=7674 (23%)	2.8 (1.6) N=33520 (100%)	
Age (years), mean (sd)	52.3 (9.5)	52.3 (9.9)	51.0 (9.1)	52.1 (9.3)	52.3 (9.0)	52.0 (9.3)	0.4
BMI (kg/m^2), mean(sd)	24.8 (4.7)	24.5 (4.3)	24.5 (4.2)	24.3 (4.2)	24.2 (4.4)	24.5 (4.4)	<0.001
Weight change since aged 20, mean(sd)	9.0 (10.4)	8.6 (9.8)	8.2 (9.7)	7.5 (9.7)	6.9 (10.2)	8.0 (10.0)	<0.001
Height (cm), mean (sd)	163.1 (7.1)	163.7 (6.9)	163.7 (6.7)	164.0 (6.7)	164.0 (6.7)	163.7 (6.9)	<0.001
Age at menarche, mean (sd)	12.93 (1.7)	12.86 (1.6)	12.81 (1.6)	12.76 (1.6)	12.72 (1.6)	12.8(1.6)	<0.001
Age at first birth, mean (sd)	25.2 (4.7)	25.9 (4.8)	25.9 (4.8)	26.1 (4.7)	26.0 (4.6)	25.8 (4.7)	<0.001
Parity, mean (sd)	1.9 (1.3)	1.9 (1.3)	1.9 (1.3)	1.9 (1.3)	1.9 (1.3)	1.9 (1.3)	0.006
Est. total breast feeding (wks), mean(sd)	19.0 (1.9)	21.2 (32.8)	24.3 (35.3)	25.1 (35.3)	27.6(38.4)	23.6 (35.0)	<0.001
Physical activity (min), mean (sd)	13 (28)	14 (28)	15 (29)	16 (30)	18 (30)	15 (29)	<0.001
Total meat (grams), mean (sd)	72 (63)	69 (63)	68 (62)	63 (61)	55 (59)	64 (62)	<0.001
Total fruit (grams), mean (sd)	224 (215)	261 (19)	273 (186)	340 (210)	444 (263)	314 (232)	<0.001
Total vegetables (grams), mean (sd)	250 (174)	285 (165)	297 (155)	333 (169)	396 (199)	315 (181)	<0.001
Total ethanol intake (grams), mean (sd)	8.2 (11.5)	8.4 (10.4)	9.6 (11.1)	8.8 (10.1)	9.5 (9.8)	8.7 (10.6)	0.1
Total energy intake (Kcal), mean (sd)	2212 (740)	2295 (726)	2338 (685)	2391 (697)	2473 (703)	2346 (716)	<0.001
Dietary vitamin A intake (µg), mean (sd)	1111 (622)	1187 (575)	1209 (532)	1288 (598)	1368 (613)	1238 (598)	<0.001
Dietary vitamin E intake (mg), mean (sd)	8.6 (4.1)	9.2 (4.1)	9.6 (4.1)	10.1 (4.2)	10.8 (4.4)	9.7 (4.3)	<0.001
Dietary vitamin C intake (mg), mean (sd)	139 (85)	155 (78)	160 (72)	181 (78)	213 (93)	171 (86)	<0.001
Vitamin C from fruit (mg), mean (sd)	43 (51)	51 (46)	54 (48)	66 (52)	88 (68)	62 (57)	<0.001
Vitamin C from veg (mg), mean (sd)	53 (42)	60 (40)	62 (37)	70 (40)	83 (49)	66 (43)	<0.001
Professional or managerial (%)	31	35	41	42	44	39	<0.001
Educated to 'A'-level or above (%)	38	46	55	58	60	52	<0.001
No children (%)	20	20	20	20	22	20	<0.001
Ever used contraceptive pill (%)	66	68	72	68	67	68	0.5
Ever used HRT (%)	29	28	27	27	28	28	0.07
Current smoker (%)	18	13	11	9	6	11	<0.001
Drinks alcohol less than once a week(%)	37	35	30	32	33	33	<0.001
Takes any supplements (%)	58	59	60	63	68	62	<0.001
Family history of breast cancer (%)	6.8	7.2	8.1	8.1	7.9	7.6	0.003
Family history of any cancer (%)[b]	38.4	39.7	38.7	38.9	40.0	39.2	0.2

[a]p= test for trend, [b]family history in first degree relatives

Table 47 Characteristics of women at baseline in the UKWCS by category of dietary vitamin C consumption

	Total dietary vitamin C consumption from FFQ by fifths						
	Lowest	2nd Fifth	3rd Fifth	4th Fifth	Highest	Total	P value[a]
Mean vitamin C mg/d (sd)	79 (18)	122 (10)	156 (10)	197 (15)	301 (87)	171 (86)	
	N=6704	N=6704	N=6704	N=6704	N=6704	N=33520	
Age (years), mean (sd)	51.2 (9.3)	51.6 (9.3)	52.1 (9.3)	52.6 (9.2)	52.7 (9.2)	52.0 (9.3)	<0.001
BMI (kg/m^2), mean (sd)	24.6 (4.6)	24.4 (4.3)	24.4 (4.2)	24.4 (4.2)	24.4 (4.5)	24.5 (4.4)	0.2
Weight change since aged 20 mean,(sd)	8.4 (10.5)	8.0 (9.9)	7.8 (9.6)	7.9 (9.7)	7.8 (10.4)	8.0 (10.0)	0.05
Height (cm), mean (sd)	163.3 (6.9)	163.7 (6.9)	163.9 (7.1)	164.0 (6.8)	163.9 (6.6)	163.7 (6.9)	<0.001
Age at menarche, mean (sd)	12.85 (1.6)	12.86 (1.6)	12.79 (1.6)	12.79 (1.6)	12.78 (1.6)	12.8(1.6)	0.02
Age at first birth, mean (sd)	25.6 (4.8)	26.0 (4.8)	26.0 (4.8)	25.9 (4.6)	25.7 (4.6)	25.8 (4.7)	0.1
Parity, mean (sd)	1.7 (1.3)	1.8 (1.3)	1.9 (1.3)	1.9 (1.3)	2.0 (1.3)	1.9 (1.3)	<0.001
Est. total breast feeding (wks), mean(sd)	18.6 (31.4)	22.6 (33.8)	24.6 (35.3)	25.4 (36.3)	26.7 (37.7)	23.6 (35.0)	<0.001
Physical activity (min), mean (sd)	12 (26)	13 (28)	14 (27)	16 (28)	20 (35)	15 (29)	<0.001
Total meat (grams), mean (sd)	61 (54)	64 (58)	65 (61)	67 (64)	66 (71)	64 (62)	<0.001
Total fruit (grams), mean (sd)	145 (93)	224 (116)	288 (138)	358 (161)	553 (329)	313 (231)	<0.001
Total vegetables (grams), mean (sd)	168 (71)	241 (84)	298 (103)	361 (124)	509 (244)	315 (181)	<0.001
Total ethanol intake (grams), mean (sd)	8.4 (11.3)	8.9 (10.9)	8.9 (10.7)	8.7 (9.8)	8.5 (10.1)	8.7 (10.6)	0.2
Total energy intake (Kcal), mean (sd)	1904 (570)	2157 (580)	2322 (596)	2501 (655)	2849 (796)	2346 (716)	<0.001
Dietary vitamin A intake (µg), mean (sd)	895 (440)	1089 (464)	1222 (509)	1361 (556)	1620 (712)	1237 (598)	<0.001
Dietary vitamin E intake (mg), mean (sd)	7.1 (3.3)	8.6 (3.6)	9.6 (3.7)	10.5 (3.8)	12.7 (4.8)	9.7 (4.3)	<0.001
Vitamin C from fruit (mg), mean (sd)	24 (16)	40 (21)	53 (26)	70 (34)	120 (90)	62 (57)	<0.001
Vitamin C from veg (mg), mean (sd)	31 (14)	48 (19)	62 (23)	78 (29)	112 (60)	66 (43)	<0.001
Professional or managerial (%)	36	39	40	41	39	39	<0.001
Educated to 'A'-level or above (%)	47	53	54	54	53	52	<0.001
No children (%)	24	21	19	19	18	20	<0.001
Ever used contraceptive pill (%)	69	70	68	67	66	68	<0.001
Ever used HRT (%)	26	26	28	29	30	28	<0.001
Current smoker (%)	16	12	10	8	9	11	<0.001
Drinks alcohol less than once a week(%)	38	33	31	30	34	33	<0.001
Takes any supplements (%)	57	58	62	64	68	62	<0.001
Family history of breast cancer (%)[b]	7.3	7.7	7.7	7.8	7.6	7.6	0.5
Family history of any cancer (%)[b]	39.0	38.7	38.3	39.9	39.8	39.2	0.2

[a]p= test for trend.
[b]In first degree relatives
sd =standard deviation

Table 48 Breast cancer risks by fruit and vegetable intake and dietary vitamin C intake at baseline in the UKWCS

	Mean (SD)	Cases/ non-cases[a]	Unadjusted HR (95% CI)	Adjusted HR (95% CI)[b]	Adjusted for family history (95% CI)[c]
Fruit & vegetable servings/d, from Q7 & Q11					
1 (0-1.4)	0.9 (0.4)	188/6450	1	1	1
2 (1.6-2)	1.9 (0.2)	176/6059	0.98 (0.80, 1.21)	1.09 (0.86, 1.38)	1.08 (0.85, 1.37)
3 (2.1-2.9)	2.5 (0.2)	191/6107	1.06 (0.87, 1.30)	1.12 (0.89, 1.42)	1.13 (0.88, 1.41)
4 (3-3.9)	3.3 (0.3)	200/6475	1.04 (0.85, 1.27)	1.05 (0.83, 1.33)	1.04 (0.82, 1.33)
5 (4 & above)	5.1 (1.3)	234/7440	1.05 (0.87, 1.28)	1.04 (0.83, 1.31)	1.04 (0.82, 1.31)
Per serving/d			1.01 (0.97, 1.05)	1.00 (0.95, 1.04)	1.00 (0.95, 1.05)
p_{trend}			0.6	0.9	1.0
Fruit & vegetable servings/d, FFQ derived					
1 (0-6.1)	4.4 (1.2)	188/6515	1	1	1
2 (6.1-8.4)	7.2 (0.7)	202/6499	1.06 (0.87, 1.30)	0.99 (0.79, 1.24)	0.99 (0.79, 1.25)
3 (8.4-10.7)	9.5 (0.7)	217/6489	1.13 (0.93, 1.38)	1.04 (0.83, 1.30)	0.99 (0.79, 1.25)
4 (10.7-14.2)	12.3 (0.9)	208/6498	1.08 (0.88, 1.31)	0.94 (0.75, 1.19)	0.93 (0.74, 1.18)
5 (14.2 & above)	19.7 (6.6)	174/6530	0.89 (0.72, 1.09)	0.85 (0.66, 1.09)	0.84 (0.65, 1.08)
Per serving/d			0.97 (0.91, 1.03)	0.97 (0.90, 1.05)	0.96 (0.88, 1.05)
p_{trend}			0.3	0.5	0.4
Fruit & veg g/d, FFQ derived					
1 lowest fifth	266g/d (72)	180/6524	1	1	1
2	433g/d (39)	198/6506	1.09 (0.89, 1.33)	0.99 (0.79, 1.24)	0.97 (0.77, 1.23)
3	566g/d (40)	220/6484	1.21 (0.99, 1.47)	1.16 (0.93, 1.45)	1.12 (0.89, 1.40)
4	730g/d (58)	212/6492	1.15 (0.95, 1.41)	1.01 (0.80, 1.28)	0.99 (0.78, 1.26)
5 highest fifth	1149g/d (360)	179/6525	0.96 (0.78, 1.18)	0.89 (0.69, 1.15)	0.87 (0.67, 1.13)
Per 80g/d			0.99 (0.97, 1.00)	0.99 (0.88, 1.01)	0.99 (0.97, 1.01)
p_{trend}			0.2	0.2	0.2

Table continued: Breast cancer risks by fruit and vegetable intake and dietary vitamin C intake at baseline in the UKWCS

	Mean (SD)	Cases/ non-cases[a]	Unadjusted HR (95% CI)	Adjusted HR (95% CI)[b]	Adjusted for family history (95% CI)[c]
Dietary vitamin C mg/d, FFQ derived					
1 lowest fifth	79mg/d (18)	182/6522	1	1	1
2	122mg/d (10)	176/6528	0.96 (0.78, 1.18)	0.96 (0.76,1.21)	0.94 (0.74, 1.20)
3	156mg/d (10)	218/6486	1.19 (0.98, 1.45)	1.16 (0.93, 1.46)	1.15 (0.91, 1.45)
4	197mg/d (15)	219/6485	1.20 (0.98, 1.45)	1.12 (0.88, 1.41)	1.08 (0.85, 1.37)
5 highest fifth	301mg/d (87)	194/6512	1.05 (0.86, 1.28)	1.05 (0.81, 1.35)	1.03 (0.79, 1.33)
Per 60mg/d			1.00 (0.96, 1.05)	1.00 (0.94, 1.06)	0.94 (0.93, 1.05)
p_{trend}			1.0	0.9	0.7
Dietary vitamin C density, FFQ derived					
1 lowest fifth	9.1/d (1.9)	178/6526	1	1	1
2	13.3/d (0.9)	190/6514	1.06 (0.86, 1.30)	1.04 (0.83, 1.31)	1.07 (0.84, 1.35)
3	16.6/d (1.0)	204/6500	1.14 (0.93, 1.39)	1.07 (0.85, 1.34)	1.09 (0.87, 1.38)
4	20.4/d (1.4)	206/6498	1.14 (0.93, 1.39)	1.08 (0.86, 1.35)	1.06 (0.84,1.33)
5 highest fifth	29.7/d (7.2)	211/6493	1.16 (0.95, 1.42)	1.09 (0.87, 1.37)	1.07 (0.85, 1.35)
Per 8mg/MJ/d			1.02 (0.96, 1.09)	1.00 (0.93, 1.08)	0.99 (0.92, 1.07)
p_{trend}			0.5	0.9	0.9

[a]Numbers of cases for the unadjusted analyses

[b]Adjusted for age, BMI, socio-economic status, parity, estimated cumulative breast feeding, age at menarche, hours exercise sweating per week, contraceptive pill use, HRT use, low alcohol use, smoking status, supplement use, total calories (other than nutrient density).

[c]Adjusted for the above, plus family history of cancer in first degree relatives (any cancer, breast cancer).

There were no significant modifying effects of menopausal status, supplement use, or family history of breast cancer on the relationship between dietary intake and breast cancer risk (Table 49). Although the interactions were non-significant in the test between supplement use and fruit and vegetable intake or dietary vitamin C intake (p=0.6, 0.2, 0.3), breast cancer time-to-event analyses were stratified by supplement use status (Table 51). Similarly, stratified analyses were tabled by menopausal status (Table 50) and by supplement use for post-menopausal (Table 52) and pre-menopausal women (Table 53).

Although different patterns of risk were seen in sub-analyses, there was little evidence of non-linear relationships, and indeed no evidence of any significant associations between breast cancer incidence and dietary intake after stratifying by supplement use or by menopausal status. Additionally, there were no differences in patterns of hazard ratios in sensitivity analyses after adjusting for family history of breast cancer and any cancer (not shown). Patterns of risk appeared different for pre- and post-menopausal women as seen in Table 50, but these differences were not statistically significant. In the restricted cubic-spline models (Figure 24), compared to risks at 60mg/d vitamin C, deviations of risk from unity for intakes between 60mg/d to 350mg/d were weak and also non-significant; confidence intervals, which were not shown, were wide. If anything, the spline model for pre-menopausal women indicated risks increased slightly, but non-significantly, with increasing vitamin C intake, though the results in Table 50 did not reflect this.

For non-supplement users, there appeared to be non-significant trends towards higher risks with increased dietary vitamin C intake, with moderate but non-significant increases in risk in the high verse low risk comparison (HR=1.38; 95% CI: 0.92, 2.06; Table 51), and similarly for post-menopausal non-supplement users (HR=1.52; 95% CI: 0.89, 2.58; Table 52). Likewise, in the predicted spline model (Figure 25) breast cancer risk tended to increase in non-supplement users with increasing dietary vitamin C intake, with the predicted risk at 350mg/d roughly 2.3 compared to 60mg/d intake, but confidence intervals were wide. Similarly, a predicted hazard ratios of 3 for postmenopausal non-supplement users was seen at this intake in spline models (not shown). The results, however, were not statistically significant since the upper confidence limits were very wide.

The hazard ratios for the nutrient density analyses for non-supplement users in Table 51 and Table 52, however, did indicate a U-shaped relationship, with women in the 2nd group having the lowest, though non-significant risks (HR=0.68; 95% CI: 0.40, 1.14; Table 52 of post-menopausal women). However, the restricted cubic spline model

showed that the relationship was reasonably flat (Figure 27), and non-significant (confidence intervals not shown).

For supplement users the relationships were largely flat (as observed in Table 51 to Table 52). This was also apparent in the spline model for total supplement users (Figure 26), and also separately for post-menopausal and pre-menopausal women (not shown). Any deviations occurring in the middle intake groups in the tables and graphs were weak and also non-significant as confidence intervals were wide. The shape of the graphs did not change when women with the top and bottom 5% or 10% of intakes were excluded (not shown).

Table 49 Assessment of modification by menopausal status, supplement use and family history of breast cancer on fruit and vegetable and dietary vitamin C intake and associations with breast cancer risk in the UKWCS

Tests for modification by	P values Unadjusted	P values Adjusted
1) Menopausal status		
Fruit & veg servings, Q7 & Q11 * Menopausal status	0.6	0.8
Dietary vitamin C mg/d * Menopausal status	0.7	0.6
Dietary vitamin C (density) * Menopausal status	0.8	0.8
2) Supplement use status		
Fruit & veg servings, Q7 & Q11 * supplement use	0.7	0.6
Dietary vitamin C mg/d * supplement use	0.1	0.2
Dietary vitamin C (density) * supplement use	0.3	0.3
Supplement use status: pre-menopausal women		
Fruit & veg servings, Q7 & Q11 * supplement use	0.6	0.6
Dietary vitamin C mg/d * supplement use	0.7	0.9
Dietary vitamin C (density) * supplement use	0.8	0.8
Supplement use status: post-menopausal women		
Fruit & veg servings, Q7 & Q11 * supplement use	0.4	0.9
Dietary vitamin C mg/d * supplement use	0.1	0.2
Dietary vitamin C (density) * supplement use	0.5	0.3
3) Family history of breast cancer status		
Fruit & veg servings, Q7 & Q11 * family history	0.9	0.9
Dietary vitamin C mg/d * family history	0.9	1.0
Dietary vitamin C (density) * family history	0.9	1.0
Family history of breast cancer: pre-menopausal women		
Fruit & veg servings, Q7 & Q11 * family history	0.8	0.5
Dietary vitamin C mg/d * family history	0.6	0.6
Dietary vitamin C (density) * family history	0.9	0.5
Family history of breast cancer: post-menopausal women		
Fruit & veg servings, Q7 & Q11 * family history	0.9	0.7
Dietary vitamin C mg/d * family history	1.0	0.9
Dietary vitamin C (density) * family history	0.7	0.5

[a]Adjusted for age, BMI, socio-economic status, parity, estimated cumulative breast feeding, age at menarche, hrs exercise sweating per week, contraceptive pill use, HRT use, low alcohol use, smoking status, supplement use, total calories (other than nutrient density).

Table 50 Breast cancer risks by fruit and vegetable intake and dietary vitamin C intake at baseline in the UKWCS by menopausal status

Total women split by	Pre-menopausal		Post-menopausal	
	cases/ non-cases[a]	HR (95% CI)[b]	cases/ non-cases[a]	HR (95% CI)[b]
Fruit & vegetable servings/day, Q7 & Q11				
1 lowest	57/2376	1	77/2368	1
2	70/2362	1.19 (0.84, 1.69)	79/2380	1.00 (0.73, 1.37)
3	76/2753	1.12 (0.79, 1.58)	83/2274	1.10 (0.80, 1.50)
4	71/2667	1.04 (0.74, 1.49)	89/2652	1.03 (0.76, 1.41)
5 highest	69/2937	0.92 (0.64, 1.32)	117/3252	1.10 (0.82, 1.48)
Per serving/d		0.95 (0.89, 1.03)		1.02 (0.96, 1.08)
p_{trend}		0.2		0.5
Dietary vitamin C mg/d, FFQ derived				
1 lowest fifth	70/2832	1	74/2279	1
2	74/2797	1.04 (0.75, 1.45)	70/2484	0.88 (0.63, 1.22)
3	78/2625	1.13 (0.81, 1.58)	97/2591	1.17 (0.85, 1.59)
4	62/2459	0.96 (0.67, 1.38)	109/2797	1.21 (0.89, 1.65)
5 highest fifth	59/2382	0.94 (0.64, 1.38)	95/2775	1.10 (0.79, 1.54)
Per 60mg/d		0.96 (0.87, 1.05)		1.01 (0.94, 1.09)
p_{trend}		0.4		0.7
Dietary vitamin C density, FFQ derived				
1 lowest fifth	71/2968	1	72/2242	1
2	76/2755	1.10 (0.79, 1.52)	81/2516	0.98 (0.71, 1.35)
3	72/2626	1.07 (0.77, 1.49)	89/2601	1.04 (0.76, 1.42)
4	69/2496	1.07 (0.76, 1.49)	95/2708	1.05 (0.77, 1.44)
5 highest fifth	55/2250	0.95 (0.66, 1.35)	108/2859	1.15 (0.85, 1.55)
Per 8mg/MJ/d		0.93 (0.83, 1.05)		1.04 (0.95, 1.15)
p_{trend}		0.3		0.4

[a]Numbers of cases for the adjusted analyses

[b]Adjusted for age, BMI, socio-economic status, parity, estimated cumulative breast feeding, age at menarche, hours exercise sweating per week, contraceptive pill use, HRT use, low alcohol use, smoking status, supplement use, total calories (other than nutrient density).

Table 51 Breast cancer risks by fruit and vegetable intake and dietary vitamin C intake at baseline in the UKWCS by supplement use

Total women split by	Non-users		Supplement users	
	cases/ non-cases[a]	HR (95% CI)[b]	cases/ non-cases[a]	HR (95% CI)[b]
Fruit & vegetable servings/day, Q7 & Q11				
1 lowest	55/2026	1	79/2718	1
2	65/1956	1.20 (0.84,1.72)	84/2786	1.01 (0.74, 1.37)
3	56/2029	1.04 (0.71,1.51)	103/2998	1.16 (0.86, 1.55)
4	64/1977	1.20 (0.83, 1.72)	96/3342	0.96 (0.71, 1.30)
5 highest	58/2018	1.04 (0.71, 1.52)	128/4171	1.03 (0.79, 1.37)
Per serving/d		0.97 (0.90, 1.05)		1.01 (0.95, 1.06)
p_{trend}		0.5		0.8
Dietary vitamin C mg/d, FFQ derived				
1 lowest fifth	58/2234	1	86/2877	1
2	63/2208	1.09 (0.76, 1.57)	81/3073	0.87 (0.64, 1.18)
3	56/2001	1.07 (0.74, 1.57)	119/3215	1.20 (0.90, 1.59)
4	61/1883	1.22 (0.84, 1.79)	110/3373	1.04 (0.77, 1.40)
5 highest fifth	60/1680	1.38 (0.92, 2.06)	94/3477	0.88 (0.64, 1.22)
Per 60mg/d		1.05 (0.96, 1.16)		0.96 (0.89, 1.04)
p_{trend}		0.3		0.3
Dietary vitamin C density, FFQ derived				
1 lowest fifth	64/2266	1	79/2944	1
2	52/2164	0.80 (0.55,1.16)	105/3107	1.21 (0.91 1.63)
3	60/2009	0.98 (0.69, 1.40)	101/3218	1.13 (0.84, 1.51)
4	62/1848	1.11 (0.78, 1.58)	102/3356	1.07 (0.80, 1.44)
5 highest fifth	60/1719	1.12 (0.79, 1.60)	103/3390	1.07 (0.80, 1.44)
Per 8mg/MJ/d		1.07 (0.95, 1.20)		0.96 (0.88, 1.06)
p_{trend}		0.3		0.4

[a]Numbers of cases for the adjusted analyses

[b]Adjusted for age, BMI, socio-economic status, parity, estimated cumulative breast feeding, age at menarche, hours exercise sweating per week, contraceptive pill use, HRT use, low alcohol use, smoking status, total calories (other than nutrient density).

Table 52 Breast cancer risks by fruit and vegetable intake and dietary vitamin C intake at baseline in the UKWCS by supplement use in post-menopausal women

Post-menopausal	Non-users		Supplement users	
	cases/ non-cases[a]	HR (95% CI)[b]	cases/ non-cases[a]	HR (95% CI)[b]
Fruit & vegetable servings/day, Q7 & Q11				
1 lowest	34/927	1	43/1441	1
2	33/934	0.98 (0.61,1.59)	46/1446	1.01 (0.66, 1.53)
3	31/869	1.00 (0.61, 1.63)	52/1405	1.16 (0.77, 1.74)
4	37/956	1.13 (0.70,1.82)	52/1696	0.97 (0.65, 1.46)
5 highest	39/1027	1.09 (0.68, 1.76)	78/2225	1.11 (0.76, 1.62)
Per servings/d		0.99 (0.89, 1.10)		1.03 (0.96, 1.11)
p_{trend}		0.9		0.4
Dietary vitamin C mg/d, FFQ derived				
1 lowest fifth	29/951	1	45/1328	1
2	34/976	1.13 (0.68, 1.87)	36/1508	0.70 (0.45, 1.09)
3	32/932	1.11 (0.66, 1.87)	65/1659	1.14 (0.77, 1.69)
4	39/964	1.35 (0.81, 2.23)	70/1833	1.09 (0.74, 1.62)
5 highest fifth	40/890	1.52 (0.89, 2.58)	55/1885	0.87 (0.56, 1.35)
Per 60mg/d		1.05 (0.94, 1.19)		0.99 (0.90, 1.09)
p_{trend}		0.3		0.8
Dietary vitamin C density, FFQ derived				
1 lowest fifth	33/913	1	39/1329	1
2	25/972	0.68 (0.40, 1.14)	56/1544	1.21 (0.80, 1.82)
3	37/958	1.02 (0.64 1.64)	52/1643	1.04 (0.68, 1.57)
4	39/911	1.14 (0.71, 1.82)	56/1797	1.01 (0.67,1.53)
5 highest fifth	40/959	1.12 (0.70, 1.78)	68/1900	1.16 (0.78, 1.72)
Per 8mg/MJ/d		1.08 (0.93, 1.26)		1.02 (0.90, 1.15)
p_{trend}		0.3		0.8

[a]Numbers of cases for the adjusted analyses

[b]Adjusted for age, BMI, socio-economic status, parity, estimated cumulative breast feeding, age at menarche, hours exercise sweating per week, contraceptive pill use, HRT use, low alcohol use, smoking status, total calories (other than nutrient density).

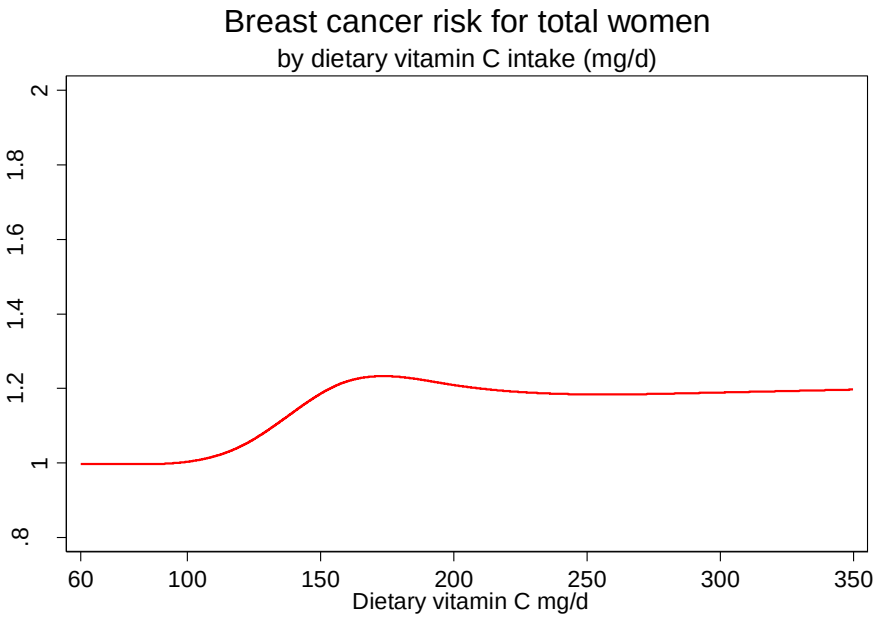
Table 53 Breast cancer risks by fruit and vegetable intake and dietary vitamin C intake at baseline in the UKWCS by supplement use in pre-menopausal women

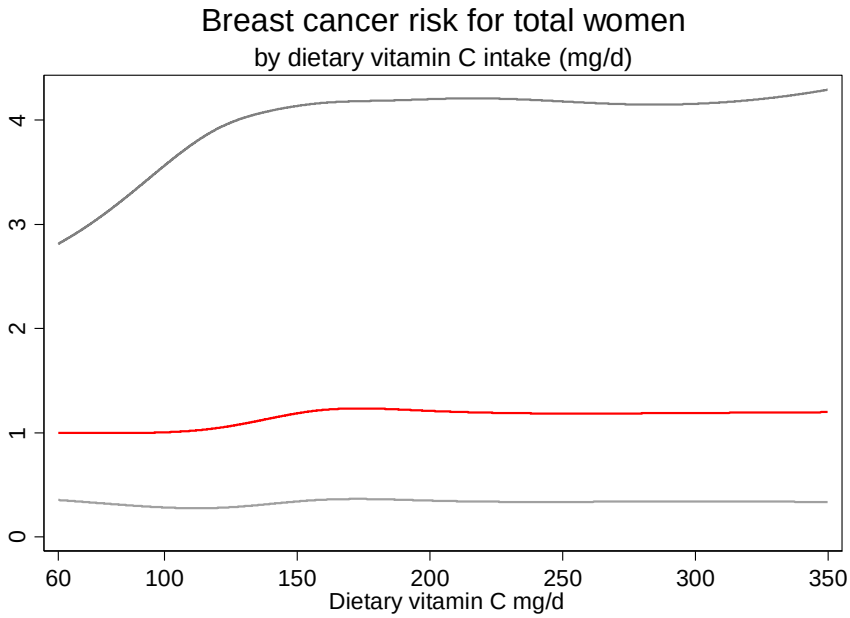
Pre-menopausal	Non-users		Supplement users	
	cases/ non-cases[a]	HR (95% CI)[b]	cases/ non-cases[a]	HR (95% CI)[b]
Fruit & vegetable servings/day, Q7 & Q11				
1 lowest	21/1099	1	36/1277	1
2	32/1022	1.50 (0.86, 2.60)	38/1340	1.00 (0.63, 1.58)
3	25/1160	1.05 (0.59, 1.89)	51/1593	1.11 (0.73, 1.72)
4	27/1021	1.27 (0.71, 2.27)	44/1646	0.91 (0.59, 1.44)
5 highest	19/991	0.89 (0.49, 1.69)	50/1946	0.90 (0.58, 1.40)
Per servings/d		0.93 (0.82, 1.06)		0.96 (0.88, 1.05)
p_{trend}		0.3		0.4
Dietary vitamin C mg/d, FFQ derived				
1 lowest fifth	29/1283	1	41/1549	1
2	29/1232	1.05 (0.62, 1.77)	45/1565	1.04 (0.68,1.60)
3	24/1069	1.02 (0.59, 1.78)	54/1556	1.22 (0.80, 1.86)
4	22/919	1.05 (0.59, 1.89)	40/1540	0.91 (0.58, 1.45)
5 highest fifth	20/790	1.13 (0.60, 2.13)	39/1592	0.86 (0.53, 1.41)
Per 60mg/d		1.03 (0.88, 1.20)		0.92 (0.82, 1.04)
p_{trend}		0.7		0.2
Dietary vitamin C density, FFQ derived				
1 lowest fifth	31/1353	1	40/1615	1
2	27/1192	0.94 (0.56, 1.59)	49/1563	1.20 (0.79, 1.83)
3	23/1051	0.89 (0.52, 1.54)	49/1575	1.19 (0.78, 1.82)
4	23/937	1.00 (0.58, 1.72)	46/1559	1.12 (0.73, 1.72)
5 highest fifth	20/760	1.07 (0.60, 1.89)	35/1490	0.91 (0.58, 1.45)
Per 8mg/MJ/d		1.02 (0.85, 1.25)		0.89 (0.76, 1.03)
p_{trend}		0.8		0.1

[a]Numbers of cases for the adjusted analyses

[b]Adjusted for age, BMI, socio-economic status, parity, estimated cumulative breast feeding, age at menarche, hours exercise sweating per week, contraceptive pill use, HRT use, low alcohol use, smoking status, total calories (other than nutrient density).

Figure 23 Adjusted restricted cubic spline models of breast cancer risks by dietary vitamin C intake before sub-grouping (confidence intervals shown in bottom graph)

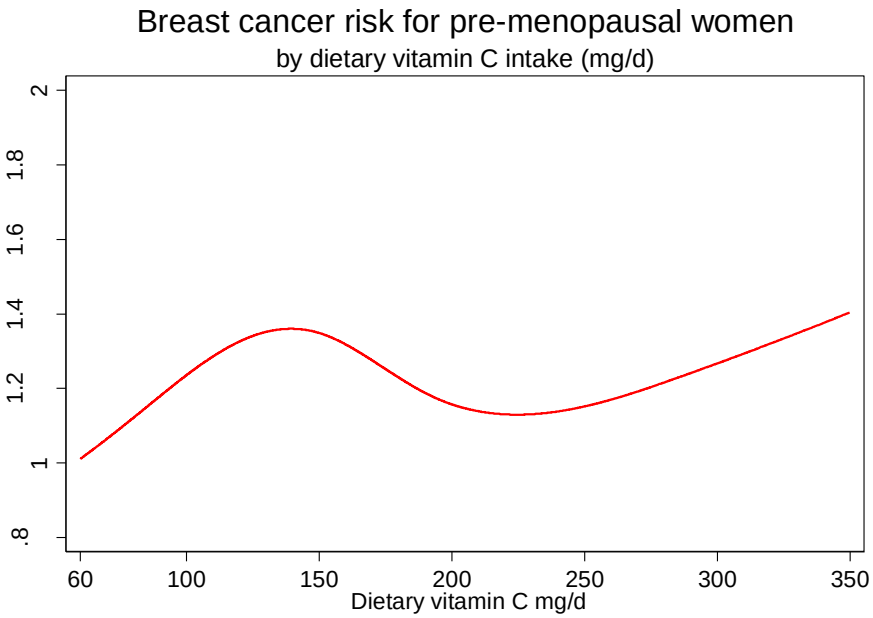




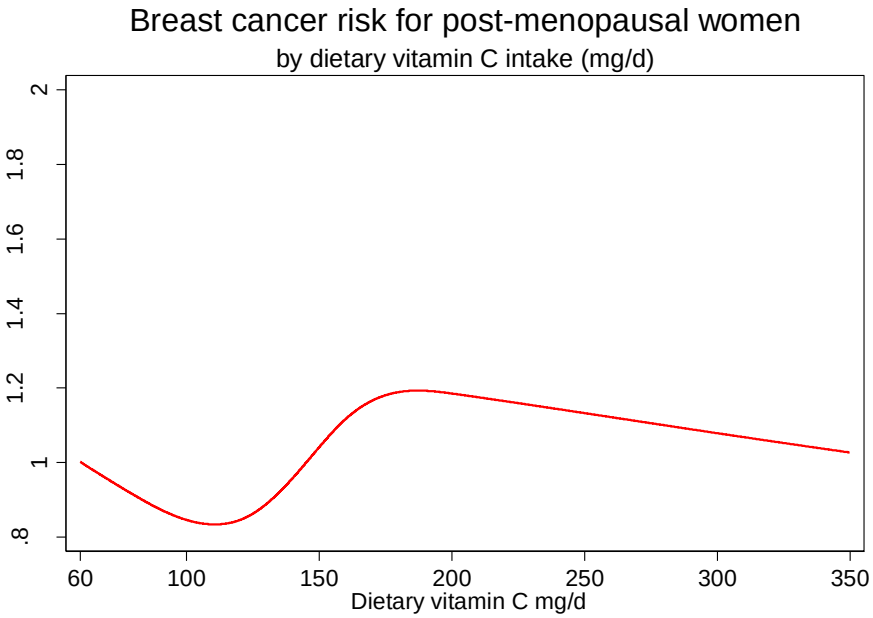
(confidence intervals shown in bottom graph)

Adjusted covariates set to mean: age = 51.8, total calories = 2341, BMI = 24.4, cumulative breast feeding wks = 24.7, age at menarche = 12.8, vigorous exercise hr/wk = 0.25, parity = 1.86

Figure 24 Adjusted restricted cubic spline models of breast cancer risks by vitamin C intake for pre-menopausal and post-menopausal women (without confidence intervals)

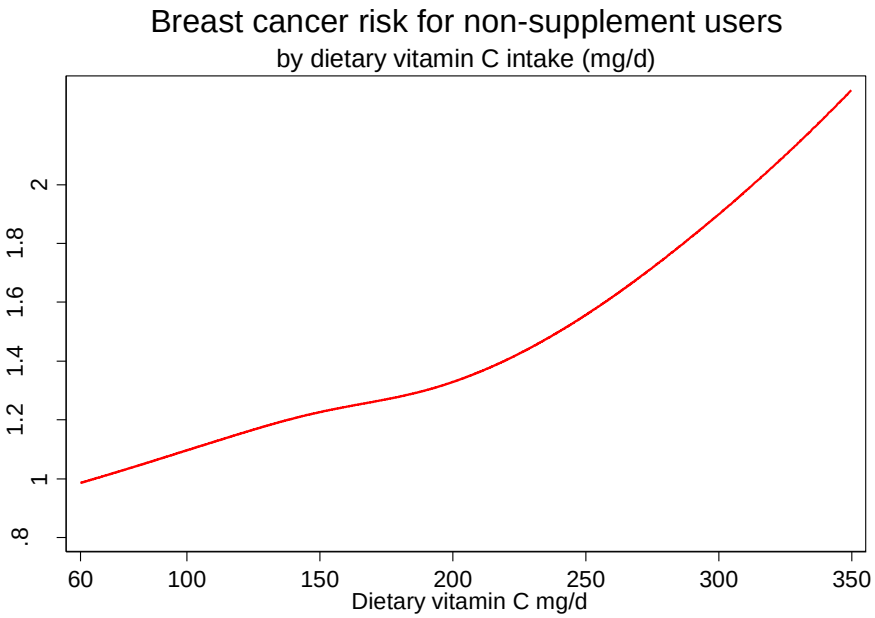


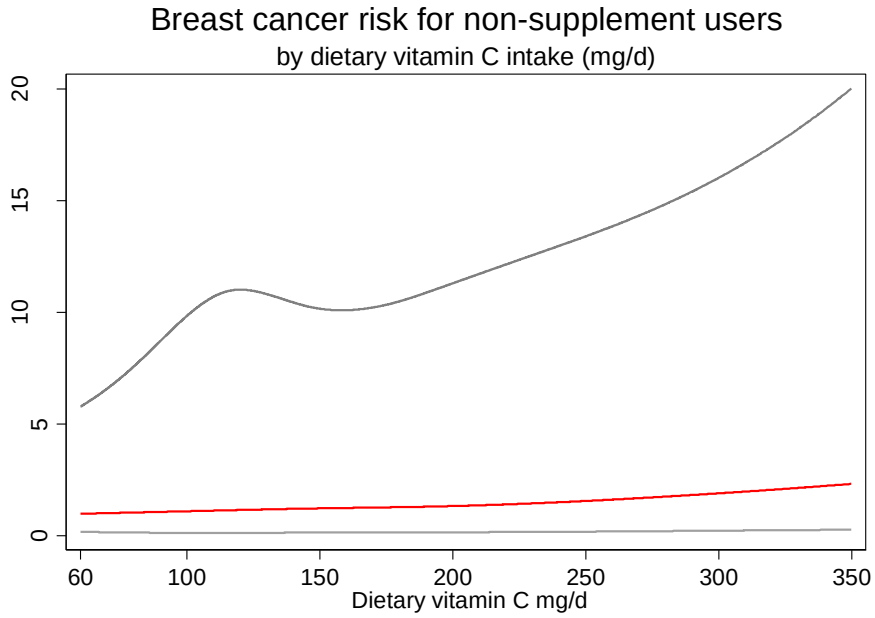
Adjusted covariates set to mean: age = 44.6, total calories = 2360, BMI = 23.9, cumulative breast feeding wks = 29.2, age at menarche = 12.8, vigorous exercise hr/wk =0.27, parity = 1.67



Adjusted covariates set to mean: age = 58.8, total calories = 2323, BMI = 25.0, cumulative breast feeding wks = 20.2, age at menarche = 12.9, vigorous exercise hr/wk = 0.24, parity = 2.05

Figure 25 Adjusted restricted cubic spline models of breast cancer risks by vitamin C intake for non-supplement users (confidence intervals shown in bottom graph)

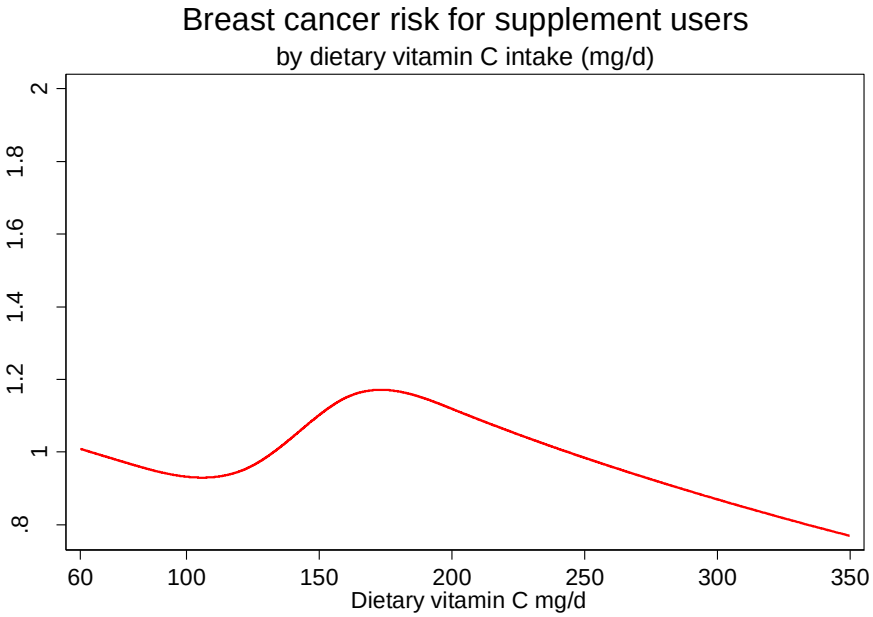


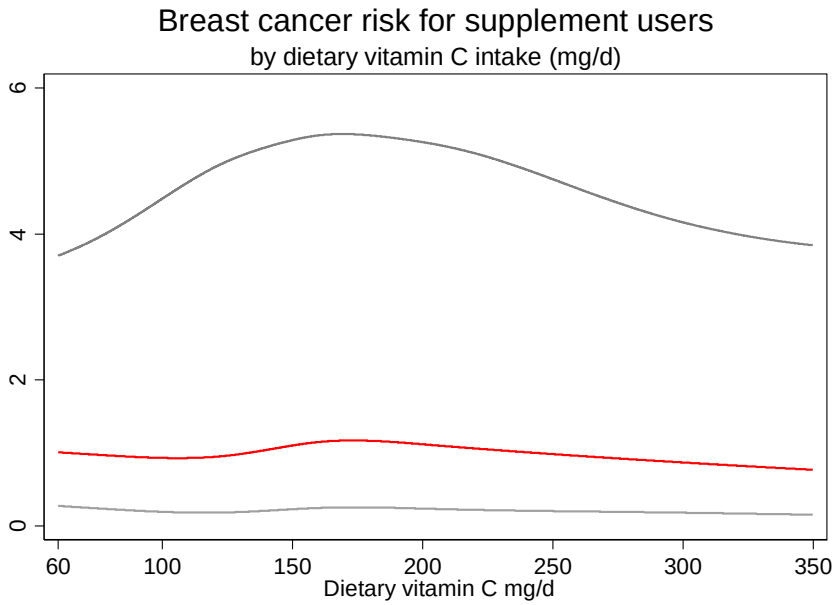


(Confidence intervals shown in bottom graph)

Adjusted covariates set to mean: age = 51.2, total calories = 2333, BMI = 24.8, cumulative breast feeding wks = 26.6, age at menarche = 12.8, vigorous exercising hr/wk = 0.24, parity = 1.93

Figure 26 Adjusted restricted cubic spline models of breast cancer risks by vitamin C intake for supplement users (confidence intervals shown in bottom graph)

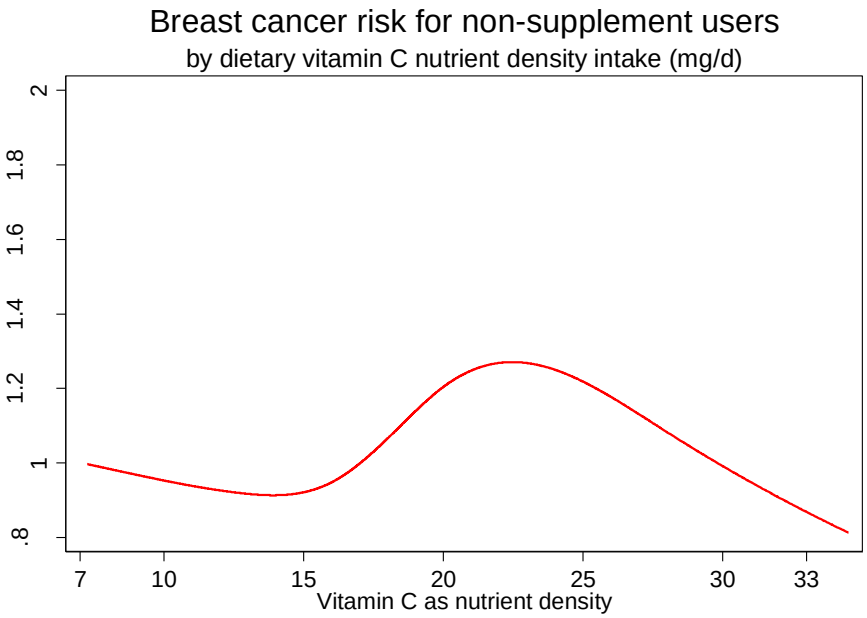




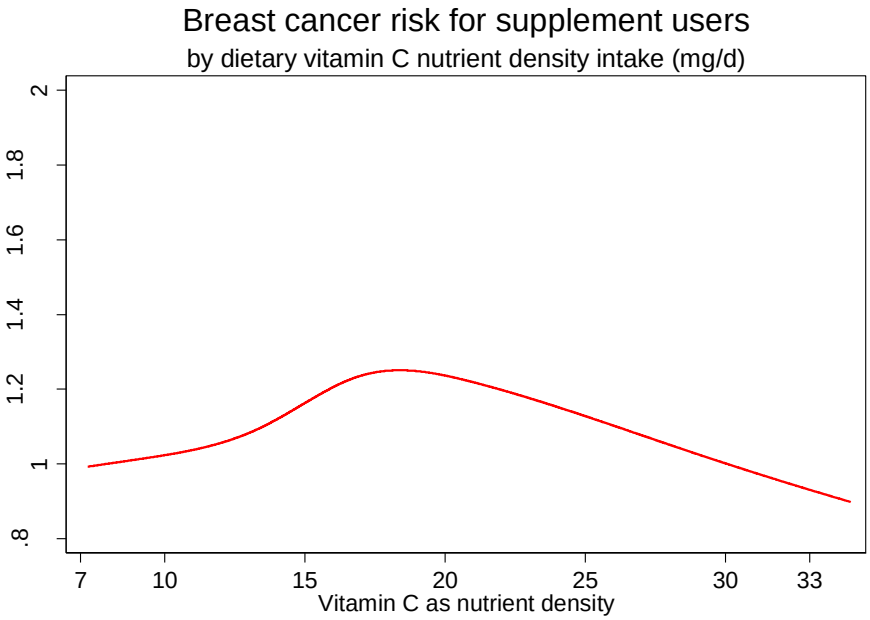
(Confidence intervals shown in bottom graph)

Adjusted covariates set to mean: age = 52.2, total calories = 2346, BMI = 24.2, cumulative breast feeding wks = 23.5, age at menarche = 12.8, vigorous exercising hr/wk = 0.26, parity 1.82

Figure 27 Adjusted restricted cubic spline models of breast cancer risks by nutrient density for non-supplement users and supplement users (without confidence intervals)



Adjusted covariates set to mean: age = 51.2, total calories = 2327, BMI = 24.8, cumulative breast feeding wks = 26.4, age at menarche = 12.8, vigorous exercise hr/wk = 0.24, parity = 1.92



Adjusted covariates set to mean: age = 51.2, total calories = 2363, BMI = 24.2, cumulative breast feeding wks = 23.5, age at menarche = 12.8, vigorous exercise hr/wk = 0.26, parity = 1.82

9.5 Discussion

The results of this UKWCS baseline analysis showed that neither total fruit and vegetable intake nor dietary vitamin C intake were significantly associated with breast cancer incidence. This is inline with previous research.[7 11 12 244 270 273] The 2007 WCRF research review and the 2008 WCRF continuous update reported no associations between breast cancer risk and fruit and vegetable or vitamin C intake.[7 169]

Although the amount of vitamin C in supplements taken by users was unknown at baseline in the UKWCS, meaning relationships with total vitamin C intake could not be assessed, the sub-analysis of women who did not take any supplements provided a clearer picture of the relationship between dietary vitamin C intake and breast cancer risk. A very weak positive dose-response relationship was noticeable for UKWCS non-supplement users in the vitamin C intake models, but this was not statistically significant. Nissen et al. (2003) reported significant evidence of a doubling in breast cancer risk with dietary vitamin C intake per 100mg/d increases in post-menopausal women in a small Danish nested case-control study, however vitamin C supplement users were included in this analysis.[245] When vitamin C supplement users were excluded in this Danish study, the risk was reduced to a moderate association and this also became non-significant.[245] Unlike our analysis other supplement users were not excluded. The recent large European wide EPIC analysis did exclude all supplement users and found no evidence of associations between dietary vitamin C intake and breast cancer risk for all pre- or post-menopausal women.[244] A significant trend of reduced risk with increasing vitamin C intake, however, was apparent for post-menopausal women using exogenous hormones.[244] These relationships should be examined in other cohorts; however, there may be insufficient power to undertake HRT sub-analyses in the UKWCS.

For supplement users in the UKWCS, the non-significant decreased risks evident at higher vitamin C intakes could be attributable to other micronutrients in the supplements which have protective effects; and, as seen in chapter 8, these women were likely to have used a variety of supplements. Alternatively, the results could be explained by residual confounding; reduced risk in supplement users may be due to other health related behaviours associated with supplement use despite attempts to adjust for them. However, since the results were non-significant they were likely to have occurred by chance. Apart from the Nissen et al. study (2003),[245] the current results support the non-significant results for dietary vitamin C analyses reported in the 2007 WCRF review,[7] and results of more recent analyses which did not exclude supplement users[270 273] (as seen in Table 2 and Table 3 in section 3.7.2). Only the Iowa

Women's Heath Study has reported dietary vitamin C analyses by supplement users only, i.e. excluding all non-users, which reported no evidence of associations.[242]

There is no simple biological explanation for these non-significant patterns observed in these UKWCS analyses. As explained in chapter 2, it is thought that via its antioxidant properties, vitamin C reduces the risk of cancer initiation by reducing DNA damage from ROS, particularly in individuals with high levels of ROS.[77] A biological explanation for increased risk with increased intake, which was the most pronounced for UKWCS post-menopausal women non-supplement users, is that antioxidant properties of vitamin C may decrease beneficial apoptosis, the ROS induced programmed death of damaged cells.[81] Such a reduction in programmed cell death could lead to the progression of cancer particularly in individuals with age-related damage.[77] Additionally, natural increases in iron stores during menopause may cause vitamin C to act as a pro-oxidant creating highly reactive and damaging hydroxyl radicals via the Fenton reaction.[76] A U-shaped relationship, which could be hypothesised from the above, was observed for vitamin C dietary intake expressed as nutrient density for non-supplement users. However, since high intakes of this water-soluble vitamin are excreted,[65] risks were expected to flatten out at high intakes, rather than increase or decrease, as seen in some of the graphed models. No previous study of vitamin C and breast cancer risk reported results analysed by nutrient density.

Given the antioxidant hypothesis, at very low vitamin C intakes, decreased risks with increasing intake up to 60mg/d would be expected. However, from FFQ recordings, only 3% of women in this predominately health conscious cohort were calculated to have vitamin C dietary intakes below the EU recommended daily allowance of 60mg/d. Indeed, the mean intake was 79mg/d (sd 18) in the reference category, the lowest fifth of intake. Furthermore, the graphed models excluded these women since they may have disproportionately influenced the extremities of the graphs. Thus, the analyses apparently could not assess the relationship between vitamin C deficiency and breast cancer risk. Nevertheless, as discussed in chapter 5, the evaluation chapter, the use of FFQs tends to overestimate intake, therefore this cohort may contain a larger percentage of women with intakes below the EU RDA than calculated.

Contrary to previous research,[247] the effect of dietary vitamin C on breast cancer risk was not modified by family history of breast cancer in first degree relatives, even after stratifying the interaction test by menopausal status. Therefore the current results do not support the hypothesis that mechanisms of breast carcinogenesis in relation to dietary intake may vary according to genetic predispositions or intergeneration transmission of other factors that may affect risk. Sensitivity analysis using breast cancer family history data from France, Spain, Cambridge and Norway centres in the

EPIC study also found no substantial differences.[244] The current results, however, may have been under powered to find an effect. It was expected that the UKWCS would have more women with a genetic predisposition than the national average since they were recruited via the WCRF. Previous analysis of pre-menopausal women at baseline found that vegetarian women had a higher risk than low meat consumers, which the authors suggested may relate to a family history of breast cancer.[82] Although, initial adjustments for family histories of cancers did not alter results in the current analyses, further sensitivity analyses could be undertaken to exclude women with a family history of breast cancer. However, misclassification of breast cancer family history may have occurred at baseline when participants did not specify the type of cancer their relatives developed.

A limitation of the study is that patterns of risk varied depending upon the tools and methods used to assess intake. Vitamin C intake was calculated from the list of fruit and vegetables ticked on the FFQ using food table nutrient compositions. As seen in section 5.3 of the thesis the agreement between servings of fruit and vegetables measured by the cross-check questions, Q7 & Q11, and that measured by the FFQ, was not good. The mean servings per day from the FFQ were considerably higher than those calculated from the cross-check questions. Indeed 11% of women (731) in group 1 of the fruit and vegetable cross-check question had not answered either the fruit or vegetable serving question; some participants may have missed these cross-check questions. Others may have misread the servings per week as servings per day and therefore under-reported their intake. Additionally, there may be a tendency for participants to underestimate in their answers to cross-check questions. Conversely, FFQs tended to encourage over-reporting of fruit and vegetables consumed, therefore the derived vitamin C amount is also likely to be overestimated especially in the high intake group. As explained in section 5.3 overestimation of fruit and vegetables by FFQs is a known problem which has been assessed in the UKWCS.[288] The authors suggest errors can be attenuated by applying a weighting factor for each subject. However, it was decided not to apply this weighting for this analysis, since it was unclear whether the cross-check answers reflected true intake or at least true ranking of fruit and vegetable servings any better than the FFQ in the UKWCS.

Using vitamin C density, i.e. dividing vitamin C intake by total energy intake, may have reduced the effect of over-reporting on the FFQ. Furthermore, Willet (1998) explains that using nutrient densities has a tendency to produce inverse association with disease if total energy intake is associated with the disease, even when the nutrient is not independently associated with the disease.[301] Willet suggests adjusting for total energy in nutrient density analyses to reduce this potential problem which could be

done in sensitivity analyses; this additional energy adjustment has been used in the next chapter on total vitamin C intake.[301]

A strength of the restricted cubic splines, over the tables, was that the splines estimated breast cancer risks at all levels of intake within a wide intake range (60-350mg/d, in comparison to 60mg/d intake), allowing any potential non-linear relationships to become apparent. Conversely, risk estimates shown in the tables were based on average intakes for each of the five intake groups so any non-linear relationships from these would have been less clear. Also the small numbers of women with extreme intakes, who could have influenced the extremities of the graphs, were excluded. Taking account of potential confounders in the cubic splines, however, was much more limited compared to the adjustments made in the tables. The graphs showed estimated risks for only the average woman in the cohort or in the sub-groups with respect to the confounding continuous variables; the mean values for these continuous variables, such as age and BMI, were noted below the graphs. Graphs for each category of the confounding categorical variables were not produced since sub-grouping the women further would have substantially reduced the power of the cubic spline analyses. Nevertheless, the conclusions from the results of the tables and from the graphs were the same; that there was no evidence of any significant linear or non-linear associations between breast cancer risk and vitamin C intake.

To explore whether breast cancer risk is influenced by nutrients in fruit and vegetables which are highly correlated with vitamin C rather than vitamin C per se, risks could be sub-analysed by vitamin C in fruit and by vitamin C in vegetables, though there may be insufficient power for further sub-analyses in the UKWCS. If different statistically significant patterns are found between fruit and vegetables with equivalent vitamin C content this would indicate that other bioactive compounds in fruit and vegetables may have more influence on breast cancer than vitamin C.

In summary the results observed from this baseline analysis of the UKWCS indicated that fruit and vegetable intake and dietary vitamin C intake are not associated with breast cancer risk.

CHAPTER 10

10 Is total vitamin C intake associated with breast cancer incidence? An analysis of diary data from the UK Dietary Cohort Consortium nested case-control study

10.1 Summary

Objectives: To determine whether total vitamin C intake is associated with breast cancer risk as few studies have included intake from both supplements and diet in their analyses. Furthermore, Food Frequency Questionnaires used in prospective studies can over-report fruit and vegetable intake, the main source of vitamin C. This is the first study to investigate breast cancer risk and vitamin C intake using food diaries.[2]

Methods: Estimated dietary vitamin C intake was derived from four to seven day food diaries pooled from five prospective studies in the UK Dietary Cohort Consortium. This nested case-control study of 851 incident breast cancer cases and 2727 matched controls examined breast cancer risk in relation to dietary vitamin C intake using conditional logistic regression adjusting for relevant covariates. Additionally, total vitamin C intake from supplements and diet was analysed in three cohorts.

Results: No evidence of associations were observed between breast cancer risk and vitamin C intake analysed for dietary vitamin C intake (OR = 1.00 per 60mg/d, 95%CI: 0.91 to 1.09, P_{trend} = 1.0) or total vitamin C intake (OR = 1.01 per 60mg/d, 95%CI: 1.00 to 1.03, P_{trend} = 0.1). Additionally, there was no significant association for post-menopausal women (OR = 1.02 per 60mg/d, 95%CI: 1.00 to 1.05, P_{trend} = 0.06). In the supplement only analysis, there was evidence of significant associations between vitamin C intake and incidence of breast cancer for pre-menopausal women (OR=2.51; 95%CI: 1.22 to 5.15; p=0.01) and total women (OR=1.39; 95%CI: 1.05 to 1.82; p=0.02).

Conclusions: This pooled analysis of individual UK women found no evidence of significant associations between breast cancer incidence and dietary or total vitamin C intake derived uniquely from detailed diary recordings. However, there was some evidence that supplements containing low doses of vitamin C were associated with an increased risk of breast cancer.

10.2 Introduction

In the previous two chapters the relationship between breast cancer risk and vitamin C intake has been examined for supplement intake only, recorded by diary at phase 2 (Chapter 8) and for dietary intake only, recorded by FFQ at baseline (Chapter 9). These analyses have produced little evidence that vitamin C intake per se, or fruit and vegetable intake were associated with breast cancer incidence in UK women. Total vitamin C intake, however, could not be assessed for the full UKWCS baseline or phase 2 datasets since both dietary and supplement vitamin C intake were not electronically captured at both collection points for the full cohort.

In summary, initial findings from retrospective case-control studies have shown that fruit and vegetable intake, the main source of vitamin C, and also vitamin C intake were inversely associated with breast cancer risk. [7 8 213] However, no conclusive evidence of a protective effect from fruit and vegetables has been produced prospectively from cohort studies.[7 10-13] Similarly, the meta-analyses of prospective cohorts using FFQs in the 2007 WCRF report showed no significant associations with dietary or supplement vitamin C intake, nor in subgroup analyses by menopausal status.[7] Only four prospective studies in this report included vitamin C from supplements as well as diet,[239 242 245 247] one of which showed an increased risk with increased total vitamin C intake.[245] Only two studies since the WCRF report was published have assessed total vitamin C intake and breast cancer risk,[270 273] one of which found a weak positive association. [270]

FFQs tend to encourage the over-reporting of fruit and vegetable consumption,[288 335 347] leading to the over-estimation of vitamin C intake,[347] Alternatively, diaries may more accurately record numbers of fruit and vegetable portions consumed individually or in mixed dishes,[347] over a period of days, though they are limited by their short-term nature.

The current analysis is one of a small number of prospective studies assessing the relationship of breast cancer risk with total vitamin C intake, which includes intake from supplements as well as from diet. Pooling of the phase 2 UKWCS nested case-control data with those from four other UK cohorts was necessary to power the analysis. This pooled analysis of the UK Dietary Cohort Consortium is the first study to investigate the relationship between breast cancer risk and vitamin C intake using food diaries, an alternative tool to FFQs used in previous analyses. (Note that this uses an additional 583 controls and 144 matched cases than the UK Dietary Cohort Consortium analysis accepted for publication.[2] These were omitted from the dataset analysed for publication due to potential selection bias, as discussed later.)

10.3 Methods

10.3.1 Subjects

Individual participant data were pooled from five established cohort studies within the UK Dietary Cohort Consortium: EPIC-Norfolk;[283] the UK Women's Cohort Study (UKWCS);[279] EPIC-Oxford;[281] Whitehall II;[284] and the MRC National Survey of Health and Development (NSHD).[285] Additional information on these cohorts is provided in section 4.1.2 of the methods chapter. Methods used were similar to those previously described for colorectal case-control analyses nested within this UK consortium.[293]

10.3.2 Case ascertainment and matching

Incident cases of breast cancer were identified from data provided by UK cancer registries based on the International Classification of Diseases (ICD) version 9 (174) or 10 (C50). Diagnoses within six months of food diary completion were excluded to ensure that latent disease without formal diagnosis was not present; otherwise disease suspected by participants could have influenced their dietary habits. Across the cohorts 851 incident cases and 2727 controls were used in the dietary vitamin C analysis. Only three cohorts (EPIC-Oxford, EPIC-Cambridge and UKWCS) were used in the total vitamin C analysis which involved 745 incident cases and 2308 controls (85% of the consortium participants); the remaining two cohorts did not have adequate supplement use data to determine the vitamin C content of supplements consumed at diary date. Within each cohort, each case was matched to randomly selected controls based on age at recruitment (± 3 years) and date of diary completion (± 3 months or as close as possible). The number of controls matched to cases was four for EPIC-Norfolk, Whitehall and NSHD, and up to five for UKWCS. In EPIC-Oxford one control was matched to each case, to within six months of case diary completion. Controls had no registry-reported cancer diagnosis at recruitment (except non-melanoma skin cancer) and were free from breast cancer at the end of the follow-up period. The mean length of follow-up for cases in the cohorts ranged from 2.4 years to 10.8 years as detailed in Table 54; these were not adjusted for in the analyses.

Table 54 Characteristics of the five cohorts from the UK Dietary Cohort Consortium included in the analyses of vitamin C and breast cancer risk

Cohort	Participants	Diary days	Years when food diary completed	Last follow up date	Mean time to diagnosis of cases	Cases	Controls	Mean(sd) dietary vit C intake	Mean(sd) total vit C intake
EPIC-Norfolk	General population in Norfolk	7 days	1993-1998	31.12.2006	6.0 yrs	365	1329	91 (50)	118 (167)
EPIC-Oxford	General population and vegetarians in the UK	7 days	1993-1998	31.12.2004	3.5 yrs	194	194	111 (61)	233 (436)
UK Women's Cohort Study (UKWCS)	Middle aged women in the UK	4 days	1999-2003	31.12.2006	2.4 yrs	186	785	117 (61)	239 (361)
Whitehall II	Civil servants in the UK	7 days	1991-1993	30.09.2005	7.8 yrs	70	275	101 (51)	_[a]
National Survey of Health and Development (NSHD)	Nationally reprehensive cohort of women who were born in one week in March 1946 in England, Wales and Scotland.	5 days	1989	31.12.2006	10.8 yrs	36	144	66 (37)	_[a]

[a]Whitehall and NSHD did not have detailed diary data of vitamin C intake from supplements

10.3.3 Dietary methods

All cohorts collected dietary information using semi-weighed food diaries or included photographs to aid the estimation of portion size. The number of days' intake recorded for each cohort is shown in Table 54; this varied from four to seven day of recording.
Food diary details were input by trained food diary analysts; the majority (88%) were entered into Data into Nutrients for Epidemiological Research (DINER) developed at Cambridge. The details were checked and nutrient data was derived using a linked system (DINERMO).[373] Diaries from 42 cases and 201 controls from the UKWCS were entered using an in-house Microsoft Access-based dietary analysis program (DANTE), which had previously been validated against DINER on a subsample of 100 randomly selected diaries, with acceptable agreement.[293] Diaries from the NSHD were entered into DIDO[295] which, after validation, proved to use portion sizes and recipes that were more concurrent with the time of NSHD diary completion. Owing to time and cost it was not possible for all diet diaries to be coded using a single data entry program. All estimated dietary vitamin C intake was based on standard tables of food composition and daily averages were calculated.[287] The underlying food tables used were the same for all coding systems. Furthermore, since cases were matched to controls within the same cohort, the different coding systems were not expected to have had any adverse effect on results.

In three cohorts (EPIC-Norfolk, EPIC-Oxford and UKWCS), in separate sections of the diaries, participants were asked to record supplement brand, name and amount per day for any supplement taken. Databases had been created to match this information against manufacturers' information for EPIC-Norfolk;[38] and UKWCS[1]. The UKWCS ingredient supplement database is described in section 4.5.3.2; this database was also used to allocate vitamin C amounts to supplements taken by EPIC-Oxford women. The two ingredient databases used included supplement descriptions and ingredient composition from product labels directly obtained from manufacturers or the participants' descriptions and/or labels. Where participants were unclear in their description, a weighted average of vitamin C from similar supplements was calculated from the database and applied.[38] For instance, separate generic averages were calculated for multivitamins, antioxidant ACE supplements and high dose vitamin C supplements. For each participant the average daily vitamin C amount consumed from all supplement types was calculated. No supplement data was analysed for Whitehall II and NSHD. For the Whitehall II study supplement data had been collected via a questionnaire but was not detailed enough to determine the vitamin C content of all supplements consumed. Since the NSHD cohort had collected supplement data at later follow-up phase, this was not included.

10.3.4 Statistical analyses

10.3.4.1 Dietary and total vitamin C analyses

Separate quintile cut points were determined for dietary intake (mg per day), dietary vitamin C intake density (mg per megajoule per day) and total vitamin C intake including supplements (mg per day). Dietary vitamin C intake density was analysed as an alternative method of controlling for potential confounding by total energy intake. Conditional logistic regression was used to model the associations between fifths of vitamin C intake and breast cancer incidence. To test for linear trends continuous intake variables per increment of approximately one standard deviation of mean intake (being 60mg/day for dietary intake and 8mg/MJ/day for intake density) were used. No supplement intakes were implausible.

Owing to the process of matching cases and controls the conditional logistic regression model automatically adjusted for date of diary completion, age (in years) and cohort. The multivariate model adjusted for exact age, parity (0, 1, 2, 3, 4+, missing), hormone replacement therapy use (current, non-current, missing), alcohol intake, total energy intake, weight (<60kg, 60-, 66-, >72kg, missing), height (<158cm, 158-, 163-, >168cm, missing), physical activity (low, low-medium, medium-high, high, missing), and menopausal status (pre, peri or post-menopausal, missing). The level of missing data ranged from 0% for alcohol and total energy intake, to 0.3% for parity to 4.2% for physical activity. Alcohol and total energy intake were ascertained from the diaries. All other covariates were collected by standard questionnaires, either self-administered or by trained researchers at or close to the time of diary completion. Sensitivity analysis was performed to adjust for variables which have weaker associations with breast cancer risk (smoking status and level of education), and also to adjust for important risk variables which had moderate levels of missing data (age at menarche (13%) and cumulative duration of breastfeeding (weeks) (15%)). This restricted the sensitivity analysis to 2811 participants. Additional sensitivity analyses also controlled for dietary vitamin E and iron which affect vitamin C bioavailability. No allowance was made for the different number of days reporting for the diaries in the different cohorts. However, the assumption of no heterogeneity across the different cohorts was formally tested by including an exposure by centre interaction term in the models. Analyses were carried out using Stata version 10 and results were based on a significance level of $p<0.05$. Note that peri-menopausal women were included in the analyses of total women but excluded from menopausal sub-analyses.

10.3.4.2 Supplement only vitamin C analyses

To test the results reported in chapter 8, which found pre-menopausal women from phase 2 of UKWCS who took supplements containing low dose vitamin C (1-60mg/d) had a significantly increased risk of breast cancer, compared to women who did not use supplement containing any vitamin C, a similar analysis was undertaken with the pooled nested case-control data from the three cohorts: EPIC-Norfolk, EPIC-Oxford and UKWCS. These were adjusted for covariates described above.

10.4 Results

10.4.1 Dietary vitamin C intake

On average the total women (2851) in the five cohorts were 56 years old and consumed 366g/d fruit and vegetables; 65% were post-menopausal, 60% had never smoked, 23% were educated to degree, HNC or HND level, and only 20% took HRT at the date of diary completion.

As observed in Table 55, total cases (851) had similar characteristics to the 2727 controls and their mean (sd) dietary vitamin C intakes were 99mg/d (55) and 101mg/d (50) respectively. Women with a higher dietary vitamin C intake tended to have a higher energy intake, consume more alcohol, dietary vitamin E and iron as well as more fruit and vegetables. Additionally, they were more active, had attained higher levels of education, or were more likely to be of higher socio-economic status or to have never smoked (Table 55).

Table 55 Characteristics of women by fifth of dietary vitamin C intake derived from food diaries in the UK Dietary Cohort Consortium

		Breast cancer		Dietary vitamin C intake (diary fifths)					
Covariates (at diary date)		Cases	Controls	1	2	3	4	5	p[a]
Cases/controls		851	2727	171/544	161/555	167/548	179/537	173/543	
Dietary vitamin C intake (mg/day)	mean (SD)	99 (55)	101 (50)	38.6 (10.1)	65.0 (6.7)	88.8 (7.4)	119.1 (10.3)	186.4(47.6)	
Fruit Intake (g/d)	mean (SD)	196 (140)	199 (143)	90 (78)	142 (89)	193 (103)	235 (121)	325 (167)	<0.001
Vegetable intake (g/d)	mean (SD)	167 (88)	175 (95)	107 (52)	146 (65)	168 (69)	196 (92)	228 (108)	<0.001
Age at diary completion (yr)	mean (SD)	56.5 (9.4)	56.0 (9.7)	55.4 (9.7)	55.9 (9.6)	56.9 (9.1)	57.1 (9.1)	56.6 (9.2)	0.03
Height (cm)	mean (SD)	162 (7)	163 (7)	161.0 (6.4)	162.0 (6.5)	162.1 (6.8)	162.8 (6.4)	163.6 (6.4)	<0.001
Weight (kg)	mean (SD)	67.2 (12.2)	68.1 (12.2)	67.7 (11.5)	67.9 (13.1)	67.6 (13.1)	67.0 (11.5)	66.8 (11.4)	0.02
Energy intake (diary, MJ/day)	mean (SD)	7.5 (1.7)	7.6 (1.7)	6.8 (1.7)	7.3 (1.6)	7.5 (1.7)	7.7 (1.7)	8.1 (1.7)	<0.001
Alcohol intake (diary, g/day)	mean (SD)	9.5 (13.8)	10.6 (13.9)	8.4 (13)	9.8 (14)	9.9 (13)	9.9 (13)	10.7 (15)	0.009
Total fat (g/d)	mean (SD)	67.5 (22.6)	69.3 (22.0)	64.5 (21.9)	68.3 (21.5)	67.3 (22.5)	69.3 (22.9)	69.7 (23.1)	<0.001
Dietary vitamin E (mg/d)	mean (SD)	9.5 (4.0)	10.0 (4.1)	8.2 (3.9)	9.2 (3.6)	9.5 (3.7)	10.2 (4.6)	11.0 (4.3)	<0.001
Dietary Iron (mg/d)	mean (SD)	11.5 (3.5)	11.9 (3.5)	9.8 (3.1)	11.1 (3.0)	11.8 (3.5)	12.2 (3.4)	13.3 (3.6)	<0.001
Parity (number of children)	mean (SD)	2.0 (4.9)	2.3 (6.0)	2.5 (6.8)	2.6 (8.2)	1.9 (1.3)	2.0 (3.9)	2.1 (6.4)	0.1
Exercise (medium - high)	n (%)	297 (37)	1021 (39)	206 (30)	246 (36)	261 (38)	297 (43)	308 (45)	<0.001
HRT use (current user)	n (%)	157 (19)	524 (20)	125 (18)	142 (20)	139 (20)	154 (22)	121 (17)	0.9
Menopausal status (post-menopausal)	n (%)	519 (63)	1801 (67)	439 (63)	460 (66)	465 (66)	488 (69)	468 (66)	0.3
Never smoked	n (%)	499 (59)	1613 (60)	341 (49)	405 (57)	445 (63)	433 (61)	488 (69)	<0.001
Education level (degree)	n (%)	202 (26)	568 (22)	75 (11)	116 (17)	142 (21)	203 (30)	234 (35)	<0.001
Social class (professional or intermediate)	n (%)	331 (51)	1272 (51)	257 (40)	304 (48)	321 (51)	356 (57)	365 (61)	<0.001

[a]p is p_{trend} over continuous exposure for continuous variables, and trend over ordered exposure groups for $\chi 2$ tests for categorical variables

The odds ratios for breast cancer according to dietary intake of vitamin C in the five cohorts are shown in Table 56 for the unadjusted and multivariate model. There was no evidence of any significant association between dietary vitamin C intake and incidence of breast cancer for total women in the five cohorts. In the adjusted analysis for total women the odds ratio of breast cancer per 60mg/day increments was 1.00 (95%CI: 0.91 to 1.09, P_{trend} = 1.0) Similarly, there was no evidence of any linear trends or significant associations between dietary vitamin C intake groups and incidence of breast cancer in the sub-analysis by post-menopausal status (OR=1.00 per 60mg/day, 95%CI: 0.89 to 1.13, P_{trend} = 1.0) or by pre-menopausal status (OR=0.98 per 60mg/day, 95%CI: 0.77 to 1.24, P_{trend} = 0.8). The results remained non-significant in sensitivity analyses after further adjustment for smoking status, age at menarche, cumulative duration of breastfeeding (weeks), and level of education. Odds ratios did not alter substantially. There was no evidence of any linear trends or significant associations between the incidence of breast cancer and dietary vitamin C expressed as intake density (Table 57).

In tests for heterogeneity there was evidence of differences between the five study centres when a study centre by dietary vitamin C intake group interaction term was included (p=0.03 total women; p=0.07 post-menopausal; pre-menopausal p=0.3), but no evidence when testing only the three largest study centres (p=0.5 total women; p=0.3 post-menopausal). When only UKWCS, EPIC-Norfolk and EPIC-Oxford, the three largest cohorts, were pooled (data not shown) there was no substantial difference in odds ratios for dietary vitamin C compared to the results for all five cohorts presented in Table 56. As observed in Table 54, the mean (sd) dietary intakes for NSHD (66mg/d (37)) was lower than EPIC-Norfolk, Whitehall II, EPIC-Oxford, and UKWCS (91mg/d (50), 101mg/d (51), 111mg/d (61) and 117mg/d (61) respectively). The lower intake for the younger, nationally representative NSHD women (mean age 43 vs. 50s in other cohorts) reflected previous findings from households with similar aged adults.[374]

Table 56 Breast cancer risks by dietary vitamin C intake recorded by diaries in the UK Dietary Cohort Consortium

Dietary vitamin C intake		Cases/	Unadjusted[a]	Adjusted[b]	Sensitivity[c]
Fifths: mean mg/day (SD)		Controls	OR (95% CI)	OR (95% CI)	OR (95% CI)
Total women					
1 (lowest):	38.6 (10.1)	171/544	1.10 (0.85, 1.42)	1.14 (0.88, 1.47)	1.09 (0.80,1.50)
2	65.0 (6.7)	161/555	1	1	1
3	88.8 (7.4)	167/548	1.02 (0.80, 1.32)	1.02 (0.79,1.31)	1.01 (0.75, 1.37)
4	119.1 (10.3)	179/537	1.12 (0.87, 1.44)	1.07 (0.82, 1.38)	1.18 (0.86, 1.60)
5 (highest):	186.4 (47.6)	173/543	1.04 (0.81, 1.35)	0.99 (0.76, 1.29)	1.10 (0.80, 1.52)
P trend per 60mg/d			0.5	1.0	0.5
Estimate per 60mg/d			1.02 (0.93, 1.12)	1.00 (0.91, 1.09)	1.04 (0.93, 1.16)
Post-menopausal					
1 (lowest)	38.5 (9.9)	100/339	1.08 (0.77, 1.52)	1.16 (0.82, 1.64)	1.17 (0.79, 1.73)
2	65.3 (6.6)	99/361	1	1	1
3	88.8 (7.3)	106/359	1.12 (0.81, 1.55)	1.16 (0.83, 1.62)	1.29 (0.88, 1.88)
4	119.1 (10.2)	112/376	1.12 (0.81, 1.56)	1.10 (0.78, 1.53)	1.26 (0.86, 1.84)
5 (highest)	186.3 (47.5)	102/366	0.99 (0.71, 1.38)	0.96 (0.67, 1.36)	1.03 (0.69, 1.53)
P trend per 60mg/d			0.4	1.0	0.7
Estimate per 60mg/d			1.03 (0.91, 1.15)	1.00 (0.89,1.13)	1.03 (0.90, 1.18)
Pre-menopausal					
1 (lowest)	38.5 (10.6)	41/136	1.32 (0.76, 2.27)	1.39 (0.76, 2.53)	1.39 (0.57, 3.37)
2	64.6 (6.9)	36/127	1	1	1
3	89.1 (7.9)	30/120	0.98 (0.54, 1.78)	1.01 (0.54, 1.90)	0.83 (0.32, 2.15)
4	119.3 (10.6)	37/101	1.25 (0.71, 2.20)	1.11 (0.59, 2.07)	1.46 (0.58, 3.69)
5 (highest)	182.1 (41.4)	37/110	1.29 (0.71, 2.28)	1.17 (0.63, 2.19)	1.24 (0.49, 3.15)
P trend per 60mg/d			0.8	0.8	0.6
Estimate per 60mg/d			1.03 (0.83, 1.28)	0.98 (0.77, 1.24)	1.09 (0.77, 1.54)

[a]Conditional logistic regression on cases and controls matched by centre, age, date of diary completion and length of follow-up

[b]As for the unadjusted model with additional adjustment for exact age, height (<158cm, 158-, 163-, 168+), weight (<60kg, 60-, 66-, 72+), physical activity, parity (0,1,2,3,4+), current HRT use, menopausal status, diary-derived alcohol consumption and total energy intake. Missing data added as a category.

[c]Sensitivity analysis, as for the adjusted model with additional adjustment for age at menarche, cumulative breast feeding, educational level and smoking status

Table 57 Breast cancer risks by dietary vitamin C density intake recorded by diaries in the UK Dietary Cohort Consortium

vitamin C nutrient density		Cases/	Unadjusted[a]	Adjusted[b]	Sensitivity[c]
Fifths:mean mg/MJ/d (SD)		Controls	OR (95% CI)	OR (95% CI)	OR (95% CI)
Total women					
1 (lowest):	5.4 (1.3)	175/540	1.04 (0.81, 1.32)	1.03 (0.80, 1.33)	1.15 (0.85,1.57)
2	8.8 (0.9)	170/546	1	1	1
3	12.0 (1.0)	166/549	0.94 (0.73, 1.20)	0.93 (0.72, 1.19)	1.05 (0.78, 1.42)
4	16.2 (1.4)	177/539	1.03 (0.80, 1.32)	1.02 (0.79, 1.32)	1.13 (0.84, 1.53)
5 (highest):	25.6 (7.0)	163/553	0.89 (0.69, 1.15)	0.90 (0.69, 1.17)	1.08 (0.78, 1.48)
P trend per 8 mg/MJ/d			0.7	0.8	0.6
Estimate per 8 units			0.98 (0.90, 1.07)	0.99 (0.90, 1.08)	1.03 (0.92,1.14)
Post-menopausal					
1 (lowest):	5.5 (1.3)	99/315	1.04 (0.74, 1.46)	1.03 (0.73, 1.46)	1.15 (0.77, 1.70)
2	8.9 (0.9)	95/344	1	1	1
3	12.0 (1.0)	104/371	0.94 (0.67, 1.31)	0.93 (0.66, 1.32)	1.02 (0.69, 1.50)
4	16.2 (1.4)	120/385	1.05 (0.75, 1.46)	1.07 (0.77, 1.51)	1.13 (0.78, 1.65)
5 (highest):	25.8 (7.1)	101/386	0.87 (0.62, 1.23)	0.90 (0.63, 1.29)	1.01 (0.67, 1.51)
P trend per 8 mg/MJ/d			0.8	0.9	0.7
Estimate/ 8 units			0.98 (0.88, 1.10)	1.00 (0.89,1.12)	1.03 (0.90, 1.17)
Pre-menopausal					
1 (lowest):	5.4 (1.4)	46/149	1.30 (0.76, 2.24)	1.32 (0.74, 2.34)	1.33 (0.57, 3.10)
2	8.8 (0.9)	42/136	1	1	1
3	12.0 (1.0)	31/107	1.02 (0.57, 1.84)	0.95 (0.52, 1.75)	0.89 (0.37, 2.18)
4	16.2 (1.4)	35/98	1.14 (0.63, 2.03)	1.04 (0.56, 1.93)	1.46 (0.64, 3.30)
5 (highest):	24.8 (6.0)	27/104	0.95 (0.52, 1.74)	0.90 (0.47, 1.74)	1.13 (0.45, 2.80)
P trend per 8 mg/MJ/d			0.7	0.7	0.6
Estimate/ 8 units			0.96 (0.78, 1.19)	0.94 (0.75, 1.20)	1.11 (0.79, 1.56)

[a]Conditional logistic regression on cases and controls matched by centre, age, date of diary completion and length of follow-up.

[b]As for the unadjusted model with additional adjustment for exact age, height (<158cm, 158-, 163-, 168+), weight (<60kg, 60-, 66-, 72+), physical activity, parity (0,1,2,3,4+), current HRT use, menopausal status, alcohol consumption and total energy intake. Missing data added as a category.

[c]Sensitivity analysis, as for the adjusted model with additional adjustment for age at menarche, cumulative breast feeding, educational level and smoking status

10.4.2 Total vitamin C intake

In the analyses of total vitamin C, cases had a somewhat higher total vitamin C intake than controls 191mg/d (sd 400) vs. 165mg/d (sd 246). As observed in Table 54 the mean vitamin C supplement intake per day for EPIC-Norfolk was significantly less than those for UKWCS or EPIC-Oxford (means mg/d (sd) were 28 (160), 122 (529) and 123 (695) respectively); likewise for total intake (means mg/d (sd) were 119 (168), 239 (361) and 234 (436) respectively). Based on diary completion date, mean total intake in autumn and winter compared to spring and summer were not significantly different (173.3 (sd 327) vs. 169.9 (sd 254) mg/d); comprising respectively of 47.1% and 52.9% of these women. The relationships between total vitamin C intake split by fifths and lifestyle characteristics are shown in Table 58 and were similar to those for dietary only intake (Table 55). The highest intake group had the highest vitamin C intake from both diet and supplements (mean (sd) 159 (76) mg/d) and 326 (561) mg/d respectively); 72% took supplements containing vitamin C and 84% of these women took them every day.

In pooling the three cohorts which recorded vitamin C intake from supplements there was also no evidence of any significant associations between total vitamin C intake and incidence of breast cancer in the adjusted analysis for the continuous estimate for all women (OR = 1.01 per 60mg/d, 95%CI: 1.00 to 1.03, P_{trend} = 0.1), or for post-menopausal women (OR = 1.02 per 60mg/d, 95%CI: 1.00 to 1.05, P_{trend} = 0.06) or for post-menopausal women (OR = 1.01 per 60mg/d, 95%CI: 0.94 to 1.09, P_{trend} = 0.8) or by fifths of total vitamin C intake (Table 59). The results remained non-significant in sensitivity analyses after further adjustment for smoking status, age at menarche, cumulative duration of breastfeeding, and level of education. There was no evidence of significant differences between the three study centres when formally tested using a study centre by fifths of total vitamin C intake interaction term, for total, post- or pre-menopausal women (p=0.7, p=0.9 and 0.5 respectively).

For both dietary and total intake no substantial differences in the estimates were found in sensitivity analyses controlling for dietary vitamin E and iron.

Table 58 Characteristics by case/control status & total vitamin C intake of women in EPIC-Oxford, EPIC-Norfolk and UKWCS nested case-control cohorts

				Total vitamin C intake (diary fifths)					
Covariates (at diary date)		Cases	Controls	1	2	3	4	5	p[a]
Cases/controls		745	2308	132/478	145/466	163/447	153/458	152/459	
Total vitamin C intake (mg/day)	mean (SD)	190.8 (399.6)	165.3 (245.8)	42.4 (11.8)	74.7 (8.4)	105.2 (9.9)	149.3 (17.5)	485.6 (541.8)	
Dietary vitamin C intake (mg/day)	mean (SD)	104.4 (59.7)	100.5 (55.5)	42.0 (11.9)	73.1 (10.5)	99.3 (18.5)	134.0 (31.6)	158.9 (75.5)	<0.001
Supplement vitamin C intake (mg/day)	mean (SD)	86.4 (391.7)	64.8 (236.2)	0.4 (3.3)	1.6 (7.3)	6.0 (16.1)	15.3 (29.9)	326 (561)	<0.001
Days/week vitamin C supplement taken	mean (SD	1.4 (2.5)	1.1 (2.6)	0.1 (0.8)	0.3 (1.2)	0.7 (2.0)	1.3 (2.4)	3.6 (2.7)	<0.001
Age at diary completion (yr)	mean (SD)	57.1 (9.6)	58.0 (9.1)	57.9 (9.2)	58.5 (9.5)	58.4 (9.1)	57.1 (8.8)	57.3 (9.5)	0.004
Height (cm)	mean (SD)	163.3 (6.5)	162.0 (6.5)	161.1 (6.1)	161.9 (6.6)	162.3 (6.8)	162.9 (6.4)	163.4 (6.5)	<0.001
Weight (kg)	mean (SD)	68.4 (12.1)	67.4 (12.1)	68.5 (11.5)	68.8 (13.3)	67.6 (12.8)	67.8 (11.8)	65.6 (10.8)	0.2
Energy intake (diary, MJ/day)	mean (SD)	7.6 (1.7)	7.4 (1.7)	6.8 (1.6)	7.3 (1.7)	7.6 (1.7)	7.8 (1.6)	7.9 (1.7)	<0.001
Alcohol intake (diary, g/day)	mean (SD)	10.5 (13.3)	9.2 (13.4)	8.0 (12.9)	9.0 (12.5)	9.7 (12.3)	10.1 (13.3)	10.7 (14.8)	0.1
Total fat (g/d)	mean (SD)	68.4 (21.9)	66.5 (22.1)	63.4 (19.9)	66.6 (22.2)	68.0 (22.4)	67.8 (20.9)	69.1 (23.7)	0.006
Non starch Polysaccharide (g/d)	mean (SD)	15.6 (5.7)	15.2 (5.6)	11.6 (4.2)	14.2 (4.7)	15.6 (5.1)	16.8 (5.3)	18.0 (6.2)	<0.001
Parity (number of children)	mean (SD)	1.8 (1.2)	2.0 (1.3)	2.1 (1.2)	2.0 (1.2)	2.0 (1.2)	2.0 (1.3)	1.8 (1.3)	<0.001
Taking supplements with vitamin C	n (%)	193 (26)	541 (23)	9 (1)	33 (5)	93 (15)	159 (26)	440 (72)	<0.001
Takes any supplement	n (%)	387 (52)	1175 (51)	179(29)	239 (39)	290 (48)	345 (56)	509 (83)	<0.001
Exercise (moderate - high)	n (%)	254 (37)	866 (39)	176 (30)	219 (37)	240 (41)	232 (40)	253 (44)	<0.001
HRT use (current user)	n (%)	144 (20)	471 (21)	113 (19)	126 (21)	124 (21)	120 (20)	132 (22)	0.4
Menopausal status (post-menopausal)	n (%)	489 (67)	1682 (74)	447 (74)	456 (76)	444 (74)	415 (69)	409 (68)	0.001
Never smoked	n (%)	440 (60)	1398 (61)	313 (52)	361 (60)	376 (62)	381 (63)	407 (67)	<0.001
Education level (degree)	n (%)	191 (27)	506 (23)	61 (10)	103 (17)	145 (25)	181 (31)	207 (36)	<0.001
Social class (professional or intermediate)	n (%)	271 (50)	1033 (50)	198 (37)	256 (47)	269 (52)	274 (54)	307 (62)	<0.001

[a]p is p_{trend} over continuous exposure for continuous variables, and trend over ordered exposure groups for $\chi 2$ tests for categorical variables

Table 59 Breast cancer risks by total vitamin C intake recorded by diaries in EPIC-Oxford, EPIC-Norfolk and UKWCS cohorts of the UK Dietary Cohort Consortium

Total vitamin C intake Fifths: mean mg/day (SD)	Cases/ Controls	Unadjusted[a] OR (95% CI)	Adjusted[b] OR (95% CI)	Sensitivity[c] OR (95% CI)
Total women				
1 (lowest): 42.4 (11.8)	132/478	0.86 (0.66, 1.14)	0.88 (0.66, 1.16)	0.96 (0.71, 1.32)
2 74.7 (8.4)	145/466	1	1	1
3 105.2 (9.9)	163/447	1.15 (0.88, 1.50)	1.13 (0.86, 1.49)	1.14 (0.85, 1.54)
4 149.3 (17.5)	153/458	1.00 (0.76, 1.31)	0.96 (0.72, 1.26)	1.07 (0.78, 1.46)
5 (highest): 485.6 (542)	152/459	1.01 (0.76, 1.35)	0.99 (0.74, 1.32)	0.98 (0.70, 1.35)
P for trend per 60mg/d		0.2	0.1	0.3
Estimate per 60mg/d		1.01 (0.99, 1.03)	1.01 (1.00, 1.03)	1.01 (0.99, 1.03)
Post-menopausal				
1 (lowest) 42.7 (11.7)	95/352	1.01 (0.72, 1.41)	1.07 (0.75, 1.51)	1.18 (0.81, 1.72)
2 74.3 (8.4)	99/357	1	1	1
3 105.4 (9.9)	112/332	1.23 (0.88, 1.71)	1.29 (0.92, 1.82)	1.39 (0.96, 2.01)
4 149.1 (17.8)	91/324	1.00 (0.70, 1.41)	0.97 (0.67, 1.39)	1.09 (0.73, 1.61)
5 (highest) 463.3 (492)	92/317	1.12 (0.78, 1.59)	1.11 (0.77, 1.59)	1.16 (0.77, 1.74)
P for trend per 60mg/d		0.09	0.06	0.2
Estimate per 60mg/d		1.02 (1.00, 1.04)	1.02 (1.00,1.05)	1.02 (0.99, 1.05)
Pre-menopausal				
1 (lowest) 41.1 (12.5)	17/74	1.04 (0.45, 2.37)	0.79 (0.31, 2.02)	1.02 (0.33, 3.14)
2 75.3 (8.1)	22/64	1	1	1
3 104.0 (9.3)	24/72	1.28 (0.56, 2.92)	1.27 (0.51, 3.11)	1.34 (0.44, 4.08)
4 149.8 (17.4)	32/78	1.26 (0.59, 2.66)	1.13 (0.49, 2.58)	1.61 (0.56, 4.66)
5 (highest) 457.5 (349)	26/85	1.46 (0.65, 3.29)	1.33 (0.55, 3.23)	1.06 (0.35, 3.20)
P for trend per 60mg/d		0.7	0.8	0.9
Estimate per 60mg/d		1.01 (0.95, 1.09)	1.01 (0.94, 1.09)	0.99 (0.91, 1.09)

[a]Conditional logistic regression on cases and controls matched by centre, age, date of diary completion and length of follow-up

[b]As for the unadjusted model with additional adjustment for height (<158cm, 158-, 163-, 168+), weight (<60kg, 60-, 66-, 72+), physical activity, parity (0,1,2,3,4+), current HRT use, menopausal status, diary-derived alcohol consumption and total energy intake

[c]Senstivity analysis as for the adjusted model with additional adjustment for age at menarche, cumulative breast feeding, educational level and smoking status

10.4.3 Supplement-only vitamin C intake

In the supplement-only analysis there was evidence of significant associations between vitamin C intake and incidence of breast cancer for total women and pre-menopausal women who took supplements containing low doses of vitamin C (1-60mg/d). As seen in Table 60, the odds ratio was higher for pre-menopausal women (OR=2.51; 95%CI: 1.22 to 5.15; p=0.01) than for total women (OR=1.39; 95%CI: 1.05 to 1.82; p=0.02). A moderate increased risk was also seen for post-menopausal women in this intake range but this was not significant (OR=1.24; 95%CI: 0.84 to 1.80). No significant associations were found in the other intake ranges (>60<500mg/d and ≥500mg/d).

Similar results were produced in sensitivity analyses after further adjustment for smoking status, age at menarche, cumulative duration of breastfeeding (weeks), and level of education. Odds ratios increased for pre-menopausal women taking supplements containing low doses of vitamin C (OR=3.34; 95%CI: 1.37, 8.17; p=0.008).

As observed in Table 61, the majority (66%) of the pre-menopausal women in this supplement intake category were from the UKWCS. Results of separate analyses for the UKWCS nest case-control for pre-menopausal women using supplements containing low dose of vitamin C were much higher than the analysis which combined the other two cohorts and the latter was not significant ((OR=4.54; 95%CI: 5.3, 13.6; p=0.007) vs. (OR=1.62; 95%CI: 0.54, 4.91; p=0.4) not tabled).

Table 60 Breast cancer risks according to vitamin C content of supplements recorded by diaries in EPIC-Oxford, EPIC-Norfolk UK and UKWCS cohorts of the UK Dietary Cohort Consortium

Supplement vit C Intake mean mg/day	Cases/ Controls	Unadjusted[a] OR (95% CI)	Adjusted[b] OR (95% CI)	Sensitivity[c] OR (95% CI)
Total women				
No vitamin C	552/1767	1	1	1
1-60mg/d	93/221	1.35 (1.03, 1.78)	1.39 (1.05, 1.83)	1.39 (1.03, 1.90)
>60<500mg/d	59/199	0.89 (0.64, 1.24)	0.89 (0.64, 1.24)	0.83 (0.56, 1.22)
≥500mg/d	41/121	0.99 (0.66, 1.48)	0.98 (0.65, 1.48)	0.90 (0.56, 1.44)
Post menopausal				
No vitamin C	383/1312	1	1	1
1-60mg/d	48/153	1.16 (0.80, 1.67)	1.24 (0.84, 1.80)	1.34 (0.89, 2.02)
>60<500mg/d	35/139	0.91 (0.60, 1.38)	0.90 (0.58, 1.37)	0.89 (0.55, 1.45)
≥500mg/d	23/78	1.04 (0.60, 1.80)	1.01 (0.58, 1.78)	0.89 (0.47, 1.68)
Pre- menopausal				
No vitamin C	81/270	1	1	1
1-60mg/d	22/39	2.34 (1.21, 4.51)	2.51 (1.22, 5.15)	3.34 (1.37, 8.17)
>60<500mg/d	8/39	0.92 (0.36, 2.35)	0.88 (0.30, 2.58)	0.90 (0.25, 3.27)
≥500mg/d	10/25	1.34 (0.51, 3.54)	1.28 (0.45, 3.67)	0.92 (0.27, 3.18)

[a]Conditional logistic regression on cases and controls matched by cohort, age and date of diary completion

[b]As for the unadjusted model with additional adjustment for exact age, height (<158cm, 158-, 163-, 168+), weight (<60kg, 60-, 66-, 72+), physical activity, parity (0,1,2,3,4+), current HRT use, menopausal status, diary-derived alcohol consumption total energy intake, and dietary vitamin C. Missing data added as a category.

[c]Senstivity analysis as for the adjusted model with additional adjustment for age at menarche cumulative breast feeding, educational level and smoking status.

Table 61 The percentage of women in each supplement category from the three cohorts of the UK Dietary Cohort Consortium in the vitamin C supplement analyses

Numbers (%)	Epic-Norfolk	UKWCS	Epic-Oxford	Total
Total				
No vitamin C	1433 (62%)	618 (26%)	268(12%)	2319 (100%)
1-60mg/d	141 (45%)	133 (42%)	40 (13%)	314 (100%)
>60<500mg/d	92 (36%)	120 (46%)	46 (18%)	258 (100%)
≥500mg/d	28 (17%)	100 (62%)	34 (21%)	162 (100%)
Total	1694 (55%)	971 (32%)	388 (13%)	3053 (100%)
Post-menopausal				
No vitamin C	1183 (70%)	389 (23%)	123 (7%)	1695 (100%)
1-60mg/d	114 (57%)	76 (38%)	11 (5%)	201 (100%)
>60<500mg/d	73 (42%)	84 (48%)	17 (10%)	174 (100%)
≥500mg/d	24 (24%)	66 (65%)	11 (11%)	101 (100%)
Total	1394 (64%)	615 (28%)	162 (8%)	2171 (100%)
Pre-menopausal				
No vitamin C	171 (49%)	155 (44%)	25 (7%)	351 (100%)
1-60mg/d	19 (31%)	40 (66%)	2 (3%)	61 (100%)
>60<500mg/d	13 (28%)	27 (57%)	7 (15%)	47 (100%)
≥500mg/d	4 (11%)	25 (72%)	6 (17%)	35 (100%)
Total	207 (42%)	247 (50%)	40 (8%)	494 (100%)

10.5 Discussion

This pooled analysis of individual participant data from five UK cohorts found no evidence of an association between incidence of breast cancer and dietary vitamin C intake recorded by food diaries. Neither was there any evidence of an association with total vitamin C intake when vitamin C from supplements was included for three of the cohorts. The non-significant results relating to dietary vitamin C intake support null results found in chapter 9 which used data from the whole of the UKWCS baseline. The current results for post-menopausal women also support the results of the 2007 WCRF meta-analyses of three cohort studies (RR=1.15 per 100mg/d, 95% CI: 0.92-1.43),[7 245 246 265] also the high versus low intake results of two US studies,[242 247] and the recent European Prospective Investigation into Cancer (EPIC) analysis involving the pooling of data from 10 European countries (highest vs. lowest quintile HR = 0.98, 95% CI: 0.87–1.11);[244] all of which used FFQs. The current results for dietary vitamin C are in conflict with significant evidence of a 12-14% reduced risk found in the meta-analysis of retrospective case-control studies which,[7] unlike our study, are prone to recall bias.

In contrast to the results from the current analysis and other studies,[239 242 247 273] the large Women's Health Initiative study[270] found significant but weak evidence of increased breast cancer risk for total intake. The advanced age of the participants in this cohort (average 64 years) might suggest that high vitamin C intake may promote the progression of cancer in older people or at later stages of the disease. Similarly positive associations with post-menopausal breast cancer for both dietary and total vitamin C intake (OR= 2.06 per 100mg/d, 95% CI: 1.45-2.91; and OR=1.08 per 100mg/d, 95% CI: 1.02-0.1.15 respectively) were found in a small Danish nested case-control study,[245] but not in the recent full analysis of this Danish cohort;[273] selection bias of controls or restricting to women who consumed vitamins from both dietary and supplement sources may have possibly influenced the earlier results. Total intake could not be assessed for the full UKWCS baseline or phase 2 datasets, since both dietary and supplement vitamin C intake was not captured at both collection points.

The doubling of breast cancer risk for pre-menopausal women consuming supplements containing low dose of vitamin C (1-60mg/d) in the supplement-only analyses supports earlier results found in chapter 8 which analysed data from the full phase 2 UKWCS dataset. This was despite a difference in calculating the average intake: the average supplement vitamin C intake used in this nested case-control was calculated over the total diary days, whereas the average used in the full dataset was calculated over the number of days taken. Possible reasons for the similar results were because diary recordings were used in both, and because a large proportion of these women in the

pooled nested case-control dataset were from the UKWCS (66%). Indeed, a significant association was found in the analysis using only UKWCS data, but not in the analyses combining EPIC-Norfolk and EPIC Oxford, though the latter may be due to low numbers involved. One reason why the result for total women consuming supplements containing low dose vitamin C in this analysis, and not in the previous analysis in chapter 8, is that pre-menopausal women made up a larger proportion of the total in this dataset than the full UKWCS phase 2 dataset.

Pooling of the cohorts was necessary since none of the separate nested case-control studies was large enough to power the analysis to detect a significant moderate effect for total vitamin C intake (about 650 cases with five matched controls were needed to detect an OR of 1.4, as calculated in the methods chapter, section 4.7.7). Pooling individual participant data in this consortium had three other advantages. Firstly, it ensured that vitamin C intake from the whole consortium could be categorised into fifths; secondly, the variations in intake across the cohorts increase the power to detect smaller effect sizes,[375] i.e. many women in EPIC-Oxford and UKWCS were vegetarians and/ or consumed supplements containing vitamin C compared to the other cohorts; thirdly, analysis and adjustment by covariates could be done in a uniform way.

The study had a few caveats. Whilst the use of missing covariate categories may have grouped dissimilar individuals and introduced some bias, its effect on the adjusted results may be considered acceptable since the level of missing data was small, and confounding was judged to be weak. To account for the possible modulation of vitamin C on cancer development due to its the role in the regeneration of vitamin E, in the absorption of iron and in the Fenton reaction,[76] sensitivity analysis adjustments were made for these dietary nutrients. Supplement intake data for these nutrients, however, was not available. The Danish studies, one of which found a positive association, controlled for both dietary and supplement intake of vitamin A and E.[245 273] In the current study data were unavailable to adjust for family history of breast cancer which has been associated with high-dose vitamin C supplement use in the UK [1]. Data were unavailable from all cohorts to exclude general supplement users from the dietary analysis; the different health behaviours of users may have influenced the results.[27] There was inadequate power to sub-analyse by HRT users, oestrogen receptor-negative or pre-menopausal breast cancers to find moderate effects.

Unfortunately, it was considered that the selection of some of the UKWCS controls was biased, in that they may have not been truly representative of the cohort, the population from which the cases were drawn, with respect to vitamin C exposure. This selection bias could have been minimised by selecting controls at random from the

whole cohort. However, the controls from the UKWCS which were coded using DINER, a system developed at Cambridge, were considered not to be a random selection since it was later found that they were more likely to be meat-eaters and less likely to be vegetarians than the cohort as a whole. It was assumed that, on average, the meat-eaters would have consumed less vitamin C than vegetarians, and therefore controls were likely to have had lower vitamin C intake than the cohort on average. An artificially inflated difference in vitamin C intake between cases and controls could have attenuated any potential associations between breast cancer risk and vitamin C intake in the results. Therefore, for the manuscript accepted for publication which used the same dietary and total analysis methods used in this chapter,[2] these 583 controls and the 144 matched cases were omitted from the dataset due to potential selection bias. However, their omission made little difference to the results, giving further support to the conclusion that vitamin C intake is not associated with breast cancer risk. Additionally, further inspection indicated that the vitamin C intake of these controls was, in fact, unlikely to be much different from the cohort as a whole; as seen in chapter 5, section 5.3.3, the vegetarians in the UKWCS on average consumed only 3% more vitamin C than non-vegetarians, the majority of whom were meat eaters. Given these facts, it was decided to include these cases and controls in the analyses for this chapter.

This is the first time the relationship between breast cancer risk and vitamin C intake has been analysed using prospective data from food diaries. Diaries can capture detailed and accurate intake over a narrow period of days due to their open format, whereas FFQs aim to reflect intake over a much longer period, normally an estimated average of the previous 12 months. Repeated diary data collections may reduce their short-term limitations but were not undertaken for the whole consortium due to expense and time taken to administer, complete and analyse. The required commitment and awareness of intake may have also influenced participants' consumption during diary recording. As discussed in section 5.4.4 of the evaluation chapter, when compared to FFQs, food diaries have shown stronger correlations with plasma vitamin C biomarkers in validity tests when collected in close temporal proximity. However this may reflect the short-term nature of both plasma vitamin C and diary data, particularly since correlations with biomarker levels re-measured several years later were similar for diaries and FFQs.[346 347 370] Furthermore, other UK validation studies have shown similar associations between biomarkers and vitamin C estimated from FFQs and diaries. [294 338] Overall correlations between biomarkers and FFQs or diaries are generally weak to moderate.[335 343] Since the absorption and storage of vitamin C is limited, particularly above 400mg/d,[65] biomarkers are unlikely to reflect

dietary vitamin C intake well. Therefore it is difficult to assess objectively whether diaries or FFQs can rank individual intake sufficiently well in order to find associations between vitamin C and cancer risk. Given the limitations, results of vitamin C analyses from either FFQs and diaries need to be treated with some caution. Additional analysis could be undertaken using dietary vitamin C FFQ data from this UK Dietary Cohort Consortium dataset, to compare them with the diary dietary vitamin C results. Nevertheless, the dietary results using diary data in this chapter supports those reported in chapter 9 using FFQ recordings.

To conclude, the supplement-only results support those found in chapter 8, that use of supplements containing low-dose vitamin C is associated with increased breast cancer risk, though as explained in chapter 8, the results may be due to the effects of other ingredients in supplements, or may be spurious. In relation to dietary or total vitamin C intake, the evidence to date from this and other prospective studies does not indicate either a beneficial or a detrimental effect of vitamin C intake on breast cancer risk.

CHAPTER 11

11 Summary discussion

The analyses in this thesis have used data from the UKWCS and other cohorts in the UK Dietary Cohort Consortium. These are some of the largest population-based prospective studies in the UK which were designed to assess associations between diet and chronic diseases. The majority of the analyses used previously unexploited supplement data to explore, for the first time, breast cancer risk in UK women in relation to:

- any supplement use, including use of any supplements over two recording periods, roughly 4 years apart (chapter 7)
- vitamin C contained in supplements, examining dose-response relationships, as well as the risk of taking supplements containing high, medium and low vitamin C content (chapter 8)
- dietary vitamin C intake derived from FFQs for supplement users and non-users; the latter sub-analysis provided a clearer picture of the relationship between breast cancer risk and dietary vitamin C intake without residual confounding by factors relating to supplement use. The use of restricted cubic spline models enabled non-linear relationships between vitamin C intake and breast cancer risk to be explored for the first time (chapter 9)
- total vitamin C intake from both diet and supplements; this was examined prospectively for the first time using food diary records instead of FFQs (chapter 10)

The combination of these objectives ensured that vitamin C intake in relation to breast cancer risk in UK women had been thoroughly examined in this thesis. The first three objectives used data from the baseline or phase 2 of the UKWCS only, whilst the fourth objective used nested case-control data from the UKWCS and other UK studies, which were part of the UK Dietary Cohort Consortium. Data from the extensive supplement ingredient database and participant supplement use database at phase 2 of the UKWCS was used for the second objective. Additionally, a large number of vegetarian women consuming high levels of dietary vitamin C from fruit and vegetables were included in the baseline analysis. This provided a wide range of vitamin C intakes for analysis. Furthermore, women from baseline and phase 2 in the UKWCS were followed-up for cancer incidences for over 11 and 7 years respectively, which was

comparable to or better than most studies detailed in the literature review chapter in section 3.7.2.

A major strength of these studies is that their prospective nature minimised reverse causality, recall bias and responder bias which can affect results of retrospective case-control studies. Selection bias, however, may have been present in the nested case-control analysis of total vitamin C, though this may have been minimal. Their large size increased the power of the analyses, particularly in the baseline and nested case-control analyses which involved substantial numbers of cases. Additionally, the study questionnaires captured many important health and lifestyle factors which were potential confounders and were adjusted for in the analyses.

The UKWCS, instigated through the World Cancer Research Fund, in general comprised of health conscious women. The results are therefore directly applicable to similar women, who would be interested to know how lifestyles choices could affect their breast cancer risk, and who may be prepared to alter their behaviours. The UK Dietary Cohort Consortium data produced results slightly more generalizable to the UK population as a whole, in addition to providing an opportunity to examine breast cancer risk in relation to total vitamin C intake recorded by diary data.

In addition to the objectives listed above, the phase 2 UKWCS data provided an opportunity to determine, for the first time, whether UK women who have a history of breast cancer were more likely to use high dose vitamin C supplements. The results showed women who had a family history of breast cancer, or who self-reported having had breast cancer, were respectively 26% and 70% more likely to use vitamin C supplements of 1000mg/d or above, (chapter 6).[1] Since these women were likely to have different health behaviours than others, women with a family history of breast cancer were adjusted for and/or excluded in breast cancer risk sensitivity analyses in this thesis.

In relation to risk, the results showed there was no evidence of statistically significant associations between general supplement use, fruit and vegetable intake and vitamin C intakes per se and breast cancer incidence in the UK in any of the analyses reported in this thesis. Similarly, no associations were found after excluding all supplement users in the dietary vitamin C analysis; previously non-supplement users had been examined in only one other cohort.[273] Moreover, the unique use of diary recorded data in dietary and total vitamin C analyses produced no evidence of associations with breast cancer risk. These results are inline with the majority of previous prospective studies from other countries reported in the 2007 WCRF review and the 2008 WCRF continuous update.[7 169] As discussed in section 3.7 of the literature review chapter, the

inclusion of the results from more recently published studies in future meta-analyses are unlikely to produce a different conclusion from the 2007 WCRF review,[14 244 270 273] despite a significantly increased breast cancer risk with high vitamin C intake from supplements reported in 2008 by a large US study.[270] Meta-analyses including these recent studies have not been done for this thesis since these are likely to be undertaken by the WCRF in their future continuous updates. Regardless, there was insufficient evidence from the UKWCS and other studies to support a U–shaped relationship between vitamin C intake and breast cancer risk, which at the start of this thesis had been hypothesised due to the potential of vitamin C to act as both an antioxidant and a pro-oxidant.

The current study did, however, find a more than doubled increased risk for pre-menopausal women taking supplements containing low dose vitamin C, up to and including 60mg/d (the EU RDA), compared to women not taking supplements containing vitamin C. Since there appears to be no biological rationale that this increased risk is specifically due to low vitamin C content, it was thought to be due to other ingredients in the supplements taken. However, in exploring the relationship with multivitamin supplements, which usually contain EU RDA amounts of vitamin C, no significant associations with breast cancer in the UKWCS were observed (although a non-significant increased risk of 50% for pre-menopausal women was found for daily users). Additionally, as seen in chapter 7, general supplement use was associated with a weak, but non-significant increased risk for pre-menopausal women. The significant finding may be spurious due to residual confounding, or possible inappropriate use of covariates or due to low numbers of pre-menopausal women involved. Alternatively multiple testing of sub-groups may have produced spurious significant associations by chance, though the pattern seen for pre-menopausal women does not support this. To avoid chance findings, lower p values for testing levels of statistical significance could have been used;[298] the p-values, nevertheless, for the doubling in risk were very low (e.g. 0.004). Instead, the significant results may be specific to this group of cases in the UKWCS. Indeed, in a previous UKWCS analysis of pre-menopausal women another result was difficult to explain; vegetarians were found to have a significantly higher breast cancer risk than low meat consumers.[82] Since few studies have examined use of supplements containing vitamin C in well-nourished pre-menopausal women more research is needed. The two studies which have assessed low level intakes of vitamin C from supplements included post-menopausal women only.[270 273]

Although the UK cohorts were of high quality and large size, the possibility that null results have been found when a true association exists should not be ruled out entirely. Various reasons for null results have been suggested by Willet, which can be related to

the current findings.[301] From the life-course perspective, vitamin C intake prior to breast tissue differentiation before first pregnancy may have a greater influence on breast cancer risk than intake in later life. Nevertheless, it is difficult to argue that midlife is not a critical period for susceptibility to the effects of vitamin C intake in relation to cancer development; midlife in general being the age when the women in these cohorts recorded intake. Considering the antioxidant theory, and given the potential accumulation of DNA damage caused by free radicals as we age, midlife appears to be a time when the antioxidant effects of vitamin C would be most beneficial in preventing cancer initiation. On the other hand, non-invasive cancer cells may be already present in many women at midlife, and these may be susceptible to further cancer progression by potentially detrimental effects of vitamin C discussed in chapter 2. Since vitamin C plays many roles in the body and interacts with other nutrients, any weak to moderate positive and negative effects these create in relation to cancer may balance out to produce a null effect overall. An additional reason for null results is vitamin C may only affect cancer risk in certain types of women and there may have been insufficient cases to power some of the sub-analyses undertaken to find statistically significant effects. Different mechanisms in the body are likely to dominate in certain sub-groups of pre- or post-menopausal women, for instance it is possible that vitamin C may have a differential effect with women who are overweight compared to those who are not; however this was not analysed in this thesis. Since subgroup analysis can produce significant associations by chance, they should be stated a priori or, as mentioned above, at least smaller p values should be used for statistical significance levels.

Although there was considerable variation in vitamin C intake across the UKWCS, the women were well-nourished, meaning there may have been insufficient women with low vitamin C status to reveal a raised risk of breast cancer in such women, if one existed. Only 3% of women in the baseline analyses had dietary intakes below the EU RDA (60mg/d) and less than 1% had intakes below the UK RNI (40%). Furthermore, simple linear dose-response relationships may not have been apparent when calculating continuous estimates over a wide intake range, due to the limits of absorption, storage, etc of vitamin C at high intake levels. Nevertheless, the restricted cubic spline graphs did not show any significant relationships, despite excluding outliers that may have undue influence. Another reason for finding a null result is that, as discussed in chapters 5 and 10, the FFQ, and 4-d and 7-d diary methods in general may be too imprecise to measure vitamin C accurately. Although Bingham et al. (1987) suggested that 36 days of weighed food records are required to correctly classify vitamin C intake at an individual level,[376] this is not practical, and besides participants are likely to change their eating habits to reduce the burden of recording. Repeat

measures over a period of years are needed to examine the effects of longer term intake, or fluctuations in intake. The largest fluctuations in nutrient intakes are likely to be due to supplements, but as seen in chapters 3 and 8, little research on this has been reported, and further research is needed. Finally, another limitation common to all the UKWCS results is that, since the cohort includes a high proportion of vegetarians, the results are not generalizable to the UK population, therefore absolute risk rates for the UK as a whole could not be calculated from the results. Sensitivity analyses could be undertaken to reweight the cohort by the percentage of vegetarians in the general population. In the 2004 NDNS survey 7% of women (and about 5% over the age of 35 years) stated they were vegetarian,[24] whereas at baseline in the UKWCS 28% stated they were vegetarian. However, a more accurate but pragmatic definition classified 18% as vegetarian (those who ate meat or fish less than once a week).[279] Previous analyses of the UKWCS, however, have found no substantial differences before or after weighting for vegetarians.

Although adjustments were made for many confounding factors, one potential limitation was that no adjustment could be made for breast screening, since this information had not been gathered. Breast screening, which has been provided to over 50 year olds since the start of the UKWCS, could have been a confounding factor, particularly in the post-menopausal analyses. This was because breast screening was likely to pick up cases sooner and because health conscious women were more likely to attend screening as well as tending to have a higher vitamin C intake. Additionally, these women may have been more likely to have had a family history of breast cancer, and potentially had a higher risk of breast cancer themselves. Future analyses of other cohorts which have gathered screening information could control for this, though prior studies however, have not done so. It is possible that given the long length of follow-up of this cohort, and many prior studies, screening may not have detected substantially more cases.

11.1 Ideas for future research

11.1.1 UKWCS

Further sensitivity analyses of the current data could be undertaken to observe the effects of excluding self-reported cancers in the baseline analyses, or the effects of excluding women with a family history of cancer, where this has not already been done. Additionally, sensitivity analyses could be undertaken to exclude women who developed breast cancer within two years, since some of these women may have suspected they had cancer and altered their diet before their diagnosis. Furthermore,

the interaction, seen in chapter 7, between general supplement use and socio-economic status in relation to breast cancer could be explored further.

Since total cancer incidence is available for the UKWCS, associations between this risk and vitamin C intake or multivitamin use could be examined. Although, there are too few women (861) who had prevalent breast cancer at baseline to examine risk of breast cancer reoccurrence, there is scope to examine changes in diet from baseline to phase 2 for the ~130 women who developed cancer between these data collections.

Within phase 2 of the UKWCS there is a range of possibilities for examining risk of cancers or other diseases in relation to other supplements, whether this is from recordings by diary or questionnaire. Supplement types recorded by questionnaire, however, are limited to those displayed in Figure 2 in chapter 2, with fish oil (26.5%), evening primrose oil/ starflower oil (18.7%) and calcium (14.2%) being used by a relatively high percentage of women. Unfortunately, vitamin D supplement use, which has been the focus of more recent attention,[377] has not been recorded by questionnaire in the UKWCS. Additionally, folic acid supplement use, which as discussed in chapter 8 has been linked to an increased risk of cancer, was only recorded by 2.2% of women on the questionnaire which would result in insufficient cases to power analyses. Nevertheless, folic acid is present in many supplements, particularly multivitamins; therefore the amount of this or other nutrients such as vitamin D in supplements recorded by diary could be determined from the UKWCS participant supplement database. Further cleaning of this database would be needed, and ideally, these analyses should be delayed to ensure sufficient cancer cases have accumulated to power the analyses.

Furthermore, breast cancer risks for UKWCS women who followed most of the eight WCRF cancer prevention guidelines, summarised at the front of the 2007 WCRF report,[7] could be compared to those who did not. Similar research has been undertaken in a US cohort where a score of 0-2 was given for adherence to each of the four American Cancer Society prevention guidelines, and subjects with total scores of 7-8 were compared to those with total scores of 0-2.[378]

Although women at phase 2 who were at raised risk or high risk of breast cancer, according to the NICE guidelines, were excluded from some of the analyses in this thesis, they comprised such a small percentage of UKWCS (2.1% and 0.8% respectively) that breast cancer risk analyses for these women were not feasible. Also, there are too few of these women to undertake a nested case-control study.

11.1.2 Other

As previously discussed, there is a need for more accurate methods of measuring dietary intake in order to examine relationships between diet and cancer risks. There is potential for cohort dietary data to be collected using innovative technologies in the future; for instance using on-line 24 hour recall questionnaires and FFQs, or using handheld devices such as the Smartphone with the 'My Meal Mate' application that is being trialled at the University of Leeds.[379] These have facilities for participants to select foods and have the potential for data to be downloaded to an automatic coding system, which would reduce data collection and coding time, as well as coding errors. Additionally, the portability and convenience of the Smartphone has an added advantage that recordings can be done in real-time and not be reliant on memory.[379] Further work, however, may be needed on the application to ensure portion sizes are correctly recorded.[379] It is expected to be less burdensome than paper diary methods, particularly for the younger generation, and therefore participants may be less likely to alter their normal intake and may be willing to produce many days of reporting necessary to correctly classify their nutrient intake, and to repeat these at future dates. In order to minimise costs, the recruitment of sufficient participants with their own personal Smartphones for a large cohort appears possible in the future since these phones are becoming widely used. Although this device may not be suitable to gather follow-up data for the women of the UKWCS, three quarters of whom will now be over the age of 60, it may be suitable for collecting data from their offspring, if required.

Future research on other cohorts needs to take account of supplements, whether this is through sub-analysis by supplement users and non-users or, for nutrient analyses, by including nutrient intake from supplements. As discussed above more research is needed relating to supplement use in pre-menopausal women, and in particular use of supplements containing low dose vitamin C such as multivitamins. Vitamin C intake sub-analyses by HRT or by BMI should be undertaken in large cohorts to test for significant results found in the pooled EPIC study and the RCT previously mentioned.[14 255] Additionally, very few studies have sub-analysed by hormone receptor status of breast cancers,[270 273] since these data have not been widely available, or there has been insufficient cases to power the analyses. In general, the prevalence of oestrogen receptor negative breast cancers may be less than 20%, and for triple negative breast cancers may be about 15%.[380] Many women who develop the latter are carriers of BRCA1 gene faults,[380] and little research has been done to identify whether lifestyle factors including diet could modify risks for these women, or others who are highly susceptible to breast cancer. Due to the small percentage of these women, nested case-control studies would be needed. A Korean case-control study reported breast

cancer risk was higher in women who were carriers of ATM (Ataxia telangiectasia mutated cells) and had low intakes of vitamin C or other antioxidants compared to those with high intake.[272] Since intake in this study was measured after diagnosis of cancer, temporal bias cannot be ruled out.

Genetic susceptibility to having low blood vitamin C levels could be researched with respect to breast cancer risk. A genetic variation (at the *SLC23A1* gene locus) in coding for a sodium-dependent vitamin C active transporter which facilitates ascorbic acid transport across intestinal cell membranes has been associated with low plasma ascorbic acid levels.[381] This genetic variant could be used as an instrumental variable within observational studies as a proxy for plasma vitamin C levels to determine the causal effect of lifetime variation in vitamin C concentrations on the risk of cancer and other chronic diseases.[381] This 'Mendelian Randomisation' method has the added benefit of being free from confounding influences since potential confounders are naturally randomised between carriers and non-carriers of the genetic variant.[382] However, this method is limited by the need for very large sample sizes.[382]

Nutrigenomics is a relatively new research discipline involving the study of genetic variations and their interactions with nutritional factors and subsequent associations with disease. Not only can genetic variation affect an individual's processing of food, but constituents of food can also change the expression of genes, i.e. the phenotype, which may then impact on the development of disease. It may be some considerable time, however, before research evidence from nutrigenomics can be employed at an individual and commercial level due to the cost and time involved in the genotype-phenotype-biomarker testing of individuals that would be necessary in order to provide tailored dietary advice.

More research using larger cohorts, nested case-control studies or RCTs is needed in relation to vitamin C intake or supplement use and risk of cancer reoccurrence, especially since cancer survivors are more likely to use supplements, as seen in chapter 6. There has been controversy surrounding evidence of benefits of high dose vitamin C supplementation for prolonged cancer survival,[87-89] indeed there is some evidence that antioxidants can potentially reduce the effectiveness of anti-cancer drugs.[77 356] Patients, therefore, should be encouraged to discuss their supplement use with their doctors in order to avoid contraindications. More recently clinical trials using intravenous vitamin C, with or without chemotherapy have been undertaken (e.g. trial reference NCT00441207 and NCT01050621). Phase I results of a recent Canadian trial failed to demonstrate an effect on patients with advanced cancer.[383]

11.2 Public health issues

The potential for antioxidants, contained in fruit and vegetables, to reduce the risk of cancers, was one of the original promoted benefits of the 5-A-day fruit and vegetable campaign. Despite the evidence in chapter 9 and in the 2007 second WCRF review,[7] that neither the increased intake of fruit and vegetables, nor dietary intake of vitamin C appear to reduce the risk of breast cancer, the public health message to consume high intakes of fruit and vegetables need not be changed since there is no convincing evidence of detrimental effects. Moreover, fruit and vegetable intake should be encouraged since there is evidence they reduce the risk of other cancers,[7] as well as heart disease.[384] Whether it is antioxidants and specifically vitamin C, or other bioactive chemicals in the fruit and vegetables that are beneficial in these cases is still unclear.

The current results also suggest, along with some but not all previous studies reported in chapter 8, that there are no detrimental effects in relation to breast cancer risk from using high dose vitamin C supplements, meaning the current results do not support a need to restrict their sale to the general public. Furthermore, after reviewing evidence on any aspect of health, both the European Food Safety Authority and the UK Expert Group on Vitamins and Minerals currently believe there is insufficient data to set an upper limit for vitamin C.[22 52] One reason why vitamin C may not have an effect on humans is that it is water-soluble and is readily excreted above levels of about 400mg/d.[65] Being water-soluble, though, does not preclude micronutrients from being toxic as there have been recommendations that other soluble micronutrients, such as folic acid, should be re-categorised as over-the-counter medicine because of concerns over toxicity.[385] Vitamins are currently sold as dietary supplements where the onus in the US has been on the food authorities to prove supplements are unsafe, rather than manufacturers needing to provide evidence of their benefits and needing to obtain licences to sell them like drugs.[385] In addition however, in Europe the EFSA has recently implemented regulations for approving or rejecting general health claims made on foods and supplements based on whether these claims are independently verified by research studies.[21]

Indeed, as seen in chapters 8 and 10, no protective effects of taking high dose vitamin C, or any dose of vitamin C, were found in terms of breast cancer risk, which in general supports previous research from cohort studies discussed in chapter 8. Results, therefore, do not support the use of vitamin C supplements for reducing breast cancer risk. As reported in chapter 2 and chapter 6, as many as a third of UKWCS women used supplements containing vitamin C, and about 10% of women frequently took high dose vitamin C. Furthermore, chapter 6 reported that women with a family history of

breast cancer or personal history of breast cancer in the UK were more likely to frequently take high doses of 1000mg/d or above.[1] Although the reasons these women took high dose supplements is unknown, it is possible they hoped they may reduce breast cancer risk. It appears, therefore, some women may be spending money and consuming supplements unnecessarily. Indeed, antioxidant supplements are sold in a multimillion pound worldwide industry. Although there are regulations to prevent specific health claims being stated explicitly, antioxidants are sold on the basis they reduce free radicals, which is widely believed, by the general public, to play a role in preventing chronic diseases including cancer. However, some researchers doubt antioxidants can provide this role and believe claims relating to them may be overstated.[386] Indeed, the EFSA has recently reported that no evidence has been provided to them to establish that having antioxidant activity/content and/or antioxidant properties exerts beneficial physiological effects on humans.[46] Any future restrictions on EU selling, however, would not prevent supplements being purchased outside the EU by UK residents via mail order.

The 2007 WCRF report, nevertheless, judged there was convincing or probable evidence from prospective studies that some antioxidants are associated with some cancers (Appendix B).[7] For instance, the antioxidant selenium was associated with a reduced risk of lung, stomach and prostate cancer,[7] although more recent analyses from the SELECT trial do not support the latter.[237 387] Furthermore, supplementing with low doses of antioxidants appears to reduce cancer risk or cancer mortality in people who may have low antioxidant status,[17] for instance men,[256] and people researched in remote parts of China who may consume insufficient nutrients via their diet.[388] Importantly, however, there has been convincing evidence that supplementation with the antioxidant β-carotene increases the risk of lung cancer in smokers.[238]

As Bjekelakovic has pointed out, dietary supplementation has moved from preventing deficiencies to being used in attempts to promote wellness and prevent disease. He maintains consumers are being taking advantage of and are not getting value for money since supplementation cannot be recommended for preventative measures in well-nourished populations.[389] Using a peer-reviewed Cochrane protocol, a systematic review and meta-analysis by Bjelakovic et al. (2007) of low-bias, primary and secondary prevention randomised trials of antioxidant supplements (published to October 2005) concluded that antioxidant supplements carotene and vitamin E, and also vitamin A may increase all-cause mortality.[351] Vitamin C and selenium were found to have no effect on mortality. Several researchers, nevertheless, have questioned the Bjelakovic et al. (2007) results, for instance, because 405 out of 815 trials reviewed were excluded since no deaths occurred.[390]

The question remains: Should vitamins be considered as drugs?[385] The evidence from this thesis for vitamin C does not support this consideration; however, the results indicate that more research evidence is needed on pre-menopausal supplement users to help answer this question. The current evidence, nevertheless, does not justify the widespread use of vitamin C supplements to reduce breast cancer risk. Indeed, the WCRF does not recommend supplements to prevent cancer and in 2009 issued a press release warning on high strength supplements in general.[362] Whether the advice has been heard and acted upon is, as yet, unknown.

The WCRF reported that a 2009 YouGov survey found media reporting of conflicting studies appears to have caused many people to ignore dietary advice relating to cancer risks, with 60% of people over the age of 55 years being cynical about dietary advice.[15] Consequently, media reporting of more important results from reviews, meta-analysis and large pooling studies are likely to be overlooked by the general public, in the midst of reports of many smaller less important results. Therefore, there may be a need for greater control over the types and amount of reporting by journalists, who may need some education in epidemiology. Additionally, academics should be encouraged to disseminate important work in popular press. Given the often inappropriate affect of the media, more government controls may be needed relating to the pricing, availability and production of food and supplements, in order to reduce the risk of cancer in general. Policy recommendations such as these have been detailed by the WCRF in their policy report.[391]

12 References

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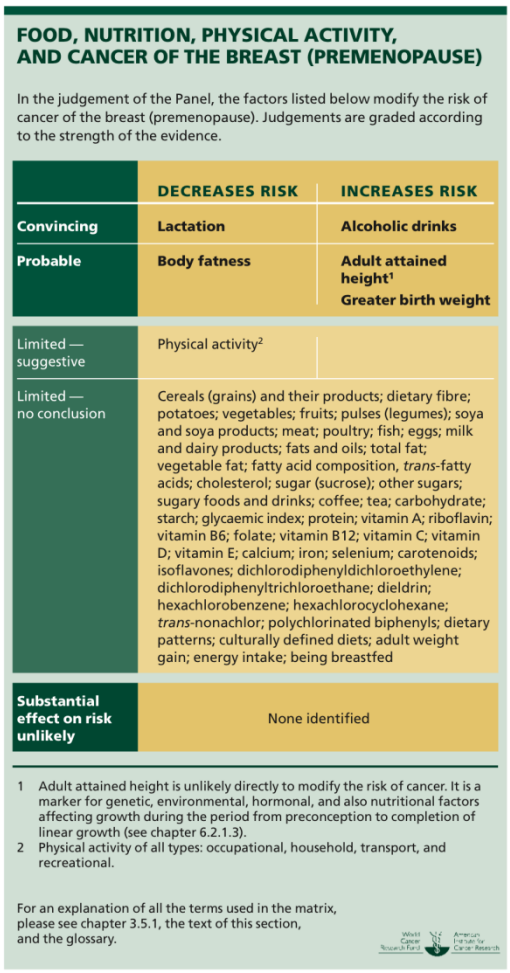
Appendix A.

Summary of research findings relating to breast cancer risks from the 2007 WCRF report on food nutrition, physical activity and cancer prevention



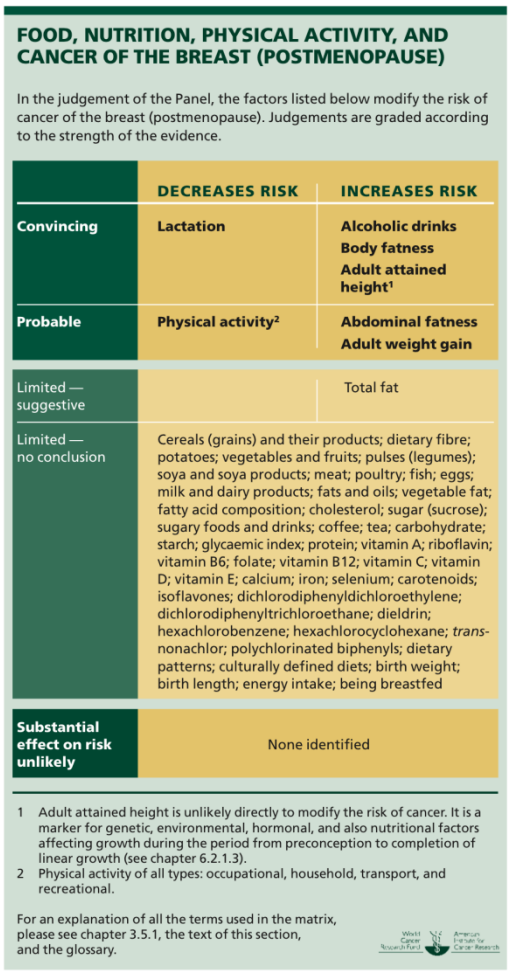
FOOD, NUTRITION, PHYSICAL ACTIVITY, AND CANCER OF THE BREAST (PREMENOPAUSE)

In the judgement of the Panel, the factors listed below modify the risk of cancer of the breast (premenopause). Judgements are graded according to the strength of the evidence.

	DECREASES RISK	INCREASES RISK
Convincing	**Lactation**	**Alcoholic drinks**
Probable	**Body fatness**	**Adult attained height[1]** **Greater birth weight**
Limited — suggestive	Physical activity[2]	
Limited — no conclusion	Cereals (grains) and their products; dietary fibre; potatoes; vegetables; fruits; pulses (legumes); soya and soya products; meat; poultry; fish; eggs; milk and dairy products; fats and oils; total fat; vegetable fat; fatty acid composition, *trans*-fatty acids; cholesterol; sugar (sucrose); other sugars; sugary foods and drinks; coffee; tea; carbohydrate; starch; glycaemic index; protein; vitamin A; riboflavin; vitamin B6; folate; vitamin B12; vitamin C; vitamin D; vitamin E; calcium; iron; selenium; carotenoids; isoflavones; dichlorodiphenyldichloroethylene; dichlorodiphenyltrichloroethane; dieldrin; hexachlorobenzene; hexachlorocyclohexane; *trans*-nonachlor; polychlorinated biphenyls; dietary patterns; culturally defined diets; adult weight gain; energy intake; being breastfed	
Substantial effect on risk unlikely	None identified	

1 Adult attained height is unlikely directly to modify the risk of cancer. It is a marker for genetic, environmental, hormonal, and also nutritional factors affecting growth during the period from preconception to completion of linear growth (see chapter 6.2.1.3).
2 Physical activity of all types: occupational, household, transport, and recreational.

For an explanation of all the terms used in the matrix, please see chapter 3.5.1, the text of this section, and the glossary.



FOOD, NUTRITION, PHYSICAL ACTIVITY, AND CANCER OF THE BREAST (POSTMENOPAUSE)

In the judgement of the Panel, the factors listed below modify the risk of cancer of the breast (postmenopause). Judgements are graded according to the strength of the evidence.

	DECREASES RISK	INCREASES RISK
Convincing	**Lactation**	**Alcoholic drinks** **Body fatness** **Adult attained height[1]**
Probable	**Physical activity[2]**	**Abdominal fatness** **Adult weight gain**
Limited — suggestive		Total fat
Limited — no conclusion	Cereals (grains) and their products; dietary fibre; potatoes; vegetables and fruits; pulses (legumes); soya and soya products; meat; poultry; fish; eggs; milk and dairy products; fats and oils; vegetable fat; fatty acid composition; cholesterol; sugar (sucrose); sugary foods and drinks; coffee; tea; carbohydrate; starch; glycaemic index; protein; vitamin A; riboflavin; vitamin B6; folate; vitamin B12; vitamin C; vitamin D; vitamin E; calcium; iron; selenium; carotenoids; isoflavones; dichlorodiphenyldichloroethylene; dichlorodiphenyltrichloroethane; dieldrin; hexachlorobenzene; hexachlorocyclohexane; *trans*-nonachlor; polychlorinated biphenyls; dietary patterns; culturally defined diets; birth weight; birth length; energy intake; being breastfed	
Substantial effect on risk unlikely	None identified	

1 Adult attained height is unlikely directly to modify the risk of cancer. It is a marker for genetic, environmental, hormonal, and also nutritional factors affecting growth during the period from preconception to completion of linear growth (see chapter 6.2.1.3).
2 Physical activity of all types: occupational, household, transport, and recreational.

For an explanation of all the terms used in the matrix, please see chapter 3.5.1, the text of this section, and the glossary.

World Cancer Research Fund /American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington, DC: AICR, 2007. P289

Appendix B.

Summary of research findings relating to supplement use from the 2007 WCRF report on food nutrition, physical activity and cancer prevention

DIETARY CONSTITUENTS AND SUPPLEMENTS, AND THE RISK OF CANCER

In the judgement of the Panel, the factors listed below modify the risk of cancer. Judgements are graded according to the strength of the evidence.

	DECREASES RISK		INCREASES RISK	
	Exposure	**Cancer site**	**Exposure**	**Cancer site**
Convincing			**Beta-carotene supplements[1]**	**Lung**
Probable	**Calcium[2]** **Selenium[3]**	**Colorectum** **Prostate**		
Limited — suggestive	Retinol[4] Alpha-tocopherol[2] Selenium[3]	Skin[5] Prostate Lung[3] Colorectum[6]	Retinol supplements[1] Selenium supplements[2]	Lung Skin
Substantial effect on risk unlikely	Beta-carotene[7]: prostate; skin (non-melanoma)			

1 The evidence is derived from studies using high-dose supplements (20 mg/day for beta-carotene; 25 000 international units/day for retinol) in smokers.
2 The evidence is derived from studies using supplements at a dose of 200 µg/day.
3 The evidence is derived from studies using supplements at 200 µg/day. Selenium is toxic at high doses.
4 The evidence is derived from studies using supplements at a dose of 25 000 international units/day.
5 Applies only to squamous cell carcinoma.
6 The evidence is derived from studies using supplements at a dose of 200 µg/day. Selenium is toxic at high doses.
7 The evidence is derived from studies using supplements (at doses of 20, 30, 50 mg for prostate, and doses of 30, 50 mg/day for skin), and foods containing beta-carotene: see chapter 4.2.

For an explanation of all the terms used in the matrix, please see chapter 3.5.1, the text of this section, and the glossary.

World Cancer Research Fund /American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington, DC: AICR, 2007. P289

Appendix C.

Search strategy for vitamin C and breast cancer

OVID MEDLINE (Jan 2006 -June 2011)

1. (vitamin$ adj5 c).ti,ab,sh.
2. Ascorbate.ti,ab,sh.
3. ascorbic acid.ti,ab,sh.
4. exp Ascorbic acid/
5. or/1-4
6. antioxidant*.ti,ab,sh.
7. anti-oxidant*.ti,ab,sh.
8. antioxidants/
9. or/6-8
10. vitamin*.ti,ab.
11. vitamins/
12. 10 or 11
13. 9 and 12
14. (micronutrient$ or nutrient$).ti,ab,sh.
15. fruit$.ti,ab,sh.
16. vegetable$.ti,ab,sh.
17. 14 and 15 and 16
18. 5 or 13 or 17
19. exp breast neoplasms/
20. exp neoplasms, hormone-dependent/
21. (breast adj25 neoplasm$).ti,ab.
22. (breast adj25 cancer$).ti,ab.
23. (breast adj25 tumo$).ti,ab.
24. (breast adj25 malign$).ti,ab.
25. (breast adj25 carcinoma$).ti,ab.
26. (breast adj25 adenocarcinoma$).ti,ab.
27. or/19-26
28. 18 and 27
29. Food/
30. Diet/
31. Nutrition Surveys/
32. Dietary Supplements/
33. diet records/
34. (diet$ or intake$ or nutri$ or food$ or supplement$).ti,ab,sh.

35. or/29-34
36. exp cohort studies/
37. exp prospective studies/
38. exp follow-up studies/
39. exp longitudinal studies/
40. exp diet surveys/
41. cohort$.ti,ab,sh.
42. longitudinal$.ti,ab,sh.
43. prospective$.ti,ab,sh.
44. controlled clinical trial.pt.
45. epidemiologic methods/
46. limit 45 to yr=1966-1989
47. exp case-control studies/
48. (case$ and control$).tw.
49. or/36-44,46-48
50. "randomized controlled trial".pt.
51. (random$ or placebo$ or single blind$ or double blind$ or triple blind$).ti,ab.
52. (retraction of publication or retracted publication).pt.
53. or/50-52
54. (animals not humans).sh.
55. ((comment or editorial or meta-analysis or practice-guideline or review or letter or journal correspondence) not "randomized controlled trial").pt.
56. (random sampl$ or random digit$ or random effect$ or random survey or random regression).ti,ab. not "randomized controlled trial".pt.
57. 53 not (54 or 55 or 56)
58. (review or review,tutorial or review, academic).pt.
59. (pooling or pooled or mantel haenszel).tw,sh.
60. meta-analysis.pt.
61. meta-analysis.sh.
62. (meta-analys$ or meta analys$ or metaanalys$).tw,sh.
63. (systematic$ adj5 review$).tw,sh.
64. (systematic$ adj5 overview$).tw,sh.
65. (quantitativ$ adj5 review$).tw,sh.
66. (quantitativ$ adj5 overview$).tw,sh.
67. (quantitativ$ adj5 synthesis$).tw,sh.
68. (methodologic$ adj5 review$).tw,sh.
69. (methodologic$ adj5 overview$).tw,sh.
70. (integrative research review$ or research integration).tw.

71. or/58-70
72. 49 or 57 or 71
73. 28 and 35 and 72

Note relating to search: it was possible that articles may have been missed which reported the analysis of vitamin C and breast cancer risk within the main body of the article but did not include the key search terms in the title, subject heading and abstract. To check this, a search was undertaken restricting key words to those indicating only cohorts (i.e. *cohort, prospective, follow-up, longitudinal),* dietary studies and breast cancer. Titles and abstracts for 303 articles were found and examined. Only four of these, over and above those found in the initial search appear to be potentially relevant; the full articles were retrieved but these did not meet the full inclusion criteria.

Appendix D.

NICE guidelines used to identify women in the UKWCS who may be at raised risk or high risk of developing breast cancer

The following NICE guidelines [319] were used to identify women in the UKWCS who may be at raised risk as those who met any of the following criteria:

- One 1st degree relative and one 2nd degree relative diagnosed with breast cancer before average age 50
- Two 1st degree relatives diagnosed with breast cancer before average age 50
- Three or more 1st or 2nd degree relatives diagnosed with breast cancer at any age
- One 1st degree male relative diagnosed with breast cancer at any age
- One 1st or 2nd degree relative with ovarian cancer at any age and one 1st or 2nd degree relative with breast cancer at any age (one should be a 1st degree relative)

Note: First-degree relatives: mother; father; daughter; son; sister; brother. Second-degree relatives: grandparent; grandchild; aunt; uncle; niece and nephew; half sister and half brother.

The following NICE guidelines[319] were used to identify women in the UKWCS who may be at high risk as those who met any of the following criteria:

- Two 1st or 2nd degree relatives diagnosed with breast cancer before average age 50
- Three 1st or 2nd degree relatives diagnosed before average age 60
- Four relatives including one 1st degree relative diagnosed at any age

Or one relative diagnosed with ovarian cancer at any age and on the same side of the family there is

- One 1st (including relative with ovarian cancer) or one 2nd degree relative diagnosed with breast cancer before age 50
- One additional relative diagnosed with ovarian cancer at any age
- Two 1st or 2nd degree relatives diagnosed with breast cancer before average age 60

Or a relative with bilateral breast cancer as follows

- One 1st degree relative with cancer diagnosed in both breasts before average age 50
- One 1st or 2nd degree relative diagnosed with bilateral breast cancer *and* one 1st or 2nd degree relative diagnosed with breast cancer before average age 60

Or one male breast cancer at any age *and* on the same side of the family there is

- One 1st or 2nd degree relative diagnosed with breast cancer before age 50
- Two 1st or 2nd degree relatives diagnosed with breast cancer before average age 60

Unfortunately the NICE guidelines could not be followed in some areas; therefore the categories are only estimates. From the UKWCS data it was impossible to determine whether aunts were from the mothers' or the fathers' side of the family. Additionally, there was no information on daughters or sons with breast cancer or any relatives with bilateral breast cancer in the UKWCS.

Appendix E.

Poster and oral conference presentations by Jayne Hutchinson

Symposium: University of Leeds LIGHT's Postgraduate Symposium. 24 January 2012

Oral presentation: Vitamin C intake from diary recordings and risk of breast cancer in the UK Dietary Cohort Consortium
(based on results from chapter 10)

Conference: IEA World Conference of Epidemiology. Edinburgh, 7-10 August 2011

Poster: Use of Supplements containing vitamin C and breast cancer risk in the UK Women's Cohort Study
(based on results from chapter 8)

Poster: Vitamin C intake from diary recordings and risk of breast cancer in the UK Dietary Cohort Consortium
(based on results from chapter 10)

Abstracts published in the Journal of Epidemiology and Community Health, August 2011, Vol 65, supplement 1

Conference: World Cancer Research Fund: Nutrition, Physical Activity and Cancer Prevention: Current Challenges, New Horizons. London 12-13 September 2010

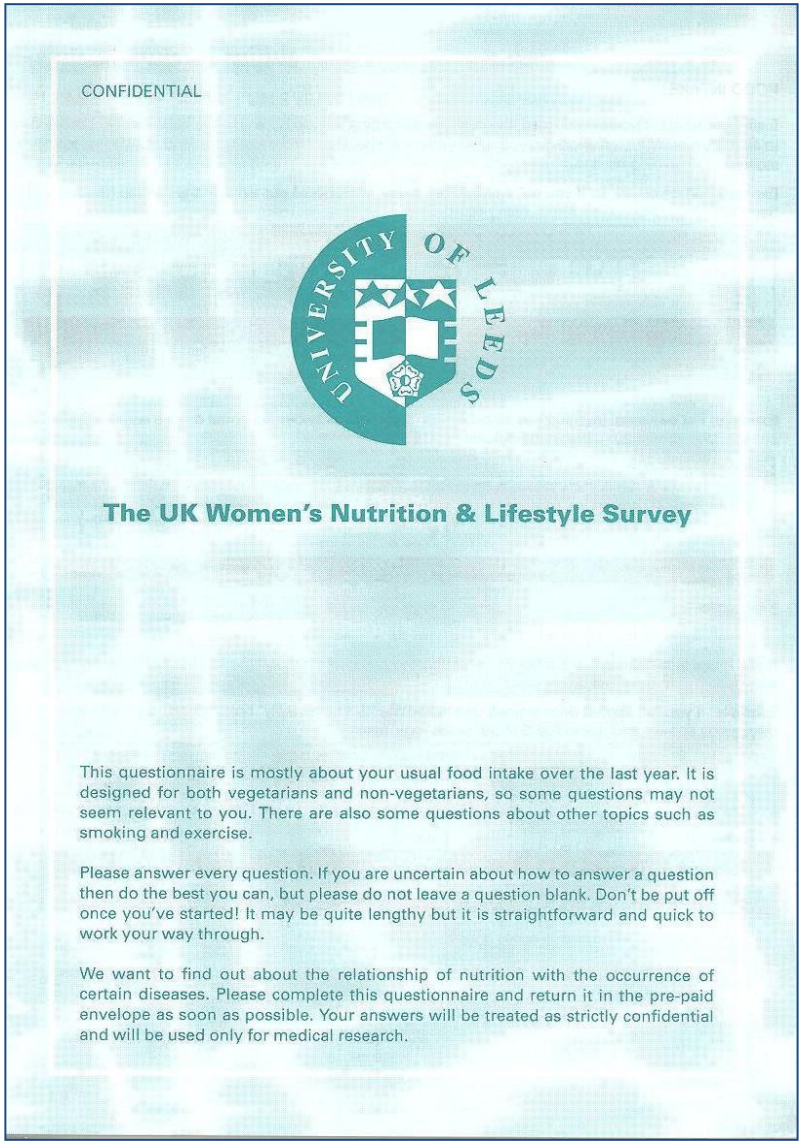
Poster: High dose vitamin C supplement use is associated with a personal and family history of cancer
(based on chapter 6)

Conference: University of Leeds Faculty of Medicine and Health Conference Day 2009: Identifying the Importance and Impact of Your Research. Weetwood Hall, Leeds 4 November 2009

Poster: Do women who take supplements have a greater risk of cancer?
(awarded poster prize)

Appendix F.

Baseline combined Food Frequency Questionnaire and Health and Lifestyle Questionnaire



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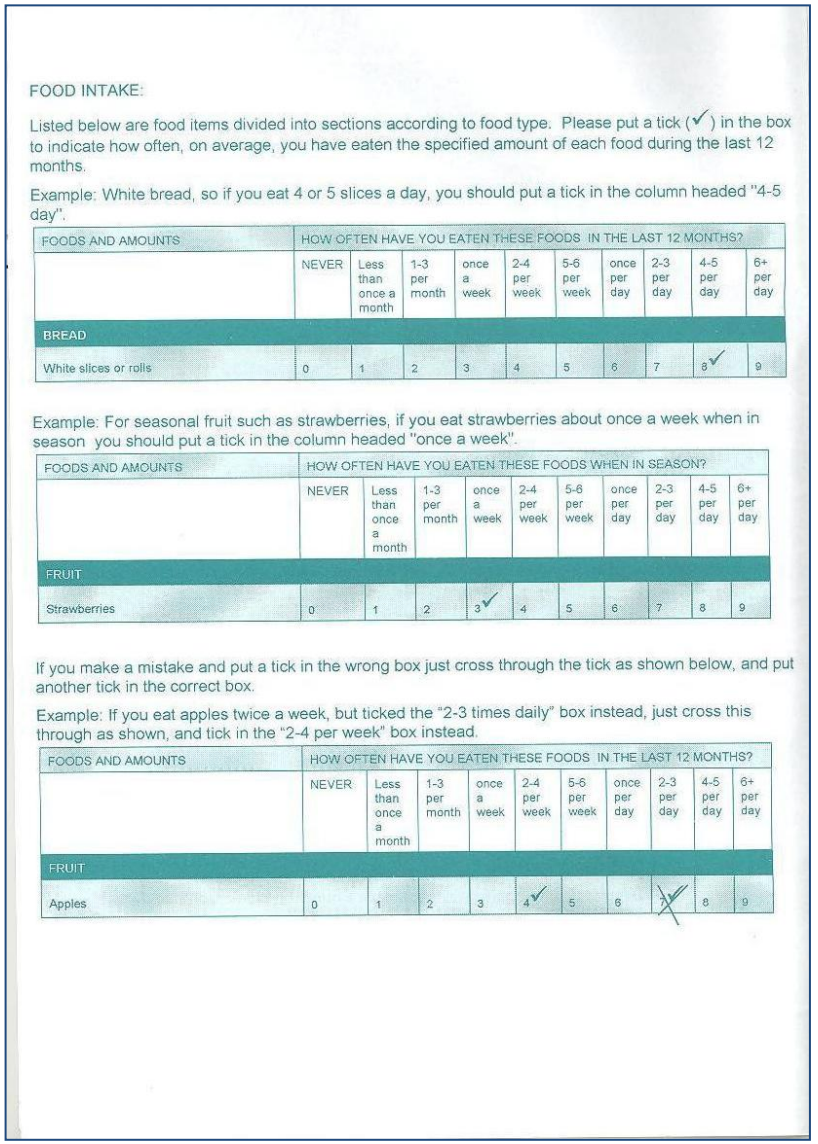
The UK Women's Nutrition & Lifestyle Survey

This questionnaire is mostly about your usual food intake over the last year. It is designed for both vegetarians and non-vegetarians, so some questions may not seem relevant to you. There are also some questions about other topics such as smoking and exercise.

Please answer every question. If you are uncertain about how to answer a question then do the best you can, but please do not leave a question blank. Don't be put off once you've started! It may be quite lengthy but it is straightforward and quick to work your way through.

We want to find out about the relationship of nutrition with the occurrence of certain diseases. Please complete this questionnaire and return it in the pre-paid envelope as soon as possible. Your answers will be treated as strictly confidential and will be used only for medical research.



FOOD INTAKE:

Listed below are food items divided into sections according to food type. Please put a tick (✓) in the box to indicate how often, on average, you have eaten the specified amount of each food during the last 12 months.

Example: White bread, so if you eat 4 or 5 slices a day, you should put a tick in the column headed "4-5 day".

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
BREAD										
White slices or rolls	0	1	2	3	4	5	6	7	8✓	9

Example: For seasonal fruit such as strawberries, if you eat strawberries about once a week when in season you should put a tick in the column headed "once a week".

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS WHEN IN SEASON?									
	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
FRUIT										
Strawberries	0	1	2	3✓	4	5	6	7	8	9

If you make a mistake and put a tick in the wrong box just cross through the tick as shown below, and put another tick in the correct box.

Example: If you eat apples twice a week, but ticked the "2-3 times daily" box instead, just cross this through as shown, and tick in the "2-4 per week" box instead.

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
FRUIT										
Apples	0	1	2	3	4✓	5	6	7✓	8	9

Please estimate how often you eat the following foods, and please answer every question.

PLEASE PUT A TICK(✓) ON EVERY LINE

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
BREAD/SAVOURY BISCUITS										
White bread & rolls	0	1	2	3	4	5	6	7	8	9
Brown bread & rolls	0	1	2	3	4	5	6	7	8	9
Wholemeal bread & rolls	0	1	2	3	4	5	6	7	8	9
Chapatis, Nan, Paratha	0	1	2	3	4	5	6	7	8	9
Papadums	0	1	2	3	4	5	6	7	8	9
Tortillas	0	1	2	3	4	5	6	7	8	9
Pitta Bread	0	1	2	3	4	5	6	7	8	9
Crispbread e.g. Ryvita	0	1	2	3	4	5	6	7	8	9
Cream crackers, cheese biscuits	0	1	2	3	4	5	6	7	8	9
BREAKFAST CEREALS										
Porridge, Readybrek	0	1	2	3	4	5	6	7	8	9
Sugar coated cereals e.g. Sugar Puffs	0	1	2	3	4	5	6	7	8	9
Non-sugar coated cereals e.g. Cornflakes, Rice Krispies	0	1	2	3	4	5	6	7	8	9
Muesli	0	1	2	3	4	5	6	7	8	9
All Bran, Bran Flakes	0	1	2	3	4	5	6	7	8	9
Weetabix, Shredded Wheat	0	1	2	3	4	5	6	7	8	9
POTATOES, RICE & PASTA										
Potatoes e.g. boiled, mashed	0	1	2	3	4	5	6	7	8	9
Chips	0	1	2	3	4	5	6	7	8	9
Jacket Potato	0	1	2	3	4	5	6	7	8	9
Roast Potatoes	0	1	2	3	4	5	6	7	8	9
Potato Salad	0	1	2	3	4	5	6	7	8	9
White Pasta e.g. Spaghetti, Green Pasta, Red Pasta, Noodles	0	1	2	3	4	5	6	7	8	9
Wholemeal Pasta, Brown Spaghetti	0	1	2	3	4	5	6	7	8	9
White Rice	0	1	2	3	4	5	6	7	8	9
Brown Rice	0	1	2	3	4	5	6	7	8	9
Wild Rice	0	1	2	3	4	5	6	7	8	9
Macaroni Cheese	0	1	2	3	4	5	6	7	8	9

Please estimate how often you eat the following foods, and please answer every question.

PLEASE PUT A TICK(✓) ON EVERY LINE

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
DAIRY & NON-DAIRY PRODUCTS										
Thick & Creamy Yoghurt (125g carton)	0	1	2	3	4	5	6	7	8	9
Low fat Yoghurt (125g carton)	0	1	2	3	4	5	6	7	8	9
Diet Yoghurt (125g carton)	0	1	2	3	4	5	6	7	8	9
Greek Yoghurt (125g carton)	0	1	2	3	4	5	6	7	8	9
Fromage Frais/Creme Fraiche (125g carton)	0	1	2	3	4	5	6	7	8	9
Dairy Desserts (125g carton)	0	1	2	3	4	5	6	7	8	9
Single/Sour Cream (tablespoon)	0	1	2	3	4	5	6	7	8	9
Double/Clotted Cream (tablespoon)	0	1	2	3	4	5	6	7	8	9
Icecream	0	1	2	3	4	5	6	7	8	9
Milk Puddings	0	1	2	3	4	5	6	7	8	9
Low-fat Cheese	0	1	2	3	4	5	6	7	8	9
Cheese e.g. Cheddar, Brie, Edam	0	1	2	3	4	5	6	7	8	9
Cottage Cheese	0	1	2	3	4	5	6	7	8	9
Cheese and Onion Pastie	0	1	2	3	4	5	6	7	8	9
Soya Cheese	0	1	2	3	4	5	6	7	8	9
Soya Yoghurt	0	1	2	3	4	5	6	7	8	9
MARGARINES/BUTTERS & SPREADS										
Butter (enough for 1 slice of bread)	0	1	2	3	4	5	6	7	8	9
Block Margarine e.g. Stork, Krona, NOT in tub (enough for 1 slice of bread)	0	1	2	3	4	5	6	7	8	9
Polyunsaturated Margarine e.g. Flora, Sunflower, Granose, in tub (enough for 1 slice of bread)	0	1	2	3	4	5	6	7	8	9
Other soft Margarine, Dairy spreads e.g. Blue Band, Clover, in tub (enough for 1 slice of bread)	0	1	2	3	4	5	6	7	8	9
Low fat spread e.g. Outline, Gold, Flora Lite, in tub (enough for 1 slice of bread)	0	1	2	3	4	5	6	7	8	9
Very low fat spread, in tub e.g. St Ivel Lowest Fat Spread (enough for 1 slice of bread)	0	1	2	3	4	5	6	7	8	9
Monounsaturated Margarine eg Mono, Olivio (enough for 1 slice of bread)	0	1	2	3	4	5	6	7	8	9

Please estimate how often you eat the following foods, and please answer every question.

PLEASE PUT A TICK(✓) ON EVERY LINE

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
SPREADS										
Marmite/Bovril/Vegemite	0	1	2	3	4	5	6	7	8	9
Peanut Butter	0	1	2	3	4	5	6	7	8	9
Chocolate/Chocolate & Nut Spread	0	1	2	3	4	5	6	7	8	9
Jam/Marmalade	0	1	2	3	4	5	6	7	8	9
Honey	0	1	2	3	4	5	6	7	8	9
Vegetable pâté	0	1	2	3	4	5	6	7	8	9
Nut Pâté	0	1	2	3	4	5	6	7	8	9
SAUCES & SOUPS										
Low Calorie Salad Cream (tablespoon)	0	1	2	3	4	5	6	7	8	9
Mayonnaise, Salad Cream Type Dressing (tablespoon)	0	1	2	3	4	5	6	7	8	9
French Type Dressing (tablespoon)	0	1	2	3	4	5	6	7	8	9
Sauces e.g. white/cheese/'Cook In'/curry	0	1	2	3	4	5	6	7	8	9
Tomato Ketchup (tablespoon)	0	1	2	3	4	5	6	7	8	9
Pickles/Chutney/Pesto sauce	0	1	2	3	4	5	6	7	8	9
Packet Soups - Meat & Veg (Bowl)	0	1	2	3	4	5	6	7	8	9
Other - Vegetable Soups (Bowl)	0	1	2	3	4	5	6	7	8	9
Other - Meat Soups (Bowl)	0	1	2	3	4	5	6	7	8	9
Low Calorie Soups (Bowl)	0	1	2	3	4	5	6	7	8	9
GRAINS (Medium serving)										
Barley	0	1	2	3	4	5	6	7	8	9
Oats	0	1	2	3	4	5	6	7	8	9
Bulgar Wheat	0	1	2	3	4	5	6	7	8	9
Wheat Germ (tablespoon)	0	1	2	3	4	5	6	7	8	9
Cous-cous	0	1	2	3	4	5	6	7	8	9
White Rice	0	1	2	3	4	5	6	7	8	9
Brown Rice	0	1	2	3	4	5	6	7	8	9
NUTS & SEEDS										
Peanuts/Pistachio Nuts	0	1	2	3	4	5	6	7	8	9
Cashew Nuts & Almonds	0	1	2	3	4	5	6	7	8	9
Pecan Nuts/Walnuts	0	1	2	3	4	5	6	7	8	9
Sunflower Seeds/ Sesame Seeds	0	1	2	3	4	5	6	7	8	9

Please estimate how often you eat the following foods, and please answer every question.

PLEASE PUT A TICK(✓) ON EVERY LINE

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
PULSES (Include when used in recipes)										
Lentils, dals	0	1	2	3	4	5	6	7	8	9
Chick Peas, Chanas	0	1	2	3	4	5	6	7	8	9
Hummus	0	1	2	3	4	5	6	7	8	9
Baked beans	0	1	2	3	4	5	6	7	8	9
Mung Beans & Red Kidney Beans	0	1	2	3	4	5	6	7	8	9
Bean Sprouts	0	1	2	3	4	5	6	7	8	9
Black Eyed Beans	0	1	2	3	4	5	6	7	8	9
Butter Beans/Broad Beans	0	1	2	3	4	5	6	7	8	9
EGGS/EGG DISHES										
Boiled/ Poached egg	0	1	2	3	4	5	6	7	8	9
Omelette, Scrambled egg	0	1	2	3	4	5	6	7	8	9
Fried egg	0	1	2	3	4	5	6	7	8	9
Quiche	0	1	2	3	4	5	6	7	8	9
VEGETABLE DISHES										
Quorn	0	1	2	3	4	5	6	7	8	9
Textured vegetable protein/ Sosmix/burger mix/soya sausages	0	1	2	3	4	5	6	7	8	9
Vegetarian Chilli/Vegetable Curry	0	1	2	3	4	5	6	7	8	9
Mixed Bean Casserole/Ratatouille	0	1	2	3	4	5	6	7	8	9
Stir-fry vegetables	0	1	2	3	4	5	6		8	9
Vegetable - Lasagne/Moussaka/Ravioli/ filled pasta with sauce	0	1	2	3	4	5	6	7	8	9
Vegetable Pizza	0	1	2	3	4	5	6	7	8	9
MEAT										
Beef e.g. roast, steak	0	1	2	3	4	5	6	7	8	9
Beef Stew/Casserole/Mince/Curry	0	1	2	3	4	5	6	7	8	9
Beefburger/Hamburger	0	1	2	3	4	5	6	7	8	9
Pork e.g. Roast, Chops, Slices	0	1	2	3	4	5	6	7	8	9
Pork Stew/Casserole	0	1	2	3	4	5	6	7	8	9
Lamb e.g. Roast, Chops	0	1	2	3	4	5	6	7	8	9
Lamb Stew/Casserole	0	1	2	3	4	5	6	7	8	9

Please estimate how often you eat the following foods, and please answer every question.

PLEASE PUT A TICK(✓) ON EVERY LINE

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
OTHER MEATS										
Chicken/Turkey roast, slices	0	1	2	3	4	5	6	7	8	9
Breadcrumbed e.g.chicken nuggets/kievs	0	1	2	3	4	5	6	7	8	9
Chicken/Turkey in creamy sauce, curry	0	1	2	3	4	5	6	7	8	9
Bacon	0	1	2	3	4	5	6	7	8	9
Ham	0	1	2	3	4	5	6	7	8	9
Corned Beef, Spam, Luncheon Meats	0	1	2	3	4	5	6	7	8	9
Sausages e.g. Beef Pork	0	1	2	3	4	5	6	7	8	9
Pies/Pasties/Sausage Rolls	0	1	2	3	4	5	6	7	8	9
Offal e.g. Liver, Kidney	0	1	2	3	4	5	6	7	8	9
Liver Pâté/Sausage, Salami	0	1	2	3	4	5	6	7	8	9
Meat - Lasagne/Moussaka/Ravioli/ filled pasta with sauce	0	1	2	3	4	5	6	7	8	9
Meat Pizza	0	1	2	3	4	5	6	7	8	9
FISH										
Fish fingers/cakes	0	1	2	3	4	5	6	7	8	9
Fried fish in batter (as in fish and chips)	0	1	2	3	4	5	6	7	8	9
White fish e.g. Cod, Haddock, Plaice, Sole, Halibut (fresh or frozen)	0	1	2	3	4	5	6	7	8	9
Oily fish e.g. Mackerel, Kippers, Tuna, Salmon, Sardines, Herring	0	1	2	3	4	5	6	7	8	9
Shellfish e.g. Crab, Prawns, Mussels	0	1	2	3	4	5	6	7	8	9
Fish Roe, Taramasalata	0	1	2	3	4	5	6	7	8	9
Fish Pie/Fish Lasagne	0	1	2	3	4	5	6	7	8	9
VEGETABLES										
Beetroot	0	1	2	3	4	5	6	7	8	9
Broccoli, Spring Greens, Kale	0	1	2	3	4	5	6	7	8	9
Brussel Sprouts	0	1	2	3	4	5	6	7	8	9
Cabbage	0	1	2	3	4	5	6	7	8	9
Carrots	0	1	2	3	4	5	6	7	8	9
Cauliflower	0	1	2	3	4	5	6	7	8	9
Celery	0	1	2	3	4	5	6	7	8	9

Please estimate how often you eat the following foods, and please answer every question.

PLEASE PUT A TICK(✓) ON EVERY LINE

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
VEGETABLES (continued)										
Coleslaw	0	1	2	3	4	5	6	7	8	9
Low-calorie Coleslaw	0	1	2	3	4	5	6	7	8	9
Courgettes, Marrow, Squash	0	1	2	3	4	5	6	7	8	9
Cucumber	0	1	2	3	4	5	6	7	8	9
Garlic	0	1	2	3	4	5	6	7	8	9
Green Beans, Runner Beans	0	1	2	3	4	5	6	7	8	9
Leeks	0	1	2	3	4	5	6	7	8	9
Lettuce	0	1	2	3	4	5	6	7	8	9
Mushrooms	0	1	2	3	4	5	6	7	8	9
Aubergine, Okra/Ladies Finger	0	1	2	3	4	5	6	7	8	9
Olives	0	1	2	3	4	5	6	7	8	9
Parsnips	0	1	2	3	4	5	6	7	8	9
Peas, Mushy peas, Mange-tout	0	1	2	3	4	5	6	7	8	9
Peppers - Red, Green, Yellow, Black etc	0	1	2	3	4	5	6	7	8	9
Swedes	0	1	2	3	4	5	6	7	8	9
Sweetcorn	0	1	2	3	4	5	6	7	8	9
Tomatoes - raw/canned/sauce	0	1	2	3	4	5	6	7	8	9
Turnip	0	1	2	3	4	5	6	7	8	9
Watercress, Mustard & Cress	0	1	2	3	4	5	6	7	8	9
FRUIT										
Apples	0	1	2	3	4	5	6	7	8	9
Avocado	0	1	2	3	4	5	6	7	8	9
Bananas	0	1	2	3	4	5	6	7	8	9
Grapes	0	1	2	3	4	5	6	7	8	9
Kiwi Fruit	0	1	2	3	4	5	6	7	8	9
Mangoes	0	1	2	3	4	5	6	7	8	9
Oranges, Satsumas, Grapefruit, etc	0	1	2	3	4	5	6	7	8	9
Papaya	0	1	2	3	4	5	6	7	8	9
Pears	0	1	2	3	4	5	6	7	8	9
Pineapple	0	1	2	3	4	5	6	7	8	9

Please estimate how often you eat the following foods, and please answer every question.

PLEASE PUT A TICK(✓) ON EVERY LINE

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
SEASONAL FRUIT How often have you eaten these fruits, when they are in season?										
Apricots	0	1	2	3	4	5	6	7	8	9
Melon	0	1	2	3	4	5	6	7	8	9
Nectarines	0	1	2	3	4	5	6	7	8	9
Peaches	0	1	2	3	4	5	6	7	8	9
Plums	0	1	2	3	4	5	6	7	8	9
Raspberries	0	1	2	3	4	5	6	7	8	9
Red currants/Black currants	0	1	2	3	4	5	6	7	8	9
Rhubarb	0	1	2	3	4	5	6	7	8	9
Strawberries	0	1	2	3	4	5	6	7	8	9
DRIED FRUIT										
Dates	0	1	2	3	4	5	6	7	8	9
Figs	0	1	2	3	4	5	6	7	8	9
Prunes	0	1	2	3	4	5	6	7	8	9
Mixed Dried Fruit e.g. Apricots, Apples, Pears, Mangoes	0	1	2	3	4	5	6	7	8	9
Currants, Raisins, Sultanas	0	1	2	3	4	5	6	7	8	9
SWEET SNACKS										
Cereal Bars/Flapjacks (one)	0	1	2	3	4	5	6	7	8	9
Fruit bars (one) eg Apricot, Date	0	1	2	3	4	5	6	7	8	9
Chocolate Snack Bars e.g. Mars, Crunchie (1 bar)	0	1	2	3	4	5	6	7	8	9
Mini chocolate snack bars, Chocolates - singles or squares (1)	0	1	2	3	4	5	6	7	8	9
Boiled Sweets, Toffees, Mints	0	1	2	3	4	5	6	7	8	9
SAVOURY SNACKS										
Crisps (1 bag)	0	1	2	3	4	5	6	7	8	9
Other fried snacks e.g. Wotsits (1 bag)	0	1	2	3	4	5	6	7	8	9
Low fat or baked snacks e.g. Low-fat Crisps (1 bag)	0	1	2	3	4	5	6	7	8	9
Bombay Mix (small handful)	0	1	2	3	4	5	6	7	8	9
Peanuts/Pistachio Nuts (small handful)	0	1	2	3	4	5	6	7	8	9
Mixed Nuts and Raisins (small handful)	0	1	2	3	4	5	6	7	8	9

7

Please estimate how often you eat the following foods, and please answer every question.

PLEASE PUT A TICK(✓) ON EVERY LINE

FOODS AND AMOUNTS	HOW OFTEN HAVE YOU EATEN THESE FOODS IN THE LAST 12 MONTHS?									
	NEVER	Less than once a month	1-3 per month	once a week	2-4 per week	5-6 per week	once per day	2-3 per day	4-5 per day	6+ per day
BEVERAGES										
Tea (cup)	0	1	2	3	4	5	6	7	8	9
Herbal Tea (cup)	0	1	2	3	4	5	6	7	8	9
Coffee - instant/ground (cup)	0	1	2	3	4	5	6	7	8	9
Coffee - decaffeinated (cup)	0	1	2	3	4	5	6	7	8	9
Coffee substitute e.g Caro/Bambu (cup)	0	1	2	3	4	5	6	7	8	9
Coffee whitener (teaspoon)	0	1	2	3	4	5	6	7	8	9
Cocoa, Hot Chocolate (cup)	0	1	2	3	4	5	6	7	8	9
Horlicks, Ovaltine (cup)	0	1	2	3	4	5	6	7	8	9
Low Calorie/Low-fat Horlicks, Ovaltine, Hot Chocolate, (cup)	0	1	2	3	4	5	6	7	8	9
Orange Juice (Pure Fruit) (glass)	0	1	2	3	4	5	6	7	8	9
Other -(100%) Pure fruit juices (glass)	0	1	2	3	4	5	6	7	8	9
Fruit Squash/Cordial - diluted (glass)	0	1	2	3	4	5	6	7	8	9
Fizzy soft drinks e.g. Coke, Lemonade (glass/can)	0	1	2	3	4	5	6	7	8	9
Low Calorie/Diet Soft Drinks (glass/can)	0	1	2	3	4	5	6	7	8	9
ALCOHOLIC BEVERAGES										
Wines (wineglassful)	0	1	2	3	4	5	6	7	8	9
Beer, Lager (half pint)	0	1	2	3	4	5	6	7	8	9
Cider (half pint)	0	1	2	3	4	5	6	7	8	9
Port, Sherry, Liqueurs (glass)	0	1	2	3	4	5	6	7	8	9
Spirits e.g. Whisky, Gin, Vodka, Brandy (single/1 measure)	0	1	2	3	4	5	6	7	8	9
BISCUITS, SWEETS & PUDDINGS										
Plain Biscuits e.g. Marie, Nice, Digestive (one)	0	1	2	3	4	5	6	7	8	9
Chocolate Biscuits (one)	0	1	2	3	4	5	6	7	8	9
Sandwich/Cream Biscuits (one)	0	1	2	3	4	5	6	7	8	9
Fruitcake (1 slice)	0	1	2	3	4	5	6	7	8	9
Sponge cakes (1 slice)	0	1	2	3	4	5	6	7	8	9
Buns/Pastries e.g. Croissants, Doughnuts, Tray Bakes, (one)	0	1	2	3	4	5	6	7	8	9
Scones/Pancakes/Muffins/Crumpets (1)	0	1	2	3	4	5	6	7	8	9
Fruit Pies, Tarts, Crumbles, (1 slice)	0	1	2	3	4	5	6	7	8	9
Sponge Puddings (1 serving)	0	1	2	3	4	5	6	7	8	9

8

1: **Other Foods**

Are there any other foods which you eat more than once a week? Yes ☐ 1 No ☐ 2

If yes, please list below

Food	Usual serving size	Number of times eaten each week

2: Would you describe yourself as a vegetarian? Yes ☐ 1 No ☐ 2

If yes, how long have you been vegetarian? ☐☐ years.

Would you describe yourself as a vegan? Yes ☐ 1 No ☐ 2

If yes, how long have you been vegan? ☐☐ years.

3: Do you use herbs and spices at least once per week when cooking food? Yes ☐ 1 No ☐ 2

Which fresh herbs and spices would you use at least once a week? Please list here

Which dried herbs and spices would you use at least once a week? Please list here

PORTION SIZE:

4: Compared to other people would you describe your typical average portion size of foods as?

Small? ☐ 1 Medium? ☐ 2 Large? ☐ 3

PULSES:

5: Do you eat pulses e.g. beans, peas, lentils etc. Yes ☐ 1 No ☐ 2

If no, please go to question 7.

6: Can you please indicate how much of the Pulses you eat are Fresh, Frozen, Canned or Dried. Please tick the appropriate boxes, e.g. 1/4 Dried, 3/4 Frozen.

	Proportion				
	Never	1/4	1/2	3/4	All
Fresh	1	2	3	4	5
Frozen	1	2	3	4	5
Dried	1	2	3	4	5
Canned	1	2	3	4	5

How do you usually cook pulses? Tick all applicable.

Steaming/Boiling/Pressure Cooking	☐ 1	Stewing/Casseroling/Baking	☐ 4
Microwaving	☐ 2	Stir Frying/Frying	☐ 5
Roasting	☐ 3	Raw/soaked/Raw-sprouted	☐ 6

VEGETABLES:

7: How many servings of vegetables or vegetable containing dishes, (excluding potatoes) do you usually eat each week? ☐☐

8: Can you please indicate how much of the vegetables you eat are Fresh, Frozen, Canned or Dried. Please tick the appropriate boxes, e.g. 1/4 Dried, 3/4 Frozen.

	Proportion				
	Never	1/4	1/2	3/4	All
Fresh	1	2	3	4	5
Frozen	1	2	3	4	5
Dried	1	2	3	4	5
Canned	1	2	3	4	5

9: Do you ever eat raw vegetables apart from salad vegetables? Yes ☐ 1 No ☐ 2

10: How do you usually cook your vegetables.? (Excluding potatoes). Tick more than one box if necessary.

Boiling	☐
Steaming	☐
Grilling/Barbecuing/Baking/Roasting (Cooked dry or using a small amount of oil)	☐
Stir Frying/Frying/Sauté	☐
Microwaving	☐
Deep frying - including in batter	☐
Casseroling//Baking in sauce	☐
Other	☐

Please describe

FRUIT:

11: How many servings of fruit or fruit containing dishes do you usually eat each week? ☐☐

Can you please indicate how much of the fruit you eat is Fresh, Canned, Dried or Stewed.

Please tick the appropriate boxes e.g. 1/4 Fresh, 3/4 Canned

	Proportion				
	Never	1/4	1/2	3/4	All
Fresh	1	2	3	4	5
Stewed	1	2	3	4	5
Dried	1	2	3	4	5
Canned	1	2	3	4	5

12: Do you ever cook the fruit you eat? Yes ☐ 1 No ☐ 2

13: If so, how do you usually cook your fruit?

Stewing	☐ 1	Poaching/Steaming	☐ 2
Baking	☐ 3	Microwaving	☐ 4
Other	☐ 5		

Please describe

MEAT: (If you **never** eat meat please go to question 16)

14: How many servings of meat or meat containing dishes do you usually eat each week? ☐☐

What do you do with the visible fat on your meat?
- Eat all/most of the fat ☐ 1
- Eat some of the fat ☐ 2
- Eat as little as possible/none ☐ 3

15: How do you usually cook meat? Tick more than one box if necessary.
- Grilling/Barbecuing/Baking/Roasting (Cooked dry or using a small amount of oil) ☐ 1
- Stir Frying/Frying ☐ 2
- Microwaving ☐ 3
- Deep frying - including in batter ☐ 4
- Casseroling/Baking in sauce ☐ 5
- Other ☐ 6

Please describe

16: How many servings of fish or fish containing dishes do you usually eat each week? ☐☐

How do you usually cook fish. Tick more than one box if necessary.
- Boiling ☐ 1
- Steaming ☐ 2
- Grilling/Barbecuing/Baking/Roasting (Cooked dry or using a small amount of oil) ☐ 3
- Stir Frying/Frying ☐ 4
- Microwaving ☐ 5
- Deep frying - including in batter ☐ 6
- Casseroling//Baking in sauce ☐ 7
- Other ☐ 8

Please describe

MILK:

17. What type of milk do you use most often? Select one only
- Full cream (Silver Top) ☐ 1
- Semi-skimmed (Red/White Top) ☐ 2
- Skimmed/fat free ☐ 3
- Channel Islands (Gold Top) ☐ 4
- Dried Milk ☐ 5
- Soya ☐ 6
- Sterilised ☐ 7
- None ☐ 8
- Other ☐ 9 Specify

If you used soya milk, please describe brand and type

18: How much milk do you drink each day, including milk with tea, coffee, milky drinks, cereals etc?
- None ☐ 1
- 1/4 Pint ☐ 2
- 1/2 Pint ☐ 3
- 3/4 Pint ☐ 4
- 1 Pint ☐ 5
- More than 1 Pint ☐ 6

BREAKFAST:

19: Are there any breakfast cereals that you normally eat that were not mentioned earlier? Yes ☐ 1 No ☐ 2

If yes, which brand and type of breakfast cereal, do you usually eat?

List the types most often used

Brand	Type

20: Do you usually take sugar on your breakfast cereal? Yes ☐ 1 No ☐ 2

If yes, how many teaspoons? ☐☐ teaspoons

21: Do you usually take sugar/honey in tea, herbal tea, coffee or coffee substitute? Yes ☐ 1 No ☐ 2

If Yes, please write the number of teaspoons per cup.
- Sugar/honey in tea ☐☐ teaspoons
- Sugar/honey in herbal tea ☐☐ teaspoons
- Sugar/honey in coffee ☐☐ teaspoons
- Sugar/honey in coffee substitute ☐☐ teaspoons

Do you use sweeteners instead of sugar or honey, Yes ☐ 1 No ☐ 2

Which brand of sweetener do you use, please specify

If yes how many tablets per day, or how many teaspoons of powder per day? ☐☐

22: On days when you eat bread, how many slices of bread or rolls do you eat ? ☐☐ slices/rolls per day

USE OF FATS:

23: Do you usually spread butter/margarine on your bread?
Yes ☐ 1 No ☐ 2 Sometimes ☐ 3

How many slices of bread/rolls/crackers do you have with spread each day? ☐☐

How much spread do you use?
- Just a scrape/thinly spread ☐ 1
- medium ☐ 2
- Thickly spread ☐ 3

24: What kind of fat do you most often use for frying, roasting, grilling etc?

Tick more than one if applicable
- Butter ☐ 1
- Lard/Dripping ☐ 2
- Vegetable Oil ☐ 3
- Solid White Vegetable Fat ☐ 4
- Margarine ☐ 5
- None ☐ 6

If you used vegetable oil, or margarine, please give type e.g. corn, sunflower

25: What kind of fat do you most often use for baking cakes etc.? Tick more than one if applicable

Butter ☐ 1 Solid White Vegetable Fat ☐ 2

Lard/Dripping ☐ 3 Margarine ☐ 4

Vegetable Oil ☐ 5 None ☐ 6

If you use margarine, please give Brand e.g. Flora, Stork

USE OF SALT:

26: How often do you add salt to food while cooking?

Always ☐ 1 Usually ☐ 2

Sometimes ☐ 3 Rarely ☐ 4

Never ☐ 5

27: How often do you add salt to any food at the table?

Always ☐ 1 Usually ☐ 2

Sometimes ☐ 3 Rarely ☐ 4

Never ☐ 5

28: Do you regularly use a salt substitute (e.g. Losalt)? Yes ☐ 1 No ☐ 2

If yes, which brand?

USE OF SUPPLEMENTS:

29: Do you take any vitamins, minerals, fish oils, fibre or other food supplements? Yes ☐ 1 No ☐ 2

If yes, please fill in details below.

Name and Brand of Supplements	How much do you take at a time	How often do you take these?			
		Daily	Weekly	Monthly	Less often
		1	2	3	4
		1	2	3	4
		1	2	3	4

SPECIAL DIETS:

30: I) Have you changed your diet over the last 12 months? Yes ☐ 1 No ☐ 2

If yes, please indicate if the change was for any of the reasons listed below?

Tick more than one box if applicable

High Blood Pressure ☐ 0

Bowel problems (e.g. irritable bowel or diverticulitis) ☐ 2

Concern over a family history of illness ☐ 4

Overweight/Obesity ☐ 6

Allergies (e.g. skin rash) ☐ 8

Stomach problems (e.g. ulcer or gastritis) ☐ 1

Concern over eating a healthy Diet ☐ 3

High Blood Cholesterol/Lipids ☐ 5

Diabetes ☐ 7

Other ☐ 9

Specify

II) Describe below how your diet has changed

Do you currently follow any of these diets? Tick more than one box if neccessary.

Low fat ☐ 1 Low salt ☐ 2 Diabetic ☐ 3

Slimming ☐ 4 Gluten free ☐ 5 High fibre ☐ 6

Other ☐ 7 Please give details

CONSUMPTION OF ALCOHOL:

31: How often, if ever do you drink alcohol?

More than once a week ☐ 1 Once a week ☐ 2

Less than once a week ☐ 3 Never drink alcohol ☐ 4

32: In a typical week, how much do you drink?

Beer or cider ☐☐ pints each week

Wine ☐☐ glasses each week

Sherry/Fortified Wines ☐☐ glasses each week

Spirits ☐☐ glasses (singles) each week

33: Five years ago, how many alcoholic drinks did you have each week?

Beer or cider ☐☐ pints each week

Wine ☐☐ glasses each week

Sherry/Fortified Wines ☐☐ glasses each week

Spirits ☐☐ glasses (singles) each week

SMOKING:

34: Which one of the following best describes you?

I smoke every day ☐ 1 I smoke occasionally, but not every day ☐ 2

I used to smoke every day, but do not smoke at all now ☐ 3 I have never smoked ☐ 4

If you have never smoked, please go to question 37.

35: Do/did you smoke?

Cigarettes ☐ 1

Cigars ☐ 2

A combination of the above ☐ 3

If you currently smoke or used to smoke cigarettes how many do/did you smoke each day?

☐☐ cigarettes

If you currently smoke or used to smoke cigarettes which brand of cigarettes do/did you usually smoke?

36: If you have stopped smoking for what period of time have you been a non-smoker?

1 year or less ☐ 1 2-5 years ☐ 2

6-10 years ☐ 3 Over 10 years ☐ 4

SIZE:

37: Approximately how much did you weigh when you were born?

☐☐ lbs or ☐☐ Kg or Don't Know ☐

38: Approximately how much did you weigh when you were 20 years old?

☐☐ stones ☐☐ pounds or ☐☐☐ Kg or Don't Know ☐

39: Approximately how much do you weigh at present?

☐☐ stones ☐☐ pounds or ☐☐☐ Kg or Don't Know ☐

40: Have you lost more than half a stone in the last year? Yes ☐ 1 No ☐ 2

Have you gained more than half a stone in the last year?
(Please ignore weight gained during pregnancy.) Yes ☐ 1 No ☐ 2

41: What is your present waist size? ☐☐ inches or ☐☐☐ centimetres or Don't Know ☐ 1

42: What is your present hip size? ☐☐ inches or ☐☐☐ centimetres or Don't Know ☐ 1

43: What is your present height? ☐ ft ☐☐ inches or ☐☐☐ centimetres or Don't Know ☐ 1

44: What size of blouse do you wear? Size ☐☐

45: What size of skirt do you wear? Size ☐☐

PHYSICAL ACTIVITY

46: In a typical week during the last 12 months, how many hours did you spend on each of the following activities? Put "0" if none

Housework, such as cleaning, washing, cooking, child care		☐☐ hours	☐☐ minutes per week
Do-It-Yourself		☐☐ hours	☐☐ minutes per week
Gardening	In Summer	☐☐ hours	☐☐ minutes per week
	In Winter	☐☐ hours	☐☐ minutes per week
Walking, including to work, shopping & leisure	In Summer	☐☐ hours	☐☐ minutes per week
	In Winter	☐☐ hours	☐☐ minutes per week
Cycling, including to work & leisure	In Summer	☐☐ hours	☐☐ minutes per week
	In Winter	☐☐ hours	☐☐ minutes per week
Other physical exercise, such as keep-fit, aerobics, jogging, tennis, swimming	In Summer	☐☐ hours	☐☐ minutes per week
	In Winter	☐☐ hours	☐☐ minutes per week

47: In a normal week, do you do any of these activities vigorously enough to cause sweating or a faster heartbeat? Yes ☐ 1 No ☐ 2

If yes, for how long each week do you do such vigorous physical activity? ☐☐ hours ☐☐ minutes per week

ILLNESS:

48: Have you ever been told by a doctor that you have, or had, any of the following conditions?
Please tick all which apply and give the age at which each condition was first diagnosed.

Heart attack, coronary thrombosis, myocardial infarction	Yes ☐ 1	No ☐ 2	at age ☐☐ yrs old
Angina	Yes ☐ 1	No ☐ 2	at age ☐☐ yrs old
Stroke	Yes ☐ 1	No ☐ 2	at age ☐☐ yrs old
High Blood Pressure (Hypertension)	Yes ☐ 1	No ☐ 2	at age ☐☐ yrs old
High Blood Cholesterol, Hyperlipidaemia	Yes ☐ 1	No ☐ 2	at age ☐☐ yrs old
Diabetes	Yes ☐ 1	No ☐ 2	at age ☐☐ yrs old
Gallstones	Yes ☐ 1	No ☐ 2	at age ☐☐ yrs old
Polyps in the large intestine	Yes ☐ 1	No ☐ 2	at age ☐☐ yrs old
Cancer	Yes ☐ 1	No ☐ 2	at age ☐☐ yrs old

If yes, what type of cancer? _____

Any other illnesses or operations?
Do not include hysterectomy or breast surgery. These are covered later in the questionnaire.

Condition/ operation / disease	Age first diagnosed
	☐☐ yrs old
	☐☐ yrs old
	☐☐ yrs old
	☐☐ yrs old

49: Are you currently receiving long-term treatment for any illness or condition? Yes ☐ 1 No ☐ 2

If yes, please give details of treatment. If no please go to question 50:

Illness or condition	Treatment	Dose	Frequency

50: Have your mother and/or father ever suffered from cancer or heart attack/heart disease?

Yes ☐ 1 No ☐ 2 Don't Know ☐ 3

If yes, please give details

51: If you have brothers and/or sisters, have they ever suffered from cancer or heart attack/heart disease?

Yes ☐ 1 No ☐ 2 Don't Know ☐ 3

If yes, please describe details

EDUCATION:

52: How old were you when you finished your full time education? ☐☐ yrs old

53: Do you have any of the following qualifications? Tick all applicable

CSE	☐ 1	"A" Level, Highers	☐ 4
GCE "O" Level	☐ 2	Teaching diploma, HNC	☐ 5
City & Guilds	☐ 3	Degree	☐ 6
Other ☐ 7 describe		None of these	☐ 8

EMPLOYMENT:

54: Have you ever had a paid job? Yes ☐ 1 No ☐ 2 ☐

If yes, please answer for your current or most recent job

What is/was your job title?

What do/did you do in your job?

What does/did the organisation you work for make or do?

Are/were you a Manager? ☐ 1 Foreman/woman? ☐ 2

Supervisor? ☐ 3 None of these? ☐ 4

Are/were you self-employed? Yes ☐ 1 No ☐ 2

Do you have a paid job at present? Yes ☐ 1 No ☐ 2

If no, how would you describe yourself?

Housewife ☐ 1 Unemployed ☐ 2

Retired ☐ 3 Student ☐ 4

Other ☐ 5 describe

When did you last have paid employment 19☐☐ (year) or Never ☐ 1

55: What is your marital status?

Married or living as married ☐ 1 Divorced ☐ 2

Widowed ☐ 3 Single ☐ 4

Separated ☐ 5

If you are not married or living as married, please go to question 57.

PARTNER'S EMPLOYMENT:

56: If married or living as married, has your partner ever had a paid job? Yes ☐ 1 No ☐ 2 ☐

If yes, please answer for your partner's current or most recent job.

What is/was your partner's job title?

What does/did the organisation your partner works for make or do?

Is/was your partner a Manager? ☐ 1 Foreman/woman? ☐ 2

Supervisor? ☐ 3 None of these? ☐ 4

Is/was your partner self-employed? Yes ☐ 1 No ☐ 2

Does your partner have a paid job at present? Yes ☐ 1 No ☐ 2

If no, how would you describe your partner?

House-husband ☐ 1 Student ☐ 2

Unemployed ☐ 3 Retired ☐ 4

Other ☐ 5 describe

57: Which of these groups would you consider you belong to?

White ☐ 1 Bangladeshi ☐ 2

Indian ☐ 3 Chinese ☐ 4

Pakistani ☐ 5 Black - Caribbean ☐ 6

Black - other ☐ 7

Other ☐ 8

MENSTRUAL & OBSTETRIC HISTORY:

58: How old were you when you had your first menstrual period ☐☐ years old

59: What is the usual length of your menstrual cycle?

(i.e. from the first day of one period to the first day of the next period e.g. 26 days)? ☐☐ days.

60: Have you ever been pregnant? Yes ☐ 1 No ☐ 2

Are you pregnant at the moment? Yes ☐ 1 No ☐ 2

How many times have you been pregnant? ☐☐

Have you ever had a miscarriage/still birth? Yes ☐ 1 No ☐ 2

If you have had children, please go to question 61. If not please go to question 63.

61: Have you had any children? Yes ☐ 1 No ☐ 2

If yes, how old were you when your first child was born ☐☐ years

If yes, how many children have you had? ☐☐ children

If none please go to question 63.

Please can you write in each child's sex and approximate birthweight.

Child	Sex of Child	Approximate Birthweight	Child's D.O.B
CHILD1:			19☐☐
CHILD 2:			19☐☐
CHILD 3:			19☐☐
CHILD 4:			19☐☐
CHILD 5:			19☐☐

62: Did you ever breast feed any of your children? Yes ☐ 1 No ☐ 2

If yes, for those children you breast-fed, please describe how long you continued breast feeding after each birth, (even only occasional breast feeding). Tick the appropriate box.

	1–6 days	1–4 weeks	1–3 months	4–6 months	6+ months	12+ months
CHILD 1:	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
CHILD 2:	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
CHILD 3:	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
CHILD 4:	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
CHILD 5:	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

63: Have you ever seen a doctor because of fertility problems? Yes ☐ 1 No ☐ 2

If yes, has a doctor ever told you that you were infertile? Yes ☐ 1 No ☐ 2

64: Have you ever used oral contraceptives (the pill)? Yes ☐ 1 No ☐ 2

If yes, how old were you when you first started to use the pill? ☐☐ years old

For how long altogether did you use the pill? ☐☐ years

Are you currently using the pill? Yes ☐ 1 No ☐ 2

If no, how old were you when you last used it? ☐☐ years old

65: Have you ever used a coil or intra-uterine device (IUD)? Yes ☐ 1 No ☐ 2

If yes, do you have a coil or IUD at present? Yes ☐ 1 No ☐ 2

66: How many "natural" menstrual periods have you had in the last 12 months?
Do not count bleeding while using the pill or HRT (Hormone Replacement Therapy)

None ☐ 1
1 to 3 ☐ 2
4 to 5 ☐ 3
6 to 9 ☐ 4
10 or more ☐ 5
Not applicable because using the Pill or HRT or currently pregnant ☐ 6

67: When did you last have a "natural" menstrual period? Do not count bleeding while using the pill or HRT (Hormone Replacement Therapy). Record as fully as possible

Date: ☐ or age ☐☐ years old Don't know ☐

68: Have you ever used HRT (Hormone Replacement Therapy) for menopause? Yes ☐ 1 No ☐ 2

If yes, how old were you when you first used HRT? ☐☐ years old

For how long altogether have you used HRT? years ☐☐ and months ☐☐

Are you currently using HRT? Yes ☐ 1 No ☐ 2

If no, how old were you when you last used HRT? ☐☐ years old

69: Have you had a hysterectomy ? If no please go to question 71.

Yes ☐ 1 Age at time of operation ☐☐ years old No ☐ 2 Don't know ☐ 3

70: Have you had an operation to remove one or both your ovaries?

Yes ☐ 1 No ☐ 2 Don't know ☐ 3

If yes, how old were you? ☐☐ years old

Were one or both ovaries removed? One ☐ 1 Both ☐ 2 Don't know ☐ 3

71: Have you ever had a breast biopsy (minor surgery to remove tissue from your breast for diagnostic purposes)? Yes ☐ 1 No ☐ 2 Don't know ☐ 3

If yes, how old were you (first occurrence)? ☐☐ years old

This page has perforations down the side so that as soon as we receive this questionnaire we will remove this sheet and keep it securely separately from the rest of the questionnaire so that the information you have given is kept anonymously.

72: Can you please give us these other details : DATE OF BIRTH

FIRST NAME(s)/FORENAME(s)

OTHER NAME(s)/FORENAME(s)

SURNAME AT BIRTH/(MAIDEN NAME)

CURRENT SURNAME/LAST NAME

73: One of the objectives of this study is to establish the relationship of nutrition with the occurrence of certain diseases such as cancer which are registered by the National Health Service (NHS). To do this we need to know your NHS number if you know it, and also details about your GP. It would be very helpful if you could fill in your NHS number (this is on your medical card). If you were born between 1939 and 1952 this is your National Identity number.

Example:

NHS NUMBER	U	N	A	Z		/	4	6	4

NHS NUMBER									

74: Please could you also fill in your G.P.'s Name and Address.

G.P. Name:

Address:

Postcode:

Telephone Number:

CONTACTING YOU:

75: If we have any queries about your answers to this questionnaire, we would like to contact you. Can you please give us your telephone number

Telephone : Dialling Code: Number: Daytime ☐

Dialling Code: Number: Evening ☐

76: We would like to write to you again about the progress of our research and to find out more information about your typical food intake.

In case you change your address and we lose contact with you, could you give us the name of two friends or relatives who would know your new address? Please inform them that you have done this.

Contact Name (1):

Contact Address:

Postcode:

Telephone Number:

Contact Name (2):

Contact Address:

Postcode:

Telephone Number:

Please will you check the name and address label overleaf, and correct the details as necessary.

Thank you for your help filling out this questionnaire and helping us with our research.

Please send your completed questionnaire in the stamped addressed envelope to:

Amanda Woodhouse,
Division of Public Health,
The Nuffield Institute for Health,
71–75 Clarendon Road, Leeds LS2 9PL.

Please phone 0113 233 4862
if you have any queries about this questionnaire.

Please write any changes to the details on the label in the spaces below

First name

Surname

Address

Postcode