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Nutrition, Inflammation and Lung Function in Middle-aged Men

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Nutrition, Inflammation and lung function in middle-aged men

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By

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Declaration

This thesis is submitted for consideration for the degree of Doctor of Philosophy. The work was carried out in the Centre for Population Sciences and the Department of Clinical Biochemistry, The Institute of Clinical Science, Grosvenor Road, Belfast. The work in this thesis was carried out by myself. The subjects in the studies presented in this thesis were recruited by others as part of the PRIME study as detailed in chapter 2. This thesis has not been submitted for a degree at any other university. Some of the studies presented in this thesis have been presented at regional, national and international scientific meetings and have been published in abstract form and as full papers in peer reviewed journals.

Kathy M McClean

Dedication

For my precious family - my husband Roy and our son Adam

Acknowledgements

There are many people who are deserving of my thanks and gratitude for their help, encouragement and friendship, which has been of great benefit to me as I have carried out this work. I have tried to make this list as full as possible, but I am aware that it most likely remains incomplete.

I would like to thank my supervisors, Professor Stuart Elborn, Professor Frank Kee and Professor Ian Young for agreeing to supervise me through this work and for their advice and mentorship throughout. I would like to thank Dr Jayne V Woodside for allowing me to use the laboratory within her department and for advice, training and guidance in the antioxidant work. I am also very grateful for the laboratory training and assistance I received from Dr. Sarah Gilchrist. Caroline Mercer's help with sample location and CRP measurement was invaluable. Charlotte Neville, Claire Butler and Thelma Craig deserve a special mention for their encouragement and friendship. I would also like to thank my parents, Evan and Joan for their unswerving support.

I am grateful to the entire Prime study team who have worked on data collection for the PRIME study since the early 1990s. They include Alun Evans, John Yarnell, Angela Scott, Kathy McCrory, Evelyn, Heather, Rosie Kearney and Annie Bingham. Dr Chris Cardwell has been extremely patient and helpful in providing statistical advice.

I am very grateful for the financial support of the Research and Development Office for Northern Ireland, without which this work could not have been undertaken.

My role in this study

1) I was involved in writing the initial proposal to the Research and Development Office (R&D) requesting a grant to fund this research. I was interviewed by a panel at R&D headquarters and was successful in obtaining funding for a three year PhD. I presented some of my findings at the R&D conference in September 2008.
2) I carried out a background literature search and spent time examining and familiarising myself with the large PRIME database. During my time in research I regularly attended the regional PRIME study meetings as part of the team.
3) I undertook some specific training in Epidemiology by attending a one week ERASMUS Summer Programme in Rotterdam in August 2005 (modules in Case-control studies and Cohort Studies) and also attended an Advanced Course in Epidemiological Analysis at the London School of Hygiene and Tropical Medicine in September 2006. I was also involved in teaching regular epidemiology tutorials to first year medical students in Queens University, Belfast (QUB).
4) I regularly attended local, regional and international conferences to present my work.
5) I examined each original Spirometry trace from baseline and follow-up and created a database to document if each trace was valid or invalid by ATS/ERS criteria. I included any reasons for invalidity. I also calculated tobacco pack-years for each man at baseline and follow-up.
6) I measured highly sensitive C-Reactive Protein for each man at baseline and follow-up from stored plasma samples. I was trained for this and assisted by Caroline Mercer in a QUB laboratory.

7) I measured antioxidant vitamins (A and E) and carotenoids in stored samples from all men at baseline who had paired valid spirometry results and at follow-up for all men with an available plasma sample. I was trained for this by Jayne Woodside and Sarah Gilchrist in a QUB laboratory.
8) I carried out all of my own statistics with advice from Chris Cardwell.

Publications arising from this work

Papers

KM McClean, F Kee, IS Young, JS Elborn. 'Obesity and the Lung:1.Epidemiology' *Thorax 2008 Jul ; 63(7):649-54. Review*

Abstracts and Spoken Presentations

Beta cryptoxanthin levels correlate with lung function in middle-aged men

K.M. McClean, JS Elborn, F. Kee, JV Woodside, I.S. Young.

Thorax 2008; 63 (Suppl VII): A58

Presented as a spoken presentation to the British Thoracic Society Conference on 5th December 2008

Beta cryptoxanthin levels correlate with lung function in middle-aged men

K.M. McClean, JS Elborn, F. Kee, J Woodside, I.S. Young

Irish Journal of Medical Science, S439, Volume 177, Supplement 13, November 2008.

Presented to the Irish Thoracic Society Conference as a spoken presentation on 7th November 2008

KM McClean, EL Petsonk, CR Cardwell, F Kee, IS Young, ML Wang, JS Elborn. 'Lung function and the distribution of weight in middle-aged men'

Am J Respir Crit Care Med; Vol 177; Abstracts Issue; April 2008: A832

Presented as a Poster Discussion to the American Thoracic Society May 2008

KM McClean, CR Cardwell, F Kee, IS Young, JS Elborn, ‘Ten year longitudinal change in lung function and C-Reactive Protein in middle-aged men in Northern Ireland’

Thorax 2007; 62 (Suppl III): A138

Presented as a poster to the British Thoracic Society conference Dec 2007

KM McClean, CR Cardwell, F Kee, IS Young, JS Elborn, ‘Longitudinal change in BMI and Lung Function in Middle-aged men in Northern Ireland

Irish Journal of Medical Science, S418, Volume 176, Supplement 10, November 2007.

Presented as a poster to the Irish Thoracic Society conference Nov 2007

KM McClean, CR Cardwell, F Kee, IS Young, JS Elborn ‘Waist-hip ratio, BMI and lung function in middle-aged men in Northern Ireland.’

Am J Respir Crit Care Med; Vol 175; Abstracts Issue; April 2007: A912

Presented as a poster at the American Thoracic Society Conference May 2007

KM McClean, CR Cardwell, F Kee, IS Young, JS Elborn, ‘CRP and Lung Function in Middle-Aged Men in Northern Ireland’.

Thorax 2006;61(Suppl 2):ii12

Presented as a spoken presentation to the British Thoracic Society Winter Meeting Dec 2006

KM McClean, CR Cardwell, F Kee, IS Young, JS Elborn, ‘Relationship between lung function and fruit consumption in smoking and non-smoking men in Northern Ireland’

Thorax 2006; 61(Suppl 2):ii82

Presented as a poster to the British Thoracic Society Winter Meeting in Dec 2006 and as a poster to the inaugural meeting of the Ulster Thoracic Society/North of England Thoracic Society in Oct 2006

KM McClean, IS Young, JS Elborn, A Evans, F Kee, G Linden, ‘Inflammation as a Risk Factor for Mortality in Men in Northern Ireland: The PRIME Study’

Spoken Presentation at the R&D Conference Sept 2007

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Abbreviations

ANOVA	Analysis of Variance
ATS	American Thoracic Society
AUC	Area under the curve
BMI	Body Mass Index
BTS	British Thoracic Society
CHD	Coronary Heart Disease
CI	Confidence interval
Cm	Centimetres
COPD	Chronic obstructive pulmonary disease
CRP	C-Reactive Protein
ECG	Electrocardiogram
ECRHS	European Community Respiratory Health Survey
ERS	European Respiratory Society
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
G	grams
IL-6	Interleukin 6
IL-8	Interleukin 8
IQ	Interquartile range
kg	Kilograms
kg/m^2	Kilograms per metre squared
m	metres
min	minutes
ml	millitres

PEF	Peak expiratory flow rate
PRIME	Prospective Epidemiological Study of Myocardial Infarction
QUB	Queen's University of Belfast
s	seconds
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
TNF	Tumour necrosis factor
TNF-α	Tumour necrosis factor alpha
μmol/L	micromoles per litre
WHO	World Health Organisation
WHR	Waist-hip Ratio
y	years

Abstract

Background

Forced expiratory volume in one second (FEV_1) is an independent predictor of all-cause mortality and therefore variables which accelerate or slow FEV_1 decline are of great potential importance to public health.

Methods

From 1991 to 1994, 2745 men aged 50 to 59 years were recruited into the Belfast cohort of the Prospective Epidemiological Study of Myocardial Infarction (PRIME). 2010 of these men were rescreened at 10 years. At both time points, the men were assessed at a clinic appointment which involved blood sampling, questionnaires, anthropometric measurements and lung function by spirometry. Each individual spirometry trace (baseline and 10 year follow-up) was reviewed for validity using ATS/ERS criteria. Plasma levels of C-Reactive Protein (CRP), antioxidant vitamins (A and E) and carotenoids were measured in the stored blood samples. All statistical analyses were performed with SPSS version 15.

Results

The 2745 men in the baseline cohort had a mean age of 54.8 years (SD 2.9) and 32.9% had never smoked. They were on average overweight with a mean body mass index of 26.3kg/m^2 and a mean waist-hip ratio of 0.94. 1779 men had valid baseline lung function measurements by spirometry. Mean percent predicted FEV_1 was 92% and mean percent predicted FVC was 96.9%. The baseline current smokers had the greatest decline in FEV_1 over 10 years of 560 ml (SD 339 ml).

Waist-hip ratio, body mass index and waist circumference were inversely associated with pulmonary function. FEV_1 decline was related to the change in distribution of weight as well as to weight gain.

Lung function and plasma C-Reactive Protein (CRP) concentrations were inversely related in both cross-sectional and longitudinal analyses. The presence of systemic inflammation was demonstrated, even in subjects with mildly reduced pulmonary function. There was also an association between annual change in CRP and annual decline in FEV_1 and forced expiratory volume in one second (FVC) in the adjusted model in this population

Plasma levels of retinol, lutein, zeaxanthin, ß-cryptoxanthin, α-carotene and ß-carotene were positively correlated with lung function in the cross-sectional analysis. There were also some longitudinal associations between baseline retinol, zeaxanthin, β-cryptoxanthin and 10 year lung function. Self-reported fruit and vegetable intake was positively correlated with both lung function and plasma levels of antioxidants.

Conclusion

In this population of middle-aged men from Northern Ireland, lung function and lung function decline as measured by FEV_1 and FVC are related to body mass index, body weight distribution, lifestyle habits (smoking and alcohol intake), social status, subclinical inflammation and diet. This data supports the current public health advice regarding healthy eating, weight control and smoking cessation.

1.0 Introduction

1.0 INTRODUCTION

1.1 Lung function and the general population

Forced expiratory volume in one second (FEV_1) is the most widely used and quoted lung function test in clinical practice. It is easily measured and has very good reproducibility (Kerstjens et al. 1997). During childhood and adolescence, FEV_1 gradually increases until it reaches a plateau phase in early adulthood before starting to decline, around the age of 25 (Brandli et al. 1996, Knudson et al. 1983). Peak FEV_1 for each individual is influenced by multiple factors including genotype (Barton et al. 2009), prematurity (Halvorsen et al. 2006), nutrition (including breastfeeding (Guilbert et al. 2007)), childhood illness (Barker et al. 1991), environmental insults (e.g. smoking and pollution) height and sex. From this peak, lung function deteriorates gradually throughout adult life, the rate of decline differing among individuals (Jiang et al. 2008). For example, lung function decline may be accelerated by environmental factors such as pollution and behavioural factors such as smoking. FEV_1 is an independent predictor of all-cause mortality (Schunemann et al. 2000, Neas, Schwartz 1998, Friedman, Klatsky & Siegelaub 1976). It is therefore potentially beneficial to individuals to slow FEV_1 decline.

1.2 The PRIME study

1.2.1 Background

The Prospective Epidemiological Study of Myocardial Infarction (PRIME) study was set up to investigate the large difference in coronary heart disease incidence between Northern Ireland and France. This difference is only partly explained by classical risk factors (Yarnell 1998). From 1991 to 1993, 10,593 men aged 50 to 59 years were recruited from the populations of Belfast in Northern Ireland and Lille, Strasbourg and Toulouse in France. Roughly equal cohorts were assembled in each centre.

2745 healthy men were recruited into the Belfast cohort from industry, various employment groups and general practice. This represented 6.5% of the total 50-59 year old male population in Belfast at that time. The sample was recruited to broadly match the social class structure of the background population. The catchment area included Belfast, Castlereagh, North-Down and Ards Health Districts in the east of Northern Ireland (see figure 1.1) and was 60 % urban with declining heavy industries and textiles.

Self-administered questionnaires covering demographic, socio-economic and dietary factors were completed at home by participants and checked by survey staff at a dedicated screening clinic. Anthropometric measurements, lung function by spirometry (Belfast cohort only) and an Electrocardiogram (ECG) were performed and a blood sample was taken. Full details of methods are given in chapter 2.0.

Figure 1.1 Map of Northern Ireland

The men were contacted annually by letter and were asked to complete and return a clinical event questionnaire.

2010 of the original cohort were re-screened 10 years later. This involved a further questionnaire, anthropometric measurements, spirometry and venous blood sample. End-points followed up were deaths, non-fatal acute myocardial infarction (MI), unstable angina pectoris, silent MI, cardiac revascularization, stable angina pectoris and non-fatal stoke events.

1.2.2 PRIME Publications

The PRIME team have published 50 scientific papers to date. Most of the results published have related to cardiovascular outcomes and include comparisons of the French and Northern Irish cohorts (see appendix for full list). A selection of the results of the studies which are relevant to this thesis are discussed below.

The 5-year follow-up data of the PRIME study confirmed a higher incidence of angina, myocardial infarction and coronary death in middle-aged men in Belfast compared to France (Ducimetiere et al. 2001). There was a strong relationship between cardiovascular morbidity and poorer material circumstances, unemployment, less full-time education and other indicators of socioeconomic disadvantage (Yarnell et al. 2005). Leisure-time physical activity net energy expenditure had a beneficial effect on coronary heart disease (CHD) incidence in Northern Ireland (Wagner et al. 2002). A nested case-referent study showed an association between depressive mood, inflammatory markers and coronary heart disease (Empana et al. 2005). A further study indicated that, in middle-aged men, physical activities of a moderate-intensity, (such as more current daily activity, walking or cycling to work) may have a favourable effect on body fat markers and body mass gain (Wagner et al. 2001).

Gey et al reported a correlation between low plasma retinol levels and coronary heart disease (Gey et al. 2009). Frequency of consumption of citrus fruit, but not other fruits were associated with lower rates of acute coronary events in both France and Northern Ireland, suggesting that geographical or related factors might affect the relationship between fruit consumption and CHD risk (Dauchet et al. 2004a). In middle-aged European men, waist-to-height ratio identified coronary risk more strongly than waist circumference, waist-to-hip ratio (WHR) or

body mass index (BMI), though the difference was marginal (Gruson et al. 2009). The relative risk of coronary artery disease associated with abdominal obesity was homogeneous in never, former and current smokers. Therefore, smokers with abdominal obesity were at high absolute risk of coronary artery disease (Chouraki et al. 2008). Alcohol consumption patterns exerted differential effects on coronary heart disease risk in middle-aged men from France and Northern Ireland. Also, the amount of alcohol consumption, rather than the type of alcoholic beverage, was related to both angina pectoris and myocardial infarction in France, whereas no relationship was found in Northern Ireland (Marques-Vidal et al. 2004).

Some 'healthy worker' effect was expected from the recruitment methods used. As for any examination on a voluntary basis, acutely ill people and those with a bad prognosis were not recruited in the PRIME study (Ducimetiere et al. 2001). Only men were included in the PRIME study and therefore the results cannot be extrapolated to women.

1.2.3 Lung function in PRIME

Only the men recruited in Belfast had lung function measurements by spirometry. This means that comparisons cannot be made with the French cohort. Prior to this thesis, there have been no published data on the lung function measurements from the PRIME study. The lung function measurements taken were Forced Expiratory Volume in one second (FEV_1) and Forced Vital Capacity (FVC).

1.3 Health and nutrition issues in middle-aged men

In the early 1990s when the PRIME study was recruiting, the leading causes of death among men on the island of Ireland were circulatory diseases (45.4%), cancer (23%) and respiratory diseases (15.2%) (see table 1.1). There were occupational class gradients in mortality rates among men for circulatory diseases, cancers, respiratory diseases, injuries and poisonings (O'Shea 1997). 1n 1996, unskilled men were twice as likely to die as higher professional men, and were eight times more likely to die from an accidental cause (Barry et al. 2001).

Table 1.1 Average annual number of deaths, by gender and cause, on the Island of Ireland (Central Statistics Office 2002a)

	1990		2000	
Principal causes	**Males**	**Females**	**Males**	**Females**
Circulatory causes	11,098	10,323	9,170	9,266
Respiratory diseases (except lung cancer)	3,725	3,517	3,683	4,338
Cancer	5,619	4,941	5,772	5,332
Road Traffic Accidents	485	179	359	117
Suicides	366	126	471	105
All other	3,138	3,234	3,603	3,802
Total deaths	24,431	22,321	23,058	22,920

In Northern Ireland, men who were born in 1950 had a life expectancy of 66 years (Central Statistics Office 2002a, Central Statistics Office 2002b). A baby boy born in 2002 has a life expectancy of 74 years (Central Statistics Office 2002a). This improvement is thought to be due to scientific and medical advances and better housing, sanitation and nutrition (Men's Health Forum in Ireland January 2004, Murphy-Lawless 2003). In spite of increased male life expectancy, men in Ireland die, on average, nearly 6 years younger than women, at all ages, and for all leading causes of death ((Men's Health Forum in Ireland January 2004, Doyal 2001). Several reasons have been suggested for this inequality between the sexes.

Men are more likely to engage in risky behaviours that can be hazardous to their health (Courtenay 2000). Risky behaviour in men has become culturally acceptable in many cases (Stakelum, Boland 2001). Men are more likely to smoke, to use 'recreational' drugs and to drink over the recommended weekly amounts of alcohol than women (Connell 2000).

Men are also less likely to seek help as sickness may be seen as an expression of weakness (Men's Health Forum in Ireland January 2004, Anonymous2001). Men have limited contact with GPs, are reluctant users of primary care services, are slower to notice signs of illness and often present late in the course of an illness (Kraemer 2000). It has been estimated that 40% of male consultations are at the prompting of a female (Men's Health Forum in Ireland January 2004).

There are concerns that men's health is under-researched, both clinically and in terms of health promotion (Men's Health Forum in Ireland January 2004). There have also been calls for more health promotion initiatives that are specifically targeted at men rather than based on the traditional population-wide approach. An All Party Group on Men's Health was formed by MPs and Peers in early 2001. This group seeks to raise awareness of issues impacting on the health of men. Issues chosen by members of the Group include male health policy, heart disease, depression, sexually transmitted infections and erectile dysfunction.

This thesis will describe the lifestyle and diet of middle-aged men in Northern Ireland, as documented in the questionnaires which were completed by the Belfast cohort at baseline (see chapter 2.0 and appendix).

Nutrition choices in middle-age are influenced by behavioural patterns formed in childhood and young adulthood, in particular, a childhood diet rich in vegetables

in associated with a healthier adult diet (Shepherd 2008a, Shepherd 2008b, Maynard et al. 2006). Socio-economic position and smoking are also important (Maynard et al. 2006). It is possible that the dietary patterns of men are influenced by the family food selection and preparation habits of their partners (Schafer et al. 1999).

Current Department of Health (UK) recommendations for a healthy diet in middle-aged men are to reduce fat, salt and sugar, control portion sizes and increase fibre content. 5 portions of fruit and vegetables per day should be included (Department of Health 2005). Fresh, frozen, chilled, canned, 100% juice and dried fruit and vegetables all count. (Fruit juice can only be counted as a maximum of one portion per day). In 1991, when the PRIME study was recruiting, advice regarding fat, salt, sugar and fibre was similar but the '5-a-day' (fruit and vegetables) recommendation was introduced in the UK in 2004 (Department of Health 2004).

1.4 Lung function, body mass index and distribution of body weight

The anthropometric data collected from the PRIME study group included height, weight, hip circumference and waist circumference. Body mass index (BMI) and waist-hip ratio (WHR) were calculated for each man and the relationship between lung function and the distribution of body weight was explored (see chapter 4.0).

A BMI of between 18.5 and 24.9 is healthy in adults and a BMI of greater than 30 is classed as obesity (World Health Organisation September 2006) (see table 1.2). Obesity is the most common metabolic disease in the world (Formiguera, Canton 2004) and it's prevention has become one of the World Health Organization's leading priorities(Lean, Lara & Hill 2006).

Table 1.2 Classification of body mass index in adults

Body mass index kg/m^2	Classification in adults
<18.5	Underweight
18.5-24.9	Healthy
25-29.9	Overweight
30-34.9	Obesity class I
35-39.9	Obesity class II
>=40	Obesity class III (Morbid Obesity)

In Europe, the prevalence of obesity has tripled in the last two decades. Between 30% and 80% of adults are overweight in most European countries, the average BMI being almost 26.5 kg/m^2 (Lean, Lara & Hill 2006). Childhood obesity is a strong risk factor for adult obesity and is associated with a higher chance of premature death and disability in adulthood (World Health Organisation September 2006). Patterns of diet and exercise developed in childhood are difficult to change.

The Framingham Heart Study showed a significant reduction in life expectancy among non-smoking obese people compared to non-smokers with a healthy BMI. At 40 years, life expectancy in females was reduced by 7.1 years and in males by 5.9 years (Peeters et al. 2003). The relative risk associated with greater BMI declines with age (Stevens et al. 1998).

Obesity is a significant health and economic burden and social class differentials are often apparent. (World Health Organisation September 2006). Obesity costs the UK economy £3.5bn every year and contributes to 30,000 deaths and 18 million days of absence from work (Haslam, Sattar & Lean 2006). Those with lower incomes may find it more difficult to access fitness facilities and to buy fresh fruit and vegetables, choosing to buy cheaper energy dense, less healthy foods (Lean, Lara & Hill 2006).

Obesity may affect respiratory function in a number of ways. It is linked to a wide range of conditions including chronic obstructive pulmonary disease (COPD), asthma, obesity hypoventilation syndrome, pulmonary embolism, aspiration pneumonia and obstructive sleep apnoea (Koenig 2001). Multiple cross-sectional studies have demonstrated an inverse relationship between FEV_1 and body mass index (Biring et al. 1999). Several longitudinal studies have shown that increases or decreases in body weight can lead to worsening or improvement of pulmonary function (Wang et al. 1997, Nishimura et al. 1995). In an 8 year Italian longitudinal study of body mass index, spirometry and diffusion in over a thousand subjects from a general population (aged greater than 24), most of those who lost weight improved their lung function and those who gained weight reduced their lung function(Bottai et al. 2002). This suggests that the detrimental effect on lung function of gaining weight may be reversible for some people (Morgan, Reger 2000, Womack et al. 2000).

Several studies agree that the effect of weight gain on lung function is greater in males than females, likely due to gender-related differences in fat distribution. i.e., the mechanical effect of male central fat distribution on the diaphragm (Carey, Cook & Strachan 1999, Chen et al. 2007).

The mechanical effects of trunchal obesity partly explain the reductions in chest wall compliance, respiratory muscle strength and function, lung volumes and peripheral airway size found in obese individuals (Rochester 1993, Sugerman et al. 1997). Obesity has a modest effect on conventional respiratory function tests until the BMI is ≥ 40 kg/m^2 (Parameswaran, Todd & Soth 2006). Abdominal fat may alter the pressure-volume characteristics of the thorax and restrict the descent of the diaphragm, thereby limiting lung expansion. This reduced ventilation at the lung bases can lead to the closure of peripheral lung units, ventilation to perfusion ratio abnormalities and arterial hypoxaemia, especially in the supine position (CARO, BUTLER & DUBOIS 1960). The expiratory reserve volume is also reduced and the work of breathing is increased (NAIMARK, CHERNIACK 1960). These changes can also lead to lower FVC measurements. Obesity can lead to respiratory muscle inefficiency and creates a heightened demand for ventilation (Parameswaran, Todd & Soth 2006).

Body mass index does not give information on body fat distribution, which is an important predictor of adverse health events like diabetes, hypertension, hyperlipidaemia and coronary events (Borkan et al. 1986).The two main types of fat distribution are central and peripheral. In central obesity most of the fat deposits are in the abdominal area, both subcutaneous and visceral. These visceral fat deposits are highly correlated with cardiovascular risk (Formiguera, Canton 2004). In peripheral obesity, the fat deposits are mainly located subcutaneously in the lower body. Waist circumference and waist-hip ratio add

vital information about the pattern of obesity. Waist circumference is a better assessor of metabolic risk than BMI because it is more directly proportional to total body fat and the amount of metabolically active visceral fat (Haslam, Sattar & Lean 2006). In a cross-sectional study of 1674 adults, waist circumference was negatively associated with FEV_1 and FVC. On average, a 1cm increase in waist circumference was associated with a 13ml reduction in FEV_1 and an 11ml reduction in FEV_1 (Chen et al. 2007). A larger waist-hip ratio is more detrimental in men than women (Harik-Khan, Wise & Fleg 2001). Total body fat and central adiposity are inversely associated with lung function, but the amount of fat-free mass correlates positively with lung function with reduced odds of a low FEV_1:FVC ratio (Wannamethee, Shaper & Whincup 2005b, Santana et al. 2001). Chapter 4.0 will explore the relationships between lung function, BMI and WHR in the PRIME cohort.

1.5 Inflammation and lung function

Body fat distribution is linked to the metabolic effects of obesity. Adiposity is a low-grade inflammatory condition and is linked to both pulmonary function and insulin resistance (Soto Gonzalez et al. 2007). Fat cells have been shown to act as a type of endocrine cell and adipose tissue can be thought of as an endocrine organ. Central type obesity can lead to an imbalance in the production of several metabolic products, adipokines and cytokines, with a variety of local and peripheral effects (Rajala, Scherer 2003). It has been suggested that visceral adipose tissue influences circulating concentrations of interleukin-6 (IL-6), tumour necrosis factor-alpha, resistin, free fatty acids, leptin and adiponectin (fat-cell derived products). These cytokines may cause systemic inflammation which is thought to be involved in the association between reduced pulmonary function and cardiovascular mortality as well as all-cause mortality (Ochs-Balcom et al. 2006).

C-reactive protein (CRP) is an acute phase protein which is produced primarily in the liver in response to interleukin-6 (Shaaban et al. 2006). CRP received its name because it binds to the C polysaccharide or cell wall teichoic acid of Streptococcus pneumoniae (Casey et al. 2008). CRP binds in a calcium-dependent manner to choline phosphate or phosphorylcholine on residues found on C polysaccharide (Volanakis, Kaplan 1971). CRP is a pentameric molecule with two faces: a recognition face, which binds to ligands on apoptotic cells, cell debris, and several microorganisms, and a reverse effector face, which facilitates interaction with host receptors (Shrive et al. 1996). Using these two functional faces, CRP can recognize pathogens and damaged host cells and can facilitate their removal by recruiting the complement cascade and phagocytic cells (Casey et al. 2008). CRP is a sensitive biomarker, the levels of which are raised in most

conditions associated with infection, inflammation or tissue damage (Hurst et al. 2006, Pepys, Hirschfield 2003). A biomarker refers to the measurement of any molecule or material (cells, tissue) that reflects the disease process (Barnes, Shapiro & Pauwels 2003).

Elevated CRP is associated with lifestyle behaviours such as smoking and obesity and is also linked to an individual's genotype (Pankow et al. 2001, Hersh et al. 2006, Sunyer et al. 2008). No CRP deficiency states have been identified in humans, suggesting that the protein is crucial to survival (Casey et al. 2008). However, at least 31 single-nucleotide polymorphisms have been identified for the human CRP gene (Carlson et al. 2005). Increased levels of CRP strongly predict the risk of cardiovascular events (Libby, Ridker & Maseri 2002) in healthy subjects (Ridker et al. 1997, Ridker 2003) and in those with pre-existing coronary artery disease (Ridker et al. 2002). CRP is a predictor of cardiovascular and overall mortality independent of conventional and other risk factors (Laaksonen et al. 2005).

There is evidence that systemic inflammation may also be related to reduced pulmonary function (Kony et al. 2004). High levels of CRP are a predictor of hospitalisation and mortality in patients with chronic respiratory failure (Cano et al. 2004). The relationship between CRP and chronic lung diseases such as COPD has been widely reported. COPD is now recognised a multiorgan systemic disease with manifestations outside the inflammation in the lungs (Stockley 2007, Broekhuizen et al. 2006). There are many common pathogenic processes between COPD and other co-morbidities such as cardiovascular disease, type 2 diabetes and osteoporosis (Stockley 2007). Systemic consequences include malnutrition with a low BMI (Schols et al. 1998, Maltais et al. 1996), impaired

muscle function (Maltais et al. 1996). Respiratory muscle strength and lung function are closely associated with body weight and lean body mass in patients with COPD(Nishimura et al. 1995). Nishimura et al also reported an association between weight gain and increasing rate of decline of FEV1. However, COPD patients have significantly less muscle mass than healthy controls, suggesting that BMI alone is insufficient to assess nutritional status in these individuals. It is possible that malnutrition may exist despite a normal BMI (Muller et al. 2006). BMI tends to be lower in COPD patients than normal controls, particularly in those who still smoke. It must be noted that there is a tendency towards increased mortality in COPD patients who are underweight. BMI tends to decrease as stage of disease increases (Ran et al. 2007). These factors emphasize the importance of keeping BMI in the normal range in the COPD population (Landbo et al. 1999) .

1.6 Antioxidants and lung function

1.6.1 Antioxidant/oxidant balance

Imbalance between the oxidant burden to the lungs (from pollutants like ozone, cigarette smoke and nitrogen dioxide) and the pulmonary antioxidant defences also has a potential impact on the rate of decline of pulmonary function. The relationship between serum antioxidants and markers of oxidative stress suggest that they may be acting as opposing members of the antioxidant/oxidant pathway (Ochs-Balcom et al. 2006). Antiproteases in the lung have a high sensitivity to oxidative damage, whether from an exogenous source like cigarette smoking or an endogenous source such as oxidants released from inflammatory cells like macrophages (Sanguinetti 1992). It is therefore logical to suggest that dietary intake, which is a major source of antioxidants, may be related to modulation of the antioxidant/oxidant balance within the lung, thereby influencing lung function decline.

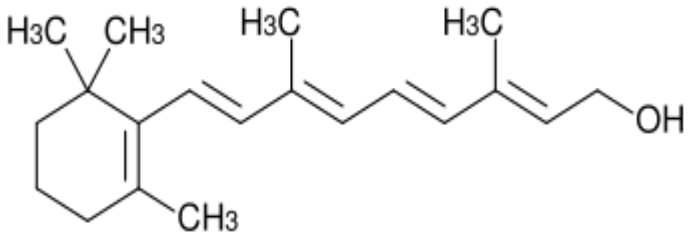
1.6.2 Plasma antioxidants

Dietary antioxidants are absorbed to varying degrees in the gut and enter the bloodstream and lymphatic system. They then enter the pulmonary circulation and are a front-line defence against endogenous and exogenous oxidant damage.

This study focuses on vitamins A and E (retinol, γ-tocopherol, α-tocopherol), and the most abundant of over 600 carotenoids which occur in nature (Monsen 2000, Schunemann et al. 2002), (lutein, zeaxanthin, ß-cryptoxanthin, α-carotene, ß-carotene and lycopene).

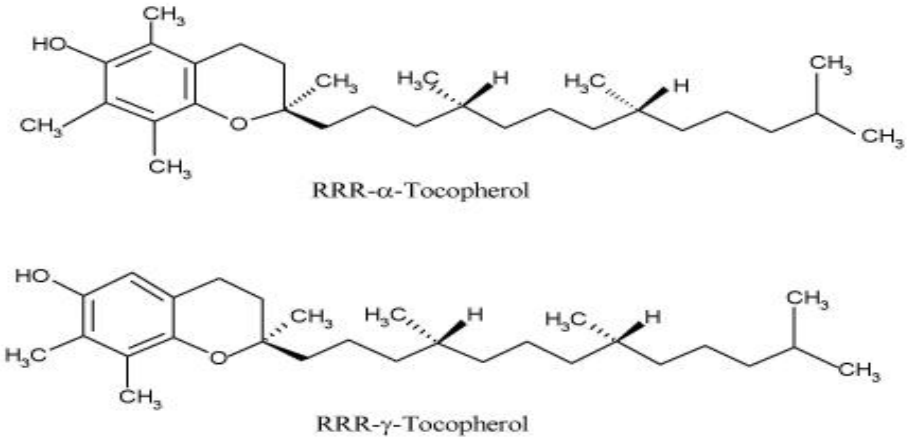
Vitamin A (**Retinol**) is fat-soluble vitamin ingested from animal sources such as liver and eggs and in precursor form from plant sources such as carrots and spinach. It plays an essential role in vision, bone and tooth development, epithelial cell function and as an antioxidant (Sommer 2008) (see figure 1.2.

Figure 1.2: Chemical structure of Retinol



Vitamin E is the generic collective name for a group of eight plant-derived, lipid-soluble substances including four tocopherols (α,ß,γ,δ) and four tocotrienols (α,ß,γ,δ). **α-Tocopherol** is the most common form of vitamin E occurring in human blood and tissues, and it has the highest biological activity among all tocopherols and tocotrienols (Traber, Packer 1995). Vegetable oils and lipid-rich plant products (e.g. nuts, seeds, grains) are the main dietary sources of vitamin E. In Western diets, vitamin E intake derives mainly from fats and oils contained in margarine, mayonnaise and desserts and from fortified foods such as breakfast cereals. Vitamin E used for food fortification and dietary supplements is mainly α-tocopherol. Diets in the United States tend to contain proportionally more **γ-tocopherol** than other Western countries due to the high consumption of soybean and corn oil which contain more γ- than α-tocopherol (Dietrich et al. 2006).

Figure 1.3 Chemical structure of vitamin E (Gao et al. 2006)



The **carotenoids** are nutrients found in coloured fruit and vegetables which have been found to have antioxidant properties in vitro (Hughes et al. 2009). They are fat-soluble pigments and can be classified into oxygenated carotenoids or xanthophylls (lutein, zeaxanthin and ß-cryptoxanthin) and hydrocarbon carotenoids or carotenes (α-carotene, ß-carotene and lycopene) (Paiva, Russell 1999). These two classes of carotenoids have differences in chemical structure and polarity which attach to distinct locations of the cell membrane and thereby differentially influence the susceptibility of lipids to oxidation (Hughes et al. 2009). ß-carotene, α-carotene and ß-cryptoxanthin are pro-vitamin A compounds (de Pee, West 1996). Among these, ß-carotene is most efficiently made into retinol. α-carotene and ß-cryptoxanthin are also converted into vitamin A, but only half as efficiently as β-carotene. Lycopene, lutein and zeaxanthin do not have vitamin A activity (de Pee, West 1996) (see figure 1.3).

1.6.3 Biomarkers of antioxidant status

There are only a few studies of lung function which have used biological markers of antioxidant status rather than dietary intake estimates. Higher levels of serum vitamin A, ß-cryptoxanthin, vitamin C, vitamin E, selenium and iron were found to be independently associated with higher levels of FEV_1 (McKeever et al. 2008). Pearson et al report a positive cross-sectional association between higher levels of serum vitamin C and selenium and FEV_1 in 18-65 year olds (Pearson et al. 2005). A study in Dutch adults aged 20-59 years found a positive cross-sectional relationship between plasma ß-carotene levels and FVC in Dutch adults aged 20-59 (Grievink et al. 1999). No relationship was found with α-tocopherol.

The Health Survey for Scotland reported that higher plasma levels of vitamin C and ß-carotene were associated with greater FEV_1, however the relationship with ß-carotene lost significance in the fully adjusted model. No relationship was found with vitamin A and E (Kelly, Sacker & Marmot 2003).

1.6.4 Food frequency questionnaires

Positive associations have been found between low dietary intake of fruit and vegetables and decreased lung function in general populations (Butland, Fehily & Elwood 2000, Tabak et al. 1999, Strachan et al. 1991)(Tabak et al. 1998). From their cross-sectional study of dietary intake and lung function in 18- 70 year olds, McKeever et al reported that higher vitamin C and magnesium intakes related to higher FEV_1 (McKeever et al. 2002). This was not found with vitamin E or A. The longitudinal data from the same study suggested that there was a less rapid decline in FEV_1 over 9 years in those with a higher average vitamin C intake. This relationship was not found with vitamins A or E. This suggests that a high dietary intake of vitamin C or foods rich in this vitamin may reduce the rate of loss of lung function in adults and thereby perhaps help to prevent lung conditions

such as chronic obstructive pulmonary disease. It may be, however, that vitamin C intake is a marker for some other unmeasured thing which positively affects lung function.

In a FFQ –based cross-sectional study in 35-79 year olds, for each of the antioxidant vitamins, pulmonary function was reported to be higher in the upper quartiles compared with the lower quartiles. The differences were greatest for vitamin C, vitamin E and lutein/zeaxanthin (Schunemann et al. 2002). A strong positive cross-sectional association has also been reported between lung function and apple intake in 45-49 year old men (Butland, Fehily & Elwood 2000). In the longitudinal analysis however, there was no association between vitamin C, E or apple intake per week and lung function. A retrospective case-control study using an FFQ about diet in the preceeding year has suggested that fruit and vegetable intake is inversely associated with Chronic Obstructive Pulmonary Disease (Watson et al. 2002). A British cohort study examined the association between the frequency of fruit consumption in the summer and the prevalence of asthma symptoms (Butland, Strachan & Anderson 1999). The subjects were 33 years old at the time of the study. It was found that the frequency of fresh fruit intake was inversely associated with frequent wheeze in current and former smokers. The association lost significance in non-smokers after adjustment for confounding variables. They concluded that association between diet and respiratory disease as indicated by fresh fruit consumption, is more evident among smokers, which is consistent with a protective antioxidant effect.

Compared to other methods such as diet records, 24-hour recalls and diet histories, food frequency questionnaires are relatively inexpensive and simple, allowing the investigation of a large number of subjects and improving the power

of epidemiological studies (Willett et al. 1985). In selecting a method, a compromise has to be made between a high response rate and the collection of accurate dietary information (Fehily et al. 1993). Food frequency questionnaires are relatively quick to complete and do not involve laborious food weighing. They can be self-administered without the need for an interview with a trained dietician or nutritionist. However data quality may be better when administered by a trained interviewer as in PRIME (Lee, Nieman). Food frequency questionnaires are easy to process because of the standard format and may be more representative of usual intake than a few days of diet records (Lee, Nieman). The reproducibility and accuracy of food frequency questionnaires has been shown in many studies (Willett et al. 1985, Freudenheim 1993, Rimm et al. 1992).

Disadvantages of the food frequency questionnaire method are that it is a subjective assessment and is dependent upon on the participant remembering their usual intake and understanding the questions. Responses may be biased by the perception of what constitutes an appropriate diet (Freudenheim 1993) and the questionnaire may not represent the usual foods chosen by all respondents. The PRIME study FFQ was designed specifically for the target age group and included the main constituents of both the French and Northern Irish diet. However, the number of food groups was limited in the PRIME study and the FFQ was not validated.

1.7 Summary of aims and hypotheses

The lung function data available at baseline and 10 year follow-up from the PRIME study (Belfast cohort) had not previously been analysed and therefore presented an excellent opportunity to explore factors influencing lung function and lung function decline in middle-aged men in Northern Ireland with a view to making possible public health recommendations.

1.7.1 Chapter 3

The hypotheses, tested in the study presented in chapter 3 of this thesis, are: that the Belfast PRIME cohort is an ethnically homogenous group of men with mean BMI, WHR and lung function results comparable to other cohorts of healthy individuals; that lung function is related to smoking habit; and moderate alcohol intake would be associated with better lung function than heavy alcohol intake or abstinence.

The aims were therefore:

a) To describe the demographic, socioeconomic, anthropometric and lung function data from the 2745 men in the Belfast cohort of the PRIME study.

b) To examine the relationship between lung function and smoking habit.

c) To examine the relationship between alcohol intake and lung function.

1.7.2 Chapter 4

The hypotheses, tested in the study presented in chapter 4 of this thesis, are: that lung function has an inverse relationship with both BMI and WHR (cross-sectional); that increases in BMI or WHR over 10 years are correlated with a steeper decline in lung function over the same time period; and that measures of body fat distribution are more closely related to lung function and lung function decline than measurement of body mass index alone.

The aims were therefore:

a) To examine the relationship between lung function, BMI and WHR (cross-sectional).

b) To examine the relationship between changes in BMI, WHR and lung function over 10 years.

1.7.3 Chapter 5

The hypotheses, tested in the study presented in chapter 5 of this thesis, are: that plasma CRP levels are inversely associated with lung function (FEV_1 and FVC); Small increases in CRP, within the 'normal' range, are associated with accelerated lung function decline; that plasma CRP levels are related to smoking history, BMI and WHR.

The aims were therefore:

a) To examine the cross-sectional relationship between plasma CRP levels and lung function.

b) To examine the relationship between 10 year change in plasma CRP and 10 year change in lung function.

c) To examine the relationship between plasma CRP levels, smoking habit, BMI and WHR.

1.7.4 Chapter 6

The hypotheses, tested in the study presented in chapter 5 of this thesis, are: that plasma levels of antioxidants and lung function are positively correlated (cross-sectional); that 10 year change in lung function and 10 year change in antioxidant levels are positively correlated; and that self-reported fruit and vegetable intake are positively correlated with lung function and plasma levels of antioxidants.

The aims were therefore:

a) To examine the relationship between plasma levels of antioxidants and lung function (cross-sectional and longitudinal).

b) To examine the relationship between fruit and vegetable intake and lung function (cross-sectional and longitudinal).

c) To examine the relationship between fruit and vegetable intake and plasma levels of antioxidants (cross-sectional).

2.0 Materials and Methods

2.0 MATERIALS AND METHODS

2.1 Participants

2.1.1 Selection of subjects (Yarnell 1998)

The Prospective Epidemiological Study of Myocardial Infarction (PRIME) study was set up to investigate the large difference in coronary heart disease incidence between Northern Ireland and France. The PRIME study recruited 10,593 men aged 50 to 59 years from the populations of Belfast, Northern Ireland, and Lille, Strasbourg and Toulouse in France from 1991 to 1993, assembling roughly equal cohorts in each centre. 2745 healthy men were recruited into the Belfast cohort.

The sample was recruited to broadly match the social class structure of the background population. The sampling frame was based firstly, on industry and various employment groups. This ensured that, with a helpful employer, subjects were given time off during the morning to be screened on factory medical premises. It had the disadvantage that the sick and unemployed were not recruited. Employment groups with more than 10% of their workforce of foreign origin were excluded. This was done to increase the homogeneity of the samples and remove the need for exclusion of subjects after the biological measurements had been made. Secondly, the sampling frame was based on health screening centres and general practices. This provided the opportunity to recruit unemployed and retired persons to the cohort. Table 2.1 describes the location of recruitment of the 2745 baseline Belfast men.

Table 2.1 Place of recruitment of the 2745 baseline Belfast subjects

Place of Work/Recruitment	Participants	Percent
SHORTS (Aeronautical Engineering)	602	22
NORTHERN IRELAND ELECTRICITY	69	2.5
BELFAST CITY HOSPITAL	21	0.8
ROYAL VICTORIA HOSPITAL	38	1.4
QUEENS UNIVERSITY	61	2.2
BRITISH TELECOM	67	2.4
HARLAND AND WOLF (Marine and Civil Engineering)	231	8.4
CIVIL SERVICE	367	13.4
BELFAST CITY COUNCIL	100	3.6
NORTHERN IRELAND HOUSING EXECUTIVE	111	4.0
FORD MOTOR COMPANY	69	2.5
BRITISH BROADCASTING CORPORATION	5	0.2
GENERAL PRACTICE	1004	36.6
Total	2745	100

2.1.2 Screening

Participation was voluntary and all subjects signed a formal consent form. Subjects were informed of the aim of the study and asked to agree to an annual follow-up. Approval was obtained from the local ethics committee. Subjects were first approached by letter. 5266 men were contacted through industry, the civil service and general practice by letter stating the aim of the study and asking to agree to an annual follow-up. There was an overall response rate to mailings of 52% (2745 men). Those who agreed to take part were given a morning appointment and asked to fast for a minimum of 12 hours. The baseline clinic appointment involved blood sampling, questionnaires, anthropometric measurements, lung function by spirometry, ECG and blood pressure measurement.

Subjects were contacted annually on the anniversary of their initial baseline examination by letter and a clinical event questionnaire was completed and returned to the centre in a pre-paid envelope. If a subject did not respond, a telephone contact was established with the subject or with his General Practitioner. If no answer was obtained, the Registrar General's data was searched to verify that he was still alive. For subjects reporting a possible clinical event, clinical information was sought directly from hospital or General Practitioner notes. Death certificates were searched for supporting clinical and post-mortem information on cause of death. Whenever necessary, death circumstances were obtained from the subject’s practitioner or family. Cardiovascular, cerebro-vascular and cancer endpoints were recorded. The Central Services Agency informed the team when any of the PRIME subjects died, moved to a health authority in Great Britain or moved outside Northern Ireland.

At ten years, the men were re-screened. This involved a clinic appointment which included further questionnaires, anthropometric measurements, a dental examination and X-ray, ECG, blood pressure measurement, vascular compliance, blood sampling and lung function by spirometry. A further consent form was completed.

2.2 Questionnaires (Dauchet et al. 2004a)(Yarnell 1998)

At baseline, self-administered questionnaires relating to demographic, socioeconomic factors and diet were completed at home by the participants and checked by the interviewer at the screening clinic. Information was collected on: level of education; occupational activity; personal history; family history; tobacco consumption; alcohol consumption; diet; drug intake; physical activity; symptoms; and psychosocial factors. The baseline questionnaires are found in the appendices and are named as follows:

-Information questionnaire

-Socio-occupational questionnaire

-Medical questionnaire

-Family questionnaire

-Food questionnaire

-Alcohol questionnaire

-Tobacco questionnaire

-Symptoms questionnaire

-Physical Activity questionnaire

-Attitude questionnaire

At the 10 year follow-up rescreening visit, further questionnaires were filled in by each individual participant and the responses were checked with clinic staff. These questionnaires were named as follows:

- Information questionnaire
- Medical Questionnaire
- Social support questionnaire
- Tobacco questionnaire
- Symptom questionnaire
- Dental questionnaire
- Family questionnaire
- Mini-mental state examination

Copies of all of the above questionnaires are included in the appendices.

2.3 Blood sampling protocol (Yarnell 1998)

Venepuncture was with minimal venous stasis with the subject seated after a 12 hour fast and at least 3 hours after the last smoke for current smokers. All samples were returned to the local laboratory (Royal Victoria Hospital site) and processed within 4 hours of venesection, with the exception of a heparinised sample collected for vitamin analysis. Plasma was prepared immediately from this sample and frozen within 15 minutes of blood collection.

The plasma and serum samples were aliquoted for immediate analysis or long-term storage at -80°C. White cells for DNA extraction were collected from the cell pellets of citrated blood. Plasma for lipid analysis was sent each week at 4°C to the co-ordinating laboratory in Lille. All other aliquots were stored locally at -80°C and sent regularly on dry ice to Lille, where they were transferred to low-temperature banks on two sites.

This protocol was repeated at the 10 year rescreening clinic when a further blood sample was taken.

2.4 Spirometry

2.4.1 Measurement of spirometry

Only the Belfast cohort had lung function measurements by spirometry. Spirometry was performed using a wedge bellows spirometer (Vitalograph S Model). All subjects were sitting and not wearing nose clips. A forced vital capacity manoeuvre was performed with the subject exhaling through a disposable cardboard mouthpiece. Several attempts were made and if the subject was able to demonstrate satisfactory technique (2 attempts with less than 10% variability), the forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) from the best three attempts were recorded. The research staff involved in spirometry measurement were trained by Respiratory medical staff. The same machine was used at baseline and at follow-up. Servicing and calibration was carried out in the Regional Respiratory Centre, Belfast. Spirometry was not included in the original protocol of the PRIME study but was added after the first 140 Belfast men had already had their initial baseline assessment. Therefore there are no spirometry results for the first 140 men in the study at baseline.

This procedure was repeated when the men attended for 10 year rescreening.

2.4.2 Standardisation of spirometry

Each individual spirometry trace (baseline and 10 year follow-up) was reviewed for validity using ATS/ERS criteria (Miller et al. 2005).

Individual spirograms were recorded as acceptable if they were:

a) free from artefacts, had good starts and showed satisfactory expiration,

b) had at least 3 acceptable manoeuvres,

c) the two largest values of FEV_1 were within 150ml of each other and,

d) the two largest values of FVC were within 150ml of each other.

The three largest values of FEV_1 and FVC, from all technically satisfactory manoeuvres, were recorded for analysis. Percent predicted results were obtained by comparing the greatest measured FEV_1 and FVC values to reference values, calculated for age, gender, height and race using the widely used equations of the European Community for Steel and Coal (ECSC)(Quanjer, Dalhuijsen & Van Zoramen 1983). The validity assessment for baseline and 10 year follow-up spirograms are detailed in Tables 2.2 and 2.3.

Table 2.2 Validity of baseline spirometry

Assessment	No. of spirometry traces	Percent Valid %
Valid	1779	64.8
Artefact present	116	4.2
No trace available	140	5.1
<3 acceptable manoeuvres	150	5.5
Two largest FEV1 or FVC values greater than 150 ml of each other	560	20.4
Total screened	2745	100

Table 2.3 Validity of 10 year follow-up spirometry (2010 men rescreened)

Assessment	No. of spirometry traces	Percent of rescreened population %
Valid	1346	67
Artefact present	3	0.1
No trace available	272	13.5
<3 acceptable manoeuvres	48	2.4
Two largest FEV1 or FVC values greater than 150 ml of each other	341	17
Total rescreened	2010	100

I decided with my supervisors to apply the ATS/ERS Criteria to the PRIME spirometry traces at baseline and follow-up as I wanted to ensure that the data used in my analyses was accurate and standardized. I felt that this was important as I had not been present when the original data was being collected and it was clear that some of the spirometry traces were invalid.

Disadvantages of this decision were that this discounted 560 traces at baseline and 341 at 10 year follow-up which had poor repeatability (i.e. the two largest values of FEV_1 or FVC were greater than 150 ml of each other). This reduced the number of men who could be included in the studies included in this thesis and may have excluded some men with reversible airways disease unnecessarily. The ATS/ERS criteria were published in 2005 which was after the PRIME study lung function data collection had been completed, however, previous guidelines actually had even stricter criteria (Anonymous1995).

2.5 Anthropometric measurements

Baseline anthropometric measurements included height, weight, and waist and hip circumferences. These were recorded with the subject dressed in undergarments and in a standing position (Marques-Vidal et al. 2000).

The height of the subject was measured to the nearest centimetre, after he had removed his shoes, standing against a stadiometer. The weight of the subject was recorded, in kilograms rounded to the nearest 200g, using electronic scales which were checked regularly.

The waist circumference was measured at a level midway between the lower rib margin and the iliac crest, slightly above the umbilicus, in centimetres to the nearest 0.0 or 0.5 cm. The subject stood with his feet fairly close together (about 12-15cm) with the weight equally distributed on each leg. A plastic metric tape was used. Before measurement, subjects were asked to breathe normally and at the time of reading the measurement, asked to breathe out gently. The tape was held firmly and its horizontal position was ensured. The observer was sitting by the participant while taking the measurement. Hip circumference was recorded as the maximum circumference over the buttocks, in centimetres to the nearest 0.0 or 0.5cm.

BMI was calculated by dividing the body weight (measured in kilograms) by the square of the individual's height (in metres). WHR was calculated by dividing the waist circumference in centimetres by the hip circumference in centimetres.

These procedures were repeated when the men returned for 10 year rescreening.

2.6 Antioxidant analysis (Vitamin A, vitamin E and Carotenoids)

All chemicals used were purchased from Sigma-Aldrich Co. Ltd (Fancy Rd, Poole, Dorset)) unless otherwise stated. Romil Super Purity Solvent (SpS) grade solvents were used throughout.

Serum concentrations of lipid soluble antioxidants were measured according to a method described by Craft et al 1992 (Craft, Wise & Soares 1992). This high performance liquid chromatography assay using diode array detection was used to assess retinol, γ-tocopherol, α-tocopherol, lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene and lycopene. A 300 µl aliquot of serum or standard was pipetted into a labelled glass tube. Ethanol (0.25 ml) containing 0.25 g/l butylated hydroxytoluene (BHT) and the internal standard α-tocopherol acetate was added to each tube. Heptane (0.5 ml) was then added to each tube. Samples were vortexed vigorously for 1 min and then centrifuged at 825 g for 5 min. The resulting heptane layer was retained and transferred to an identically labelled glass tube and a second heptane extraction was performed. All standard tube contents were retained and dried.

The combined heptane layers were evaporated to dryness in a centrifugal evaporator under vacuum. The standards and samples were reconstituted in methanol (150 µl), vortexed and assayed by a Thermo Separation Products automated HPLC system with a diode array detector. The mobile phase consisted of 97% methanol and 3% tetrahydrofuran, which was filtered and degassed using an inline degasser. The mobile phase was pumped at a flow rate of 1 ml/min. The column used was a Vydac 201TP C_{18}, 5 µm (4.6 x 250 mm) (ThermoFinnegan). The assay was standardised using serum samples of

known concentration from the National Institute of Standards and Technology (NIST), Gaithersburgh, MD, USA. The intra-assay CV for each of the lipid-soluble antioxidants was <10%. The detection limits for retinol and the tocopherols were 0.05 µmol/l and 0.005 µmol/l for carotenoids.

The baseline samples had been in storage for an average of 15 years before being defrosted and analysed. The follow-up samples had been in storage for 3 years.

The PRIME study blood samples which had been stored for future vitamin C analysis were not available at the time of writing.

2.7 C-Reactive Protein Measurement

Ultra-sensitive C-Reactive Protein was measured using a latex-enhanced immunoturbidimetric assay (Randox Pharmaceuticals, UK), Quantex Biokit Reagents and the Instrumentation Laboratory (ILab 600) automated random access Clinical Chemistry Analyser (Instrumentation Laboratories Ltd, Warrington, UK) and ILab 600 computer software. See figure 2iv. This test is standardised on the International Reference Material CRM470 using the recommended test parameters. An intra-assay CV of 1.70% was calculated for 10 runs.

The baseline samples had been in storage for an average of 15 years before being defrosted and analysed. The follow-up samples had been in storage for 3 years. The same kit was used for both sets of samples and they were standardised on the same reference material and measured within a year of each other.

2.8 Lipids

Serum total cholesterol was estimated by a laboratory technician using an enzymatic Cholesterol Oxidase Phenol-4-Aminoantipyrine Peroxidase (CHOD-PAP) kit, (Boehringer Mannheim, Mannheim, Germany). All cholesterol assays were carried out on the Cobas Fara auto-analyser. As well as employing internal quality control procedures, total cholesterol was subject to external quality control through the WHO reference laboratory in Prague.

2.9 Data Analysis

2.9.1 Smoking status

Smoking status at each screening was defined in three categories: current smoker, ex-smoker and never smoker. The definitions for the three categories were outlined in the PRIME study operations manual as follows. A smoker was defined as any subject who had ever smoked for at least three consecutive months with a minimum daily consumption of 1 cigarette or tobacco equivalent. An ex-smoker was defined as any smoker whose consumption in the previous 7 days was less than 7 cigarettes or tobacco equivalents. A current smoker was defined as any smoker who had consumed more than 7 cigarettes or tobacco equivalents in the previous 7 days. A non-smoker was defined as any subject who did not meet the definition of a smoker. Pack years of smoking exposure was estimated for each subject from the detailed tobacco history in the questionnaires. Equivalent pack-years were used for those who smoked cigarillos, cigars and pipes (Boffetta et al. 1999).

Smoking status was reassessed at 10 year re-screening via a questionnaire.

2.9.2 Social status

Social status was based on two variables; educational group and material conditions.

Educational group was determined by the number of years spent in full-time education. A score for material conditions variable was devised which was based on proxy indicators (the number of cars, baths and/or showers, toilets and on home ownership) (Wagner et al. 2003)(Yarnell et al. 2005). Low material conditions were defined by rental accommodation with only one (or less) car, bath/shower, or toilet; high material conditions were defined as home ownership with two or more cars and, either two or more baths/showers, or two or more toilets; the remaining subjects were classified as living in mid-(range) conditions.

Social status was not reassessed during the 10 year rescreening process.

2.9.3 Alcohol intake

This study aimed to evaluate mean weekly alcohol consumption by individualizing the different categories of alcoholic beverages defined by the type of beverage (wine, beer, etc.) and the alcohol content. This was evaluated during an interview, by means of an administered questionnaire which was integrated as much as possible with the validation of the self-administered food questionnaire. Alcohol drinking was assessed as usual weekly intake of wine, beer, cider and spirits, reported by each subject through a standardized interview which focused on different days of the week and different periods within the day. Daily intake of alcohol was estimated from the average number of grams of ethanol in a typical serving of each type of alcoholic beverage (9.6-12 g for wine, 12-21g for beer, 3.8 g for cider and 3.2-19.2 g for spirits) (Marques-Vidal et al. 1996). The present consumption was taken as that in the week preceding the examination. If, for some reason the consumption for that week was different to the average consumption, an earlier week which would correspond to a more usual intake was referred to instead.

Regular consumption was defined by the intake of at least 3 usual amounts per week, for a period of at least 3 consecutive months, whatever the type of beverage consumed. The amount of alcohol consumed per week was calculated in grams per week. A categorical variable was also produced relating to the government guidelines on alcohol consumption that were current at the time of the study (Anon 1991). The three groups were: teetotal, 168g per week or less (21 units or less) and greater than 168g per week.

Alcohol intake was not reassessed during the 10 year rescreening process.

2.10 Statistics

All data were tested for normality of distribution with a plotted histogram. Where the data was skewed, values are quoted as median and interquartile range, or $\log_{10}$values were used. For parametric data, values are quoted as mean and standard deviation, unless otherwise stated. The independent samples t-test was used to compare two groups and Analysis of Variance (ANOVA) for comparisons involving more than two groups. For non-parametric data, the chi-square test for independence was used to determine if two categorical variables were related, the Mann-Whitney U test was used to compare two groups and the Kruskal-Wallis test was used for comparisons involving more than two groups. Non-parametric correlations were performed using Spearman's rank correlation. In the various cross-sectional analyses, the baseline dependent variables (FEV_1 and FVC) were modelled using simple linear regression and adjustment was made for various confounding variables. Longitudinal associations between baseline variables and 10 year change in FEV_1 or FVC in ml were analysed by linear regression using absolute values of change in FEV_1 or FVC in ml over 10 years as the dependent variable and change in the variable of interest as the main predictor. Adjustment was made for baseline, FEV_1 or FVC, height, age, smoking history, body mass index, educational group and material conditions.

This thesis includes 4 types of longitudinal analysis:

- Baseline variables and their relationship to 10 year lung function
- Baseline variables and their relationship to change in 10 year lung function
- Change in variable over 10 years and 10 year change in lung function
- Change in variable over 10 years and 10 year lung function

On discussion with my supervisors and statistician, I feel that each of these approaches have value. Analysing baseline variables and their relationship to 10 year lung function is may be helpful in identifying potential predictors of lung function decline. Analysis of change in variables with respect to change in lung function may suggest (but not prove) causation.

In each analysis in this thesis, adjustment was made for significant confounding variables. My approach was to include the variables which from my initial analyses were found to be closely and significantly related to the outcome (lung function) when analysed individually. In my initial analyses I found significant relationships between lung function and smoking (p84), alcohol (p88), educational group, material conditions, body mass index and waist-hip ratio (p101), height and age. I therefore adjusted for these confounding variables as part of every analysis.

Statistical methods specific to the various studies will be discussed in the respective chapters. All analyses were performed with SPSS 15 (SPSS Inc., Chicago, Illinois, USA), unless otherwise stated.

3.0 Cohort characteristics

3.0 COHORT CHARACTERISTICS

3.1 Introduction

In epidemiology, a cohort is defined as 'any designated group of individuals who are followed up or traced over a period of time' (Last 1995, Rothman 2002). The individuals who were recruited into the Belfast cohort of the PRIME study from 1991-1993 had several characteristics in common: age (50-59y), sex (male), nationality (British or Irish) and geographical location. The purpose of this chapter is to explore the characteristics of this cohort and to study the relationships between lung function and lifestyle related exposures such as smoking and alcohol intake.

3.1.1 Hypotheses

a) The Belfast cohort contains an ethnically homogenous group of men with mean BMI, WHR and lung function results within normal limits.

b) Lung function is closely related to smoking habit.

c) Moderate alcohol intake is associated with better lung function than heavy alcohol intake or abstinence.

3.1.2 Aims

The aims of this study are to:

a) Describe the demographic, socioeconomic, anthropometric and lung function data from the 2745 men in the Belfast cohort of the PRIME study.

b) Examine the relationship between lung function and smoking habit.

c) Examine the relationship between alcohol intake and lung function.

3.2 Methods

3.2.1 Participants

2745 men aged 50-59 were recruited into the Belfast cohort of the PRIME study between 1991 and 1994 as described fully in chapter 2.1.

3.2.2 Data Collection

Each subject completed a questionnaire on demographics, socio-economic factors and dietary habits. Anthropometric measurements included height, weight, and waist and hip circumferences. Only the Belfast cohort had lung function measurements by spirometry. 2010 of the original cohort were re-screened 10 years later. This involved a further questionnaire, anthropometric measurements, spirometry and venous blood sample.

3.2.3 Data analysis

Smoking status was defined in three categories: current smokers, ex-smokers and never smokers. Pack years of smoking exposure was also estimated for each subject from the detailed tobacco history in the questionnaires. Social status was based on two variables, educational group and material conditions as previously described in chapter 2.9.

Data analysis was carried out using SPSS (Version 15). All data were tested for normality of distribution with a plotted histogram. Alcohol intake was skewed to the right so values were quoted as median and interquartile range and/or $\log_{10}$values were used. For parametric data, values are quoted as mean and standard deviation or 95% confidence intervals, unless otherwise stated. Analysis of variance was used to compare the three smoking groups and the three groups of alcohol intake. The independent samples t-test was used to compare the two groups of men who had been baseline smokers. For non-parametric data, the

Mann-Whitney U test was used to compare two groups and the Kruskal-Wallis test was used for comparisons involving more than two groups. In the various cross-sectional analysis of alcohol intake and lung function, the baseline dependent variables (FEV_1 and FVC) were modelled using simple linear regression and adjustment was made for various confounding variables.

3.3 Results

3.3.1 Demographics

The 2745 men in the baseline cohort were from the greater Belfast area and had a mean age of 54.8 years (SD 2.9). The demographic features of this group are outlined in table 3.1. Most of the men were married or co-habiting (85.6%) and 99.3% were British or Irish.

Table 3.1 Demographic features of the 2745 Belfast men at baseline in the PRIME study

		n	%
Marital status	Single	181	6.6
	Married/co-habiting	2349	85.6
	Widowed	69	2.5
	Divorced/separated	146	5.3
Nationality	British	2137	77.9
	Irish	588	21.4
	Other	20	0.7
Educational group	<10 yrs education	795	29
	10 yrs education	752	27.4
	11-12 yrs education	595	21.7
	13-14 yrs education	282	10.3
	>15 yrs education	321	11.7
Alcohol group	Teetotal	1095	39.9
	168g or less per week (21 units)	782	28.5
	>168g per week	868	31.6
Smoking history	Never smoker	904	32.9
	Former smoker	970	35.3
	Current smoker	871	31.7
Material conditions	Low	1075	39.2
	Mid-range	589	21.4
	High	1081	39.4
Occupation(HMSO Feb 1990)	Managers and Administrators	469	17.1
	Professional Occupations	304	11.1
	Associate Professional and Technical	254	9.3
	Clerical and Secretarial	176	6.4
	Craft and Related	755	27.5
	Personal and Protective	159	5.8
	Sales	38	1.4
	Plant and Machine Operatives	376	13.7
	Other	169	6.2
	Unemployed	45	1.6

36.6% of the baseline men were recruited via their local GP surgery and the remaining 63.4% from local industry and the public sector.

3.3.2 Anthropometric data

The 2745 baseline men were, on average, overweight with a body mass index of 26.3kg/m^2 and a waist-hip ratio of 0.94 (see table 3.2).

Table 3.2 Baseline anthropometric data from the 2745 Belfast men

Measurement	Mean	SD
Body mass index kg/m^2	26.3	3.4
Height (m)	173.9	6.8
Weight (kg)	79.4	11.6
Waist-hip ratio	0.94	0.05
Waist circumference (cm)	91.2	9.4
Hip circumference (cm)	96.9	6.8

3.3.3 Baseline lung function

When the individual spirometry traces were validated as detailed in section 2.4.2, 1779 men (64.8%) had valid spirometry traces, 826 men (30.1%) had invalid spirometry traces and 140 men (5.1%) did not have any spirometry carried out.

There was no significant difference between the groups in waist circumference, weight, BMI or waist-hip ratio (see table 3.3). There was a statistically significant difference in height and in hip measurement between the no spirometry group and the invalid spirometry group. Age and smoking status was significantly different in all three groups but particularly between the no spirometry group and the other two groups. There was no difference between the invalid spirometry group taken as a whole and the valid spirometry groups in any of the above measurements except for age (Student's t test, p=0.002). When the invalid spirometry group is split into those who had the two largest values of FEV_1 (n=560, 'WIDE') or FVC were greater than 150ml of each other and those who had artefact present or < 3 acceptable manoeuvres (n=266, 'OTHER'), the 'OTHER' group had a lower mean waist-hip ratio and less current smokers.

Table 3.3: *Comparison of baseline men with valid, invalid and absent spirometry results*

	No spirometry n=140	Invalid spirometry***		Valid spirometry n=1779	Analysis of variance (ANOVA)
		WIDE n=560	OTHER n=266		
	Mean(SD)	Mean (SD)	Mean (SD)	Mean(SD)	
Age (years)	56.9 (2.7)	54.8 (2.9)	55.1 (2.9)	54.6 (2.9)	**p<0.001**
Waist circumference(cm)	92.1 (8.6)	91.7 (9.4)	91.0 (9.6)	91.2 (9.5)	p=0.44
Height(cm)	172.9 (6.1)	174.3 (6.7)	174.3 (7)	173.7 (6.9)	**p=0.03***
Weight(kg)	77.4 (9.9)	80.1 (11.2)	78.9 (12)	79.4 (11.7)	p=0.09
Hip measurement (cm)	98.2 (6.8)	97.3 (6.7)	96.7 (6.5)	96.7 (6.9)	**p=0.02***
BMI(kg/m2)	25.9 (3.0)	26.3 (3.3)	26.0 (3.6)	26.3 (3.5)	p=0.39
Waist-hip ratio	0.94 (0.04)	0.94 (0.06)	0.91 (0.8)	0.94 (0.06)	p=0.6
Current smokers	26.4%	32.3%	27.4%	32.6%	**p<0.0001 ****

* Difference between groups significant at the 0.05 level ** Kruskal-Wallis test

***WIDE = the two largest values of FEV1 or FVC were greater than 150ml of each other. OTHER= Artefact present or < 3 acceptable manoeuvres

1779 men had valid baseline lung function measurements by spirometry. Mean percent predicted FEV_1 was 92% and mean percent predicted FVC was 96.9% (see table 3.4). The men who were excluded because the two largest values of FEV_1 or FVC were greater than 150ml of each other had a lower mean FEV_1 and FEV_1/FVC ratio (see table 3.5).

Table 3.4 Baseline lung function data from the 1779 Belfast men with valid baseline spirometry[1]

Lung function	Mean	SD
Best FEV_1 (L)	3.13	0.68
%Predicted FEV_1	91.9	17.6
Best FVC (L)	4.12	0.82
%Predicted FVC	96.9	16.3
FEV_1/FVC ratio	0.76	0.10

Table 3.5 Baseline lung function data from the 560 Belfast men with baseline spirometry in which the two largest values of FEV_1 or FVC were greater than 150ml of each other (WIDE).

Lung function	Mean	SD
Best FEV_1 (L)	3.01	0.67
%Predicted FEV_1	88.0	17.8
Best FVC (L)	4.15	0.81
%Predicted FVC	97	16.2
FEV_1/FVC ratio	0.73	0.12

[1] Percent predicted results were obtained by comparing the greatest measured FEV_1 and FVC values to reference values, calculated for age, gender, height and race using the equations of the European Community for Steel and Coal (ECSC)(Quanjer, Dalhuijsen & Van Zoramen 1983)

3.3.4 Smoking history

3.3.4.1 Baseline

31.7 % of the baseline cohort of Belfast men were current smokers at the time of screening and 32.9% had never smoked in their lifetime. The mean age of smoking initiation in this population of men was 17.1 years (SD 5.09). 91% of the current and former smokers reported 'inhaling' their cigarette smoke.

Of the 871 current smokers at baseline, 682 men smoked cigarettes alone, 86 men smoked cigars alone, 93 men smoked a pipe alone and 10 men smoked cigarillos only. 14 men smoked a mixture of the above (pipe and cigarettes, 5; pipe and cigars, 3; cigars and cigarettes, 4 and cigar and cigarillos, 2).

There was an inverse relationship between smoking history and lung function (see table 3.6). The current smoker group had the lowest FEV_1 and FVC levels. The highest FEV_1 and FVC levels were found in the never smoker group. Former smokers were slightly older than current and never smokers.

BMI was significantly different in each group with former smokers having the greatest mean BMI and current smokers having the lowest mean BMI. Mean WHR was highest in former smokers but the lowest in never smokers. Alcohol intake was highest in the current smoker group and lowest in the never smokers. Never smokers on average had spent longer in full-time education and were in a higher social class than former and never smokers.

Table 3.6 Baseline characteristics stratified by smoking status

	Never smokers n=904 Mean (SD)	Former smokers n=970 Mean (SD)	Current smokers n=871 Mean (SD)	ANOVA p
Age started smoking yr	N/A	17.0 (4.6)	17.3 (5.6)	**0.02***
Pack years smoking history yr	N/A	25.2 (12.3-42.0)***	36.8 (27-46.6))***	**<0.0001***
BMI kg/m^2	26.2 (3.2)	26.9 (3.5)	25.6 (3.5)	**<0.0001**
WHR	0.93 (0.05)	0.95 (0.05)	0.94 (0.06)	**<0.0001**
Age at screening yr	54.7(2.9)	55.0 (2.9)	54.7 (2.9)	**0.013**
Alcohol group				
Teetotal	54.4%	37.3%	27.7%	
Moderate(<168g/wk)	26.7%	30.1%	28.6%	**<0.0001****
Heavy (>168g/wk)	18.9%	32.6%	43.7%	
>15 yrs education %	15.7%	10.7%	8.6%	**<0.0001****
Highest social class%	48.1%	39.3%	30.4%	**<0.0001****

*Independent samples t-test ** Kruskal-Wallis test *** Median and IQ range

Lung function was closely related to smoking history both cross-sectionally and longitudinally. The never smoker group had the highest mean FEV_1 and FVC and the current smokers had the lowest mean FEV_1 and FVC levels at baseline. Current smokers also had the lowest mean FEV_1/FVC ratio at baseline (see table 3.7).

Table 3.7 Relationship between smoking status and lung function in the 1779 baseline men with valid spirometry

Outcome	Characteristic	Crude		Adjusted*		
		Mean lung function outcome in ml Mean (SD)	p-value (trend across cats)	Difference in mean outcome (95% CI)	p-value (trend across cats)	R^2
Baseline FEV_1 in ml	**Smoking status by category**					
	Never, n= 571	3314 (664)	<0.0001	0 (ref. cat)	<0.0001	0.31
	Former, n=628	3141 (666)	(<0.0001)	-130 (-196,-64)	(<0.0001)	
	Current, n=580	2931 (643)		-296 (-365, -227)		
Baseline FVC in ml	**Smoking status by category**					
	Never, n= 571	4254 (832)	<0.0001	0 (ref. cat)	<0.001	0.34
	Former, n=628	4122 (793)	(<0.0001)	-70 (-148, 7)	(<0.0001)	
	Current, n=580	3988 (804)		-166 (-248, -85)		

* Adjusted for baseline age, height, BMI, alcohol intake, WHR, educational group and material conditions

There was no significant difference between the smoking groups in percentage of men who produced valid spirometry traces at baseline (p=0.19) (see table 3.8), i.e. smoking history was not related to the ability to produce a valid spirometry trace.

Table 3.8 Validity of baseline spirometry traces by smoking group

	Never smokers N=904	Former smokers n=970	Current smokers n=871	p (Kruskal-Wallis test)
% Valid spirometry traces	63.2%	64.7%	66.6%	0.19

3.3.4.2 Follow-up

Of the 871 baseline smokers, 304 were not re-screened, 350 were still smoking at follow-up, 217 stopped in the 10 year follow-up period. Of the 304 who were not rescreened, 33 had died of a smoking associated cancer, 68 had died of other causes and 203 men were alive but were not rescreened (refused or not contactable).

Table 3.9 Comparison of the baseline smokers who continued to smoke and those who gave up during the 10 years of follow-up

	Kept smoking N= 350 Mean (SD)	Stopped smoking N=217 Mean (SD)	p (t-test)
Baseline BMI kg/m2	25.4 (3.4)	25.7 (3.1)	0.23
Baseline WHR	0.94 (0.06)	0.94 (0.06)	0.64
Baseline % predicted FEV_1	87.5 (16.3)	87.4 (17.4)	0.94
Baseline % predicted FVC	94.9 (15.6)	96.5 (15.9)	0.28
Baseline FEV_1/FVC	0.74 (0.09)	0.73 (0.1)	0.09
10 year BMI	26.2 (4.1)	27.8 (3.5)	**<0.0001**
10 year WHR	0.94 (0.08)	0.96 (0.07)	**0.01**
10 year % predicted FEV_1	80.9 (16.4)	82.0 (17.8)	0.53
10 year % predicted FVC	87.9 (13.6)	86.1 (17.3)	0.21
10 year FEV_1/FVC	0.71 (0.09)	0.76 (0.25)	**0.01**
Change in FEV_1 over 10 years mls	544 (424)	485 (459)	0.2

The baseline smokers who gave up in the 10 year follow-up period, had significantly greater BMI and WHR at 10 years than the group who continued to smoke (see table 3.9). They had significantly less obstructive spirometry and a tendency towards better FEV_1 than the group who continued to smoke.

At 10 year follow-up, of the 2010 Belfast men re-screened, only 19.8% of the men were current smokers, 45% were former smokers and 35% were never smokers.

Of the 397 men who were current smokers at follow-up, 5 had started to smoke during the 10 year follow-up having previously been never smokers and 42

restarted smoking during the 10 year follow-up, having been former smokers at baseline.

Table 3.10 10 year follow-up characteristics stratified by smoking status

	Never smokers N=904	Former smokers n=970	Current smokers n=871	ANOVA p
BMI	27.2 (3.8)	28.1 (3.63)	26.4 (4.1)	**<0.0001**
WHR	93.3 (7.0)	95.0 (7.6)	94.4 (7.9)	**<0.0001**
FEV_1 L (SD)	2.92 (0.58)	2.71 (0.62)	2.54 (0.58)	**<0.0001**
%predicted FEV_1	92.7 (16.5)	86.4 (17.7)	81 (16.1)	**<0.0001**
FVC L (SD)	3.83 (0.69)	3.60 (0.73)	3.54 (0.65)	**<0.0001**
%predicted FVC	94.5 (14.6)	89.5 (15.5)	88.1 (13.5)	**<0.0001**
FEV_1/FVC ratio	0.77 (0.08)	0.76 (0.13)	0.72 (0.09)	**<0.0001**

At 10 year follow-up, the former smoker group had the highest BMI and the current smokers had the lowest BMI (see table 3.10). The former smokers group also had the highest mean WHR, although the never smokers had the lowest WHR. As before, FEV_1, FVC and the FEV_1/FVC ratio were lowest in the current smoking group and highest in the never smoking group.

Lung function decline over 10 years was examined for the 932 men with valid baseline and 10 year follow-up spirometry. The baseline current smokers had the greatest decline in FEV_1 over 10 years at 56 ml/y compared to 44.3 ml/y for never smokers (see table 3.11).

Table 3.11 Relationship between baseline smoking history and lung function decline over 10 years in the 932 men with valid baseline and 10 year follow-up spirometry

	Never smokers n=329 Mean (SD)	Former smokers n=357 Mean (SD)	Current smokers n=246 Mean (SD)	p ANOVA
Decline in FEV_1 over 10 years	443 (361)	444 (333)	560 (339)	**<0.0001**

3.3.5 Alcohol

39.9% of the baseline population of 2745 men were teetotal, 28.5% drank 21 units of alcohol (168g) or less per week and 31.6% drank more than 21 units (168g) per week (see table 3.12). The heavy drinking group had a significantly greater mean WHR than the other two groups. The heavy drinking group contained the highest proportion of current smokers, the highest proportion of men in the lowest social class and with the least education. The moderate drinker group had spent the most time in education on average.

Table 3.12. Baseline anthropometric and socioeconomic data stratified by alcohol intake

	Teetotal Mean (SD)	Moderate <168g/week Mean (SD)	Heavy >168g/week Mean (SD)	p ANOVA
Age yr	54.9 (2.9)	54.9 (2.9)	54.6 (2.9)	0.01
BMI kg/m^2	26.3 (3.5)	26.3 (3.3)	26.2 (3.5)	0.9
WHR	0.94 (0.06)	0.94 (0.05)	0.95 (0.05)	<0.0001
Alcohol g/wk	0	84.2 (49-84)*	318.1 (227-475)*	<0.0001**
>15 yrs education	12.1%	14.5%	8.8%	<0.0001**
Lowest social class	33.9%	35.5%	49.1%	<0.0001**
Current smokers	22%	31.8%	43.9%	<0.0001**

***** Median (IQ range) ** Kruskal-Wallis

There was a 'J' shaped association between alcohol and lung function in the cross-sectional study. The highest mean FEV_1 and FVC levels were in the moderate alcohol intake group and the lowest levels were in the heavy alcohol intake group. This pattern remained significant after adjustment for age, height, BMI, smoking history, educational group and material conditions (see table 3.13).

Table 3.13 Relationship between alcohol intake and lung function in the 1779 baseline men with valid spirometry

Outcome	Characteristic	Crude		Adjusted*		
		Mean lung function outcome in ml Mean (SD)	p-value (trend across cats)	Difference in mean outcome (95% CI)	p-value (trend across cats)	R^2
Baseline FEV_1 in ml	**Alcohol intake by category**					
	Teetotal, n=718	3137 (700)	<0.0001	0 (ref. cat)	0.02	0.30
	168g/wk or less, n=472	3213 (669)	(0.03)	83.4 (16.8,150)	(0.99)	
	>168g/wk, n=589	3049 (641)		-6.2 (-70.3, 58)		
	Log_{10}alcoholintake	-260 (-351,-169) †	<0.0001	-136(-218,-55) †	0.001	0.32
Baseline FVC in ml	**Alcohol intake by category**					
	Teetotal, n=718	4117 (847)	<0.0001	0 (ref. cat)	0.001	0.33
	168g/wk or less, n=472	4243 (829)	(0.07)	110.3 (32, 189)	(0.43)	
	>168g/wk, n=589	4027 (752)		-39.4 (-115, 37)		
	Log_{10}alcoholintake	-279 (-390, -169)	<0.0001	-162 (-258, -66)	0.001	0.34

*justed for baseline age, height, BMI, smoking history, educational group and material conditions
† Increase in lung function outcome per unit ten-fold increase in alcohol intake (based upon log_{10}alcoholintake)

FEV_1 /FVC ratio was the same in each group of alcohol intake (0.76 (SD 0.1)).

There was no significant relationship between baseline alcohol intake and subsequent decline in lung function over 10 years in either the crude (p=0.31) or adjusted (p=0.57) model.

There was a crude inverse relationship between baseline alcohol group and 10 year FEV_1 but this was lost on adjustment for confounding variables.

3.3.6. Diet

The baseline diet of the cohort is discussed fully in chapter 6.0

3.4 Discussion

The Belfast cohort of the PRIME study was an ethnically homogenous group of men with a mean age of 54.8 years. Most were married (or co-habiting) and employed at the time of recruitment. The mean BMI of the group was in the overweight category (26.3 kg/m^2) and mean lung function was within normal limits (FEV_1 3.13L and FVC 4.12L, FEV_1/FVC 0.76). Time spent in education, alcohol intake, smoking history, material conditions and occupation varied widely among the group. It is interesting that only 11.7% of the men had had a university education but 11.1% were in professional occupations and 17.1 % were in managerial and administrative roles.

3.4.1 Generalisability of data.

The PRIME study only included men so the results cannot be generalised to both sexes. The sick and unemployed are unrepresented so there is likely a healthy worker effect. However, it must be noted that many of the men would have been exposed to various industrial gases and chemicals which may have affected their lung function. It is difficult to compare the data directly to the NI Census data from 1991 as the published census data is not fully categorised by age and sex. The proportions of married and single men can be ascertained and these are very similar to the PRIME figures (Northern Ireland Statistics and Research Agency).

There was a 52% response rate to the initial mailings so there may be some selection bias, however this response rate is similar to that obtained in the MONICA (MONitoring CArdiovascular disease) surveys (Evans et al. 2001). No information is available about the non-responders so it is difficult to quantify this. Caution must therefore be used in translating findings from this study to a different population.

3.4.2 Smoking

Most of the 871 baseline current smokers smoked cigarettes alone, but 22% smoked cigars, pipe, cigarillos or a mixture of tobacco products. Current smokers had the lowest FEV_1, FVC and FEV_1/FVC ratio at baseline and the steepest FEV_1 decline over 10 years (56 ml/y). Never smokers had the best mean baseline lung function and smallest decline in lung function over 10 years (43.3ml/y). The findings for current smokers are in line with other studies of the effect of cigarette smoking on lung function (Kerstjens et al. 1997, Anthonisen, Connett & Murray 2002) although the rate of decline in never smokers is a little higher than expected. This may potential reflect unmeasured environmental toxin or industrial gas or chemical exposure.

The current smokers at baseline had the lowest mean body mass index, however the never smoker group had the lowest waist-hip ratio, supporting the suggestion that weight distribution may be different in smokers and non-smokers (Canoy et al. 2005). Of the 567 baseline smokers who were rescreened at 10 years, 217 (38.3%) had stopped smoking in the intervening period. This group had a higher 10 year BMI and WHR than the group who continued to smoke. This phenomenon of weight gain following smoking cessation is also well-described and may be linked post-cessation endocrine changes as well as increased food intake (Berlin 2009) Lung function after smoking cessation is significantly influenced by weight gain, an effect more marked in men than women. This may be because men tend to preferentially increase abdominal fat compared with women when the gain weight (Shimokata et al. 1989). In a study by Williamson et al, black patients, those under the age of 55 and people who smoked 15 cigarettes or more per day were at higher risk of major weight gain after quitting smoking (Williamson et al. 1991). Data from the third National Health and Nutrition Examination Survey showed that weight gain associated with the

cessation of smoking over a ten year period was 4.4kg for men and 5.0kg for women (Flegal et al. 1995). The deleterious effects of weight gain are small, however in comparison with the beneficial effects of smoking cessation (Wise et al. 1998). This issue should be addressed by smoking cessation programmes.

Smoking cessation is associated with a rate of decline that is similar to that noted in never smokers and is considerably less than that of continuing smokers (Anthonisen, Connett & Murray 2002, Lange et al. 1989, Xu et al. 1992). No information was gathered in PRIME about methods of achieving smoking cessation or the reasons why individuals decided to quit. This would have been helpful in assessing the impact of anti-smoking policies and campaigns. Short- and long-term health-care savings may be realized if smoking cessation is made a priority

3.4.3 Alcohol

There are few published reports on the effect of alcohol on lung function. Modest alcohol intake has been shown to be associated with reduced risk of cardiovascular disease (Marques-Vidal et al. 1996), cerebrovascular disease (Berger et al. 1999), hearing loss (Sisson et al. 2005) and dementia (Ruitenberg et al. 2002).

The data from the PRIME study confirms previously reported findings that heavy alcohol intake and smoking habits often coincide (Sisson et al. 2005). In addition, a 'J'-shaped association between alcohol and lung function was demonstrated. The moderate alcohol intake group had the highest lung function levels and the lowest lung function levels were found in the heavy alcohol intake group. This pattern did not change after adjustment for smoking history, age, height,

educational group and material conditions. Specifically, mean FEV_1 in the moderate alcohol group was 83.4ml higher than in the teetotal group and 89.4 ml higher than the heavy alcohol intake group. FEV_1/FVC was not related to alcohol intake in this study.

It has been suggested that the beneficial effects of moderate alcohol consumption in cardiovascular disease prevention are related to the increase in HDL cholesterol levels associated with alcohol intake (Marques-Vidal et al. 1996). Raised HDL cholesterol levels have been associated with regression of atherosclerosis suggesting that high levels may modify the inflammatory cascade which could potentially be beneficial in the respiratory system (Walter 2009). Adjusting for HDL cholesterol levels did not affect the relationship in our study, suggesting that this is not the mechanism in the respiratory system. There may be confounding factors which we have not considered which would account for the observed relationship. Since the moderate drinkers had the highest education levels it may be that diet and lifestyle factors may account for the association with better lung function. Education may enable individuals to make more informed choices about food and particular lifestyles (Shohaimi et al. 2004). This will be explored further in chapter 6.0.

3.4.4 Conclusion

Lifestyle choices such as smoking and alcohol intake affected lung function in this population. Smoking accelerated lung function decline. Heavy alcohol intake was related to poor lung function but moderate alcohol intake was associated with higher mean FEV_1 levels than abstinence.

4.0 Distribution of body fat and lung function decline

4.0 DISTRIBUTION OF BODY FAT AND LUNG FUNCTION DECLINE

4.1 Introduction

4.1.1 Obesity and lung function

Weight is an important determinant of the level of lung function (Sugerman et al. 1997, Cook et al. 1994). Obese individuals have a tendency towards increased dyspnoea, decreased exercise capacity and decreased muscle mass which can create a vicious cycle of inactivity and weight gain leading to further deconditioning (McClean et al. 2008). The mechanical effects of truncal obesity and the metabolic effects of adipose tissue (Ochs-Balcom et al. 2006) are important factors contributing to the clear inverse relationship between obesity and forced expiratory volume in one second (FEV_1) reported in many cross-sectional studies (Wannamethee, Shaper & Whincup 2005a). Several longitudinal studies have demonstrated that increases in body weight can lead to a reduction in pulmonary function (Wang et al. 1997, Nishimura et al. 1995, Thyagarajan et al. 2008, Chen, Horne & Dosman 1993). It has also been reported that losing weight can lead to an improvement in lung function (Bottai et al. 2002). However, less is known about the influence of weight distribution on lung function.

4.1.2 Body mass index and waist-hip ratio

Body mass index (BMI) is easily calculated from weight and height measurements but it does not give information on body fat distribution (Wannamethee, Shaper & Whincup 2005a). Waist and hip circumference are also simple to measure and add important information about the pattern of weight distribution which is an important predictor of adverse health events like diabetes, hypertension, hyperlipidaemia and coronary events (Klein et al. 2007). Current obesity guidelines recommend the measurement of waist circumference in persons with a BMI between 25.0 and 34.9 and propose highly increased risk cut-off points for waist circumference of 102 cm in men and 88cm in women and for waist-to-hip ratio of 1.0 and 0.85, respectively (Pischon et al. 2008).

Waist-hip ratio (WHR) is positively associated with risk of death (Pischon et al. 2008). In central obesity most of the fat deposits are in the abdominal area, both subcutaneous and visceral. These visceral fat deposits are highly correlated with cardiovascular risk (Formiguera, Canton 2004). Consequently, it has been suggested that waist circumference is a better assessor of metabolic risk than BMI because it is more directly proportional to total body fat and the amount of metabolically active visceral fat (Haslam, Sattar & Lean 2006). Cross-sectional studies have shown a negative relationship between waist circumference, WHR and lung function (Wannamethee, Shaper & Whincup 2005a, Canoy et al. 2004), particularly in men (Harik-Khan, Wise & Fleg 2001). It has been suggested that the pattern of fat distribution, measured by waist-hip ratio is a better predictor of pulmonary function than weight or BMI (Harik-Khan, Wise & Fleg 2001, Ochs-Balcom et al. 2006) but there are few longitudinal studies investigating this relationship. Reductions in fat-free mass and increase in sagittal abdominal diameter are significant predictors of lung function decline (Rossi et al. 2008) and there is an inverse relationship between annual change in FEV_1 over 7 years and

changes in weight, BMI, waist circumference, hip circumference and WHR in men (Carey, Cook & Strachan 1999)

This present study investigates relationships between decline in FEV_1, forced vital capacity (FVC) and change in BMI, WHR, waist circumference and hip circumference in a cohort of working middle-aged men.

4.1.3 Hypotheses

a) Lung function has an inverse relationship with both BMI and WHR (cross-sectional).

b) Increases in BMI or WHR over 10 years will be correlated with a steeper decline in lung function over the same time period.

c) Measures of body fat distribution are more closely related to lung function and lung function decline than measurement of body mass index alone.

4.1.4 Aims

a) Examine the relationship between lung function, BMI and WHR (cross-sectional).

b) Examine the relationship between changes in BMI, WHR and lung function over 10 years.

4.2 Methods

4.2.1 Participants

2745 men were recruited into the Belfast cohort of the PRIME study as described in chapter 2.0.

4.2.2 Data Collection

Each subject completed a questionnaire to provide details of demographic characteristics, socio-economic factors and tobacco consumption. Responses were checked by medical staff at a targeted clinic. Baseline anthropometric measurements included height, weight, and waist and hip circumferences. These were recorded with the subject dressed in undergarments (Marques-Vidal et al. 2000). BMI was calculated by dividing the body weight (measured in kilograms) by the square of the individual's height (in metres). The waist circumference was measured at a level midway between the lower rib margin and the iliac crest, slightly above the umbilicus, in centimetres to the nearest 0.0 or 0.5 cm. Hip circumference was recorded as the maximum circumference over the buttocks, in centimetres to the nearest 0.0 or 0.5cm. WHR was calculated by dividing the waist circumference in centimetres by the hip circumference in centimetres. 2010 of the original cohort were re-screened 10 years later. This involved a further questionnaire, anthropometric measurements, spirometry and venous blood sample.

4.2.3 Data analysis

Data analysis was carried out using SPSS (Version 15). In the cross-sectional analysis, the baseline dependent variables (FEV_1 and FVC) were modelled using simple linear regression and multiple regression to adjust for initial age, height, material conditions, educational group and smoking history (never, former or current). FEV_1 and FVC change over the 10 years of follow-up was modelled using simple linear regression and multiple linear regression to adjust for age, height, material conditions, educational group, baseline lung function and smoking history. Non-linear associations were investigated by the use of categories (quartiles).

4.3 Results

4.3.1 Cross-sectional analysis

4.3.1.1 Baseline data

At baseline, 1779 men had a valid spirometry measurement and a record of waist circumference, hip circumference, weight and height. Spirometry tests for 966 men were either missing (140 tests) or did not meet quality criteria. For 560 tests, the difference between the largest FEV_1 and the next largest FEV_1 was greater than 150 ml, for 266 tests, there were fewer than 3 acceptable manoeuvres. The mean age was 54.6 years (SD 2.9). Mean FEV_1 and FVC were within normal ranges (see table 4.1). 99.2% of the men were British or Irish. 32.9% had never smoked and 31.7% were current smokers.

Table 4.1 Baseline data of the 1779 PRIME men who had a valid spirometry measurement

Measurement	Mean (SD)
Age (years)	54.6 (2.9)
Waist circumference (cm)	91.2 (9.5)
Hip circumference (cm)	96.7 (6.9)
Waist-hip ratio	0.94(0.06)
Height (cm)	173.7 (6.9)
Weight (kg)	79.4 (11.7)
BMI (kg/m^2)	26.3 (3.5)
FEV_1 (L)	3.13 (0.68)
%Predicted FEV_1	91.9 (17.6)
FVC (L)	4.12 (0.82)
%Predicted FVC	96.9 (16.3)
FEV_1/FVC ratio	0.76 (0.1)

4.3.1.2 Valid and Invalid spirometry

There were no significant differences in body mass index, waist-hip ratio, waist circumference, height, weight, smoking history, and material conditions between the group of men with valid baseline spirometry and the group of men with invalid spirometry. The men with valid baseline spirometry were on average 0.6 years younger and had an average hip circumference 0.56cm smaller than the men with invalid spirometry (see table 4.2).

Table 4.2 Differences between the groups of men with valid and invalid spirometry at baseline

	Valid spirometry n=1779 Mean (SD)	Invalid spirometry n=966 Mean (SD)	p
Age (years)	54.56(2.85)	55.2(2.93)	<0.001 (t-test)
Hip circumference (cm)	96.7 (6.88)	97.26 (6.67)	0.04 (t-test)
>10 years of education %	47.5	41.5	0.02 (Chi-squared test)

4.3.1.3 Relationship between BMI, WHR and FEV_1

There was a clear inverse relationship between WHR and FEV_1 at baseline (see table 4.3). FEV_1 was on average 20 ml lower with each 0.01 increment in WHR ($p<0.001$). This association was slightly attenuated after adjustment for various covariates but was also significant when WHR was considered as a categorical variable ($p<0.0001$). In contrast, there was no evidence of a linear association between baseline BMI and FEV_1 ($p=0.1$). There was some evidence of a difference in mean baseline FEV_1 between BMI categories ($p<0.0001$). Men in both the underweight and obese categories had significantly lower mean values for baseline FEV_1 and baseline FVC than men who had a normal body mass index or were overweight.

Although waist circumference was not associated with baseline FEV_1 in the crude model, after adjustment for confounding factors (in particular, height), an association became apparent. FEV_1 was on average 4ml lower with each centimetre increase in waist circumference. There was evidence of a relationship between hip circumference and baseline FEV_1 in unadjusted data but after adjustment for confounding variables this association was not significant.

Table 4.3. Cross-sectional relationships between lung function and measures of body weight distribution, n=1779

Outcome	Characteristic	Crude		Adjusted*		
		Mean lung function outcome in ml Mean (SD)	p-value (trend across cats)	Difference in mean outcome (95% CI)	p-value (trend across cats)	R^2
Baseline FEV1 in ml	**BMI by category**					
	Underweight <18.5, n=11	2473(602)		-382 (-723,-40)		
	Healthy 18.5-25, n=642	3138(715)	**<0.0001**	0 (ref.cat.)	**0.002**	
	Overweight 25-30, n=914	3161(666)	(0.23)	70 (12,128)	(0.37)	0.30
	Obese > 30kg/m^2, n=212	2983(589)		-34 (-123,56)		
	WHR by category					
	Healthy <0.90, n=388	3262 (657)	**<0.0001**	0 (ref. cat.)	**<0.0001**	
	0.9-0.94, n=487	3213 (691)		-2 (-78,75)		
	0.94-0.97, n=410	3084 (658)	**(<0.0001)**	-70 (-150,10)	**(<0.0001)**	0.30
	>0.97, n=494	2972 (679)		-145 (-222,-68)		
	BMI (kg/m2)	-8 (-17, 1) †	0.10	0 (-8, 8) †	0.99	0.30
	Waist-hip ratio	-20 (-26,-14) †	**<0.001**	-10 (-16, -6) †	**<0.0001**	0.31
	Waist circumference (cm)	0 (-4, 3) †	0.89	-4 (-7, -1) †	**0.007**	0.30
	Hip circumference (cm)	13 (9, 18) †	**<0.001**	0 (-4, 4) †	0.87	0.30
Baseline FVC in ml	**BMI by category**					
	Underweight <18.5, n=11	4211(844)		-268 (-671,135)		
	Healthy 18.5-25, n=642	4128(806)	**<0.0001**	0 (Ref.cat.)	**<0.0001**	
	Overweight 25-30, n=914	3844(702)	**(<0.0001)**	-5 (-74,64)	(0.012)	0.33
	Obese > 30kg/m2, n=212	3614(684)		-196 (-301,-90)		
	WHR by category					
	Healthy <0.90, n=388	4308(835)		0 (Ref. cat.)		
	0.9-0.94, n=487	4262(814)	**<0.0001**	20 (-69, 109)	**<0.0001**	
	0.94-0.97, n=410	4074(756)	**(<0.0001)**	-109 (-203,-15)	**(<0.0001)**	0.34
	>0.97, n=494	3873(785)		-254 (-344,-164)		
	BMI (kg/m2)	-29 (-40, -18) †	**<0.0001**	-17 (-26, -8) †	**<0.0001**	0.33
	Waist-hip ratio	-31 (-37, -24) †	**<0.0001**	-19(-25,-13) †	**<0.0001**	0.34
	Waist circumference (cm)	-4 (-8, 0) †	0.07	-9 (-13,-6) †	**<0.0001**	0.34
	Hip circumference (cm)	13 (8, 19) †	**<0.0001**	-6 (-11, -1) †	**0.02**	0.33

*Adjusted for age, height, smoking history, educational group and material conditions.

† Increase in lung function outcome per unit increase in variable

4.3.1.4 Relationship between BMI, WHR and FVC

Table 4.3 also displays the coefficients for various cross-sectional models of baseline FVC. When investigated as a predictor variable in an adjusted model, each of the body weight variables (BMI, WHR, waist and hip circumference) demonstrated a significant inverse relationship with FVC.

When WHR and BMI were added as variables to the same linear model of initial FVC, the FVC showed a reduction of 18ml (95%CI -25, -12) for each 0.01 increment in WHR, ($p<0.0001$, R^2 0.34), while the effect of BMI was non-significant ($p=0.78$).

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4.3.2 Longitudinal analysis

4.3.2.1. Comparison of baseline values of those alive and dead at 10 year follow-up.

Of the 1779 men with valid baseline spirometry measurements, 144 died before rescreening. Men who survived to 10 years had higher baseline values of FEV_1, FVC and FEV_1/FVC ratio and a lower number of baseline pack years of smoking compared to those who died (see table 4.4). There was no significant difference in BMI or WHR between the two groups. An additional 496 men were not rescreened because they refused, were ill or were not able to be contacted, while the quality of 209 follow-up spirometry tests was insufficient or could not be validated.

Table 4.4 Comparison of baseline lung function and anthropometric measurements between those dead and alive at 10 year follow-up

	DEAD n=144 Mean(SD)	ALIVE n=1635 Mean(SD)	p
FEV_1 at baseline (L)	2.74 (0.77)	3.16 (0.66)	<0.0001
FVC at baseline (L)	3.77 (0.92)	4.15 (0.80)	<0.0001
%Predicted FEV_1	82.8 (21.5)	92.7 (17.0)	<0.0001
%Predicted FVC	90.7 (19.1)	97.5 (15.9)	<0.0001
FEV_1/FVC ratio at baseline	0.73 (0.12)	0.76 (0.09)	0.001
BMI kg/m^2	26.0 (3.9)	26.3 (3.47)	0.38
Waist-hip ratio	0.95 (0.06)	0.94 (0.05)	0.1
Waist circumference (cm)	91.2 (10.2)	91.2 (9.42)	0.93
Hip circumference (cm)	96.0 (7.3)	96.8 (6.84)	0.21
Pack years smoking (yrs)	34.6 (28.3)	23.8 (27.3)	<0.0001

4.3.2.2 Description of dataset

The following analyses relate to the 930 men who had 2 valid spirometry measurements (baseline and 10 year re-screening) (See table 4.5).

Table 4.5 Demographic, anthropometric and lung function data at baseline and 10 year follow-up for the 930 men with longitudinal lung function data

	Baseline	**10 yr Follow-up**
	Mean (SD)	Mean (SD)
Age (years)	54.4 (2.8)	64.1 (2.8)
BMI (kg/m2)	26.2 (3.2)	27.6 (3.6)
Waist circumference (cm)	90.9 (9.0)	95.6 (10.0)
Hip circumference (cm)	96.8 (6.3)	101.5 (6.7)
Waist-hip ratio	0.94 (0.05)	0.94 (0.06)
Baseline FEV_1 (L)	3.24 (0.64)	2.77 (0.61)
% predicted FEV_1	94.5 (16.3)	88.0 (17.5)
Baseline FVC (L)	4.24 (0.77)	3.66 (0.70)
% predicted FVC	98.9 (15.1)	90.8 (14.8)
FEV_1/FVC ratio	0.77 (0.09)	0.75 (0.07)
Smoking History	%	%
Never smoker	35.4	35.7
Former smoker	38.3	46.0
Current smoker	26.3	18.3
Social history		
>10 years in education	46.7	

There were no significant differences in waist circumference, hip circumference, WHR, BMI, height, weight, smoking history, educational group or material conditions between the 930 men with and the 1080 men without longitudinal lung function data. There was a significant difference between the two groups in age (the group with valid follow-up spirometry were younger by 1.1years, $p<0.001$).

Current smokers at re-screening had a mean FEV_1 decline over the 10 year follow-up period of 58ml per year (SD 33ml). Former smokers had a mean FEV_1 decline of 47 ml per year (SD 35ml) and never smokers had a mean FEV_1

decline of 46 ml (SD 36.5ml) per year over the ten year period. There was evidence of a difference in decline in lung function between the three smoking groups (ANOVA P-value=0.001). The time between baseline and follow-up averaged 9.72 years (SD 0.83yrs).

4.3.2.3 Baseline measures of distribution of body weight as a predictor of 10 year lung function as measured by FEV_1

Waist-hip ratio at baseline was associated with FEV_1 at 10 years follow-up. In the crude model there was a reduction of 22 ml in 10 year FEV_1 (95% CI -29, -15, p<0.001), for each 0.01 increment WHR at baseline. After adjustment for smoking history, age, height, educational group and material conditions, the pattern was similar - there was a 11 ml reduction in 10 year FEV_1 for each 0.01 increment in baseline WHR (95% CI -17, -4, p=0.0011, R^2 = 0.3).

Baseline BMI was associated with 10 year FEV_1 in the crude analysis with a 19ml reduction in 10 year FEV_1 per kg/m^2 increase in baseline BMI (95%CI -31, -7, p=0.003), however a significant relationship was not observed after adjustment for covariates (see table 4.6). There was evidence (p=0.003) of a difference in mean 10 year FEV_1 by baseline BMI category. In particular, men in the obese category at baseline had significantly lower 10 year FEV_1 levels than the men with normal BMI at baseline.

When both baseline WHR and baseline BMI were added to the model as continuous variables, the relationship between 10 year FEV_1 and baseline WHR was maintained but the relationship between 10 year FEV_1 and baseline BMI remained non-significant. When both baseline WHR and baseline BMI are fitted to the model as categorical variables, both baseline WHR (p=0.02) and baseline BMI (p=0.01) are significantly associated with 10 year FEV_1.

Table 4.6. Relationships between baseline BMI, WHR, WC and HC and 10 year FEV_1 and FVC in ml, n=930

Outcome	Baseline Characteristic	Crude		Adjusted*		
		Mean 10 yr lung function outcome in ml Mean (SD)	p-value (trend across cats)	Difference in mean 10 yr outcome (95% CI)	p-value (trend across cats)	R^2
10 year FEV_1 in ml	**Baseline BMI by category**					
	Underweight <18.5, n=2	1900 (919)		-329 (-1056, 398)		
	Healthy 18.5-25, n=339	2805(630)	**<0.0001**	0 (Ref.cat.)	**0.003**	0.3
	Overweight 25-30, n=491	2789 (609)	**(0.012)**	48 (-24, 12)	(0.22)	
	Obese > 30kg/m2 n=98	2553 (532)		-162 (-280, -44)		
	Baseline WHR by category					
	Healthy <0.90, n=211	2931 (636)	**<0.0001**	0 (Ref.cat)	**0.006**	0.30 1
	0.9-0.94, n=277	2834 (614)	**(<0.0001)**	-21 (-114, -62)	**(0.001)**	
	0.94-0.97, n=212	2702 (551)		-62 (-163, 40)		
	>0.97, n=230	2601 (606)		-162 (-261, 73)		
	BMI (kg/m2)	-19 (-31,-7) †	**0.003**	-10 (-21, 1) †	0.063	0.3
	Waist-hip ratio	-22 (-29,-15) †	**<0.0001**	-11 (-17, -4) †	**0.001**	0.3
	Waist circumference (cm)	-5 (-9, 0) †	0.29	-6 (-10,-2) †	**0.002**	0.3
	Hip circumference (cm)	7 (0,13) †	**0.04**	-5 (-10,1) †	0.2	0.3
10 year FVC in ml	**BMI by category**					
	Underweight <18.5, n=2	3000 (282)	**<0.0001**	-12 (-819, 794)	**<0.0001**	0.33
	Healthy 18.5-25, n=339	3777 (695)	**(<0.0001)**	0 (Ref.cat.)	**(<0.0001)**	
	Overweight 25-30, n=491	3643 (705)		-51 (-131, 29)		
	Obese > 30kg/m2, n=98	3360 (569)		-291 (-422, -161)		
	WHR by category					
	Healthy <0.90, n=211	3896 (698)	**<0.0001**	0 (Ref. cat)	**<0.0001**	
	0.9-0.94, n=277	3763 (673)	**(<0.0001)**	-56 (-159, 47)	**(<0.0001)**	
	0.94-0.97, n=212	3537 (626)		-182 (-294, -70)		
	>0.97, n=230	3437 (675)		-270 (-380, -161)		
	BMI (kg/m2)	-39 (-53, -25) †	**<0.0001**	-27 (-38, -15) †	**<0.0001**	0.33
	Waist-hip ratio	-31 (-39, -22) †	**<0.0001**	-18 (-25, -11) †	**<0.0001**	0.34
	Waist circumference (cm)	-9 (-14, -4) †	**<0.0001**	-12 (-16, -8) †	**<0.0001**	0.34
	Hip circumference (cm)	4 (-4, 11) †	0.32	-13 (-19,-6) †	**<0.0001**	0.33

*Adjusted for age, height and smoking history at follow-up and educational group and material conditions at baseline.

† Increase in lung function outcome per unit increase in variable

4.3.2.4 Baseline measures of distribution of body weight as a predictor of 10 year lung function as measured by FVC

Waist-hip ratio at baseline was associated with FVC at 10 years follow-up. In the crude model there was a reduction of 31 ml in 10 year FVC (95% CI -39,-22, p<0.001) for each 0.01 increment in WHR at baseline. After adjustment for smoking history, age, height, educational group and material conditions, the pattern was similar. There was an 18 ml reduction in 10 year FVC per 0.01 increment in baseline WHR (95% CI -25, -11, p<0.0001, R^2 = 0.34).

Baseline BMI was associated with 10 year FVC in the crude analysis with a 39ml reduction in 10 year FVC per kg/m^2 increase in baseline BMI (95%CI -53, -25, p<0.001). This relationship was attenuated to a 27ml reduction in 10 year FVC per kg/m^2 increase in baseline BMI (95%CI -38, -15, p<0.001) on adjustment for covariates. When both baseline WHR and baseline BMI were added to the model, the relationships were maintained (see table 4.7).

Table 4.7. Linear models of 10 year FEV_1 and FVC, coefficients for baseline WHR and BMI, n=930

	Crude		Adjusted*		
Baseline variable	Increase in 10 year FEV_1 per unit change in baseline variable ml (95%CI)	P	Increase in FEV_1 per unit change in baseline variable ml (95% CI)	P	R^2
BMI kg/m^2	2 (-13, 16)	0.81	-1 (-13, 12)	0.09	0.3
Waist-hip ratio	-23 (-31, -14)	**<0.0001**	-11 (-18, -3)	**0.006**	
	Increase in 10 year FVC per unit change in baseline variable ml (95%CI)	P	Increase in FVC per unit change in baseline variable ml (95% CI)	P	R^2
BMI kg/m^2	-15 (-32, 1)	0.065	-15 (-29, -2)	**0.03**	0.34
Waist-hip ratio	-26 (-35, -16)	**<0.0001**	-13 (-21, -4)	**0.003**	

*Adjusted for age, height and smoking history at follow-up and material conditions and educational group at baseline.

4.3.2.5 Longitudinal changes in BMI, waist-hip ratio and lung function

In the linear model (adjusted for covariates) there was an inverse relationship between change in BMI, change in WHR, change in waist circumference and both change in FEV_1 and FVC (see table 4.8). FEV_1 decline was 1ml per year greater per 0.01 change in WHR over the 10 year study period ($p<0.0001$). Rate of decline of FEV_1 increased by 2.5mls per year, per kg/m^2 increase in BMI over 10 years, ($p<0.0001$). Change in BMI and change in WHR were correlated (correlation coefficient $r = 0.345$, $p<0.0001$). There was no relationship between change in hip circumference and change in FEV_1.

The group of men whose BMI had increased the most over 10 years demonstrated the highest average FEV_1 decline per year over the same period. The relationship was linear across quintiles of BMI change ($p<0.001$). This pattern was also evident for the group of men which had the greatest increases in WHR (after adjustment for covariates).

When change in WHR over 10 years and change in BMI over 10 years were included in the model together as continuous variables and the model adjusted for covariates, both remained associated with change in FEV_1 ($p=0.008$ and 0.001 respectively) and with change in FVC ($p<0.0001$ for both). This pattern was also evident when both WHR and BMI were put into the model as categorical variables.

When both change in waist circumference and change in BMI were added to the adjusted model, only change in waist circumference remained associated with change in FEV_1 and change in FVC.

Table 4.8 Relationships between change in lung function and change in measures of body fat distribution over 10 years

Outcome	Characteristic	Crude		Adjusted*		
		Mean change in lung function outcome in ml per year Mean (SD)	p-value (trend across cats)	Difference in mean change in outcome in ml per year (95% CI)	p-value (trend across cats)	R^2
Change in FEV1 in ml	**Change in BMI by category n=186 per group**					
	Reduction <0.0	-41.5 (35.6)	**0.006 (<0.0001)**	0 (Ref. cat)	**<0.0001**	0.19
	Small increase 0.0 to 1.0	-46.6 (40.3)		-3.1 (-9.7, 3.5)	**(<0.0001)**	
	Moderate increase 1.0 to 1.7	-48.9 (33.3)		-5.7 (-12.4, 0.9)		
	Large increase 1.7 to 2.7	-50.8 (33.0)		-8.2 (-14.8, -1.6)		
	Greatest increase >2.7	-54.8 (32.9)		-15.6 (-22.3, -9)		
	Change in WHR by category n=186 per group					
	Moderate Reduction <-0.04	-48.5 (35.0)	0.08 (0.08)	0 (Ref.cat.)	**0.001**	0.19
	Small reduction -0.04 to 0.0	-46.1 (34.7)		-0.8 (-7.5, 5.8)	**(0.001)**	
	Small increase 0.0 to 0.02	-44.1 (32.6)		2.3 (-4.4, 9.1)		
	Moderate increase 0.02 to 0.04	-50.2 (33.8)		-5.0 (-11.8, 1.8)		
	Large increase >0.04	-53.7 (40.0)		-11.4 (-18.3, -4.5)		
	Change in BMI (kg/m2)	-2.8 (-4.0,-2.0) †	**<0.0001**	-2.5 (-3.6, -1.3) †	**<0.0001**	0.17
	Change in WHR	-0.4 (-0.9,0.4) †	0.08	-0.9 (-1.3, -0.4) †	**<0.0001**	0.17
	Change in Waist circumference (cm)	-0.4 (-0.8, -0.1) †	**0.02**	-0.6 (-1.0, -0.3) †	**<0.0001**	0.17
	Change in Hip circumference (cm)	-0.3 (-0.8, 0.2) †	0.19	-0.3 (-0.8, 0.2) †	0.21	0.16
Change in FVC in ml	**Change in BMI by category**					
	Reduction <0.0	-45.8 (45.1)	**<0.0001 (<0.0001)**	0 (Ref. cat.)	**<0.0001 (<0.0001)**	0.29
	Small increase 0.0 to 1.0	-53.9 (49.2)		-4.1 (-12.4, 4.3)		
	Moderate increase 1.0 to 1.7	-57.1 (50.7)		-9.0 (-17.4, -0.6)		
	Large increase 1.7 to 2.7	-63.5 (45.5)		-15.8 (-24.1, -7.5)		
	Greatest increase >2.7	-73.8 (42.8)		-28.2(-36.6, -19.9)		
	Change in WHR by category					
	Moderate Reduction <-0.04	-57.5 (44.3)	**0.001 (0.001)**	0 (Ref. cat)	**<0.0001 (<0.0001)**	0.29
	Small reduction -0.04 to 0.0	-49.5 (48.8)		1.0 (-7.4, 9.4)		
	Small increase 0.0 to 0.02	-54.7 (47.4)		-3.1 (-11.6, 5.4)		
	Moderate increase 0.02 to 0.04	-63.6 (46.8)		-13.4 (-22, -4.8)		
	Large increase >0.04	-68.6 (48.7)		-21.5(-30.1, -12.8)		
	Change in BMI (kg/m2)	-5.2 (-6.9, -3.6) †	**<0.0001**	-4.8 (-6.2, -3.3) †	**<0.0001**	0.29
	Change in Waist-hip ratio	-1.0 (-1.6, -0.4) †	**0.001**	-1.8 (-2.3, -1.2) †	**<0.0001**	0.3
	Change in Waist circumference (cm)	-0.9 (-1.4, -0.4) †	**<0.0001**	-0.8 (-1.1, -0.5) †	**<0.0001**	0.3
	Change in Hip circumference (cm)	-0.5 (-1.2, 0.1) †	0.125	-0.9 (-1.4, -0.4) †	**<0.0001**	0.3

* Adjusted for age, height, material conditions and educational group, lung function at baseline and baseline value of the relevant anthropometric measurement and smoking history at follow-up.
† Increase in lung function outcome per unit increase in variable

4.4 Discussion

In this population of middle-aged Northern Irish men, we have demonstrated cross-sectional and longitudinal relationships between lung function, WHR, BMI and waist circumference. WHR, BMI and waist circumference are inversely associated with pulmonary function. FEV_1 decline is related to the change in distribution of weight as well as to weight gain.

4.4.1 Cross-sectional analysis

In the cross-sectional analysis of baseline data, FEV_1 was on average 20 ml lower with each 0.01 increment in WHR ($p<0.001$). BMI appeared to have a non-linear relationship with lung function, in that men in both the underweight and obese categories had lower mean FEV_1 levels than men in the overweight and healthy categories. Mean FEV_1 in the overweight category was higher than in the healthy category. The 11 men in the underweight category at baseline had the lowest mean FEV_1. Both WHR and BMI had inverse cross-sectional relationships with FVC. Waist circumference, which was highly correlated with WHR ($r=0.74$) as expected, was also inversely related to FEV_1 and FVC in the adjusted model. It is interesting to note that at baseline 339/930 (36.5%) of the men had a 'healthy range' BMI (18.5–25 kg/m^2) but only 211/930 (22.7%) of the men had an 'acceptable' waist-hip ratio of <0.9 (Welborn, Dhaliwal & Bennett 2003). This means that 128 of the men who were classed as having a 'healthy' BMI did not have an 'acceptable' WHR. This suggests that measuring BMI alone could be misleading.

4.4.2 Longitudinal analysis

Data from 930 men were examined in the longitudinal analysis. Baseline WHR was associated with FEV_1 at 10 year follow-up. Baseline BMI, however, was not related to 10 year FEV_1 in the adjusted model, except for those in the obese

category. Both baseline WHR and baseline BMI were associated with FVC at 10 year follow-up. In this population of Northern Irish men, WHR at baseline is has a more linear association with FEV_1 and FVC at 10 year follow-up than BMI.

This study demonstrates a positive longitudinal association between weight gain and loss of lung function in this population of middle-aged men. This relationship has been found in other studies of younger men and women with the greater effect being in men (Thyagarajan et al. 2008, Chen, Horne & Dosman 1993, Pistelli et al. 2008). The finding of a positive longitudinal association between increase in WHR and loss of lung function is less well-described and adds to the importance of weight loss advice, particularly when the WHR is high.

The relationship between WHR and mortality has recently been described (Pischon et al. 2008). After adjustment for confounders including BMI, mortality risk rose with increasing waist circumference and waist-hip ratio. In particular, a 2-inch increase in waist circumference raised the risk for death by 1.17 among men and 1.13 among women, and a 0.1 unit increase in waist-to-hip ratio raised mortality risk by 1.34 in men and 1.24 in women.

4.4.3 Limitations of study

It is reassuring that men with valid spirometry measurements were comparable at baseline to those without valid measurements in terms of BMI, WHR, smoking history and material conditions. However, given that over 600 men at follow-up either died, were too ill, or refused examination, we must consider how their omission might bias our conclusions. It is more likely that these men would have been among those who lost relatively more respiratory function, but the magnitude of the relationship between change in WHR and change in respiratory

function would likely depend on the nature of any intercurrent illness. Either that illness or the mens' behavioural response might have caused weight loss. In that event, we feel it is less likely that we would have over-estimated the association.

4.4.4 Obesity

The men included in this study were recruited from the workplace and are therefore essentially a ‘healthy’ cohort. Weight gain has a greater potential impact on those who already have a respiratory illness. Obesity may increase the prevalence, incidence and severity of asthma, while weight loss in obese individuals improves asthma outcomes (Akerman, Calacanis & Madsen 2004). Obesity-related systemic inflammation and asthma are both associated with raised levels of similar cytokines (e.g. tumour necrosis factor α and interleukin 6). There has been a suggestion that the ‘cytokine environment’ of obesity may modify the therapeutic response to glucocorticoids (Sin, Sutherland 2008). Obesity is also the major risk factor for obstructive sleep apnoea (OSA) (Resta et al. 2001). Individuals with OSA are at greater risk of hypertension, stroke and other cardiovascular diseases (McClean et al. 2008).

Obesity has multiple detrimental effects on the respiratory system. Weight loss can reverse many of these problems. Challenges in achieving weight loss include the genetic predisposition to weight gain, high fat and sugar diets and reduced physical activity. Our ‘obesogenic’ environment needs to change: processed foods are cheaper and more convenient and more people live in urban areas and travel by car.

The NICE guidelines on obesity emphasise that energy intake from food should not exceed the energy expended through everyday activities and exercise (National Institute for Health and Clinical Excellence December 2006). National

policies are required to provide more opportunities for physical activity and greater accessibility of healthy foods. All sections of society need to be involved – media, private businesses, food producers, supermarkets and government sectors. Local authorities are encouraged to provide play areas, cycling and walking routes. Local shops should be advised regarding the promotion of healthy foods and drinks. Schools and nurseries need to help to develop life-long healthy eating and physical activity patterns (National Institute for Health and Clinical Excellence December 2006).

4.4.5 Conclusion

This study supports the use of WHR in addition to BMI in assessing potential lung function decline. WHR is a simple well-standardized measurement which should be easy to include in population studies (Harik-Khan, Wise & Fleg 2001) and in clinical assessments and may improve interpretation of longitudinal changes in lung function.

5.0 Inflammation and Lung function

5.0 INFLAMMATION AND LUNG FUNCTION

5.1 Introduction

5.1.1 C-Reactive protein - background

CRP is a sensitive biomarker, the levels of which are raised in most conditions associated with infection, inflammation or tissue damage (Hurst et al. 2006, Pepys, Hirschfield 2003) for example, bacterial pneumonia, rheumatoid arthritis, Crohn's disease and neoplasic conditions. In the respiratory system, CRP has protective functions in innate immune responses against bacteria (Mold, Rodic-Polic & Du Clos 2002).There is evidence that systemic inflammation may also be related to reduced pulmonary function (Kony et al. 2004). High levels of CRP are a predictor of hospitalisation and mortality in patients with chronic respiratory failure (Cano et al. 2004).

5.1.2 Cross-sectional associations

Cross-sectional studies have previously shown an inverse relationship between CRP and lung function (Kony et al. 2004, Fogarty et al. 2007, Mendall et al. 1996), even in subjects without pulmonary disease and in never smokers (Aronson et al. 2006). Increased CRP levels have also been associated with bronchial hyperresponsiveness (Kony et al. 2004). CRP is increased in those with Chronic Obstructive Pulmonary Disease (COPD) (Barnes, Shapiro & Pauwels 2003, Gan et al. 2004) and is a marker for impaired energy metabolism, reduced functional capacity and distress due to respiratory symptoms in these patients (Broekhuizen et al. 2006, Yende et al. 2006). This suggests that disease within the lung may provoke a systemic inflammatory response (Hancox et al. 2007). The lungs are a primary defence organ against toxins and pollutants (Hancox et al. 2007) and the abundant inflammatory macrophages in the lung

may be a source of a low grade systemic inflammation in the absence of overt pulmonary disease (Aronson et al. 2006).

5.1.3 Longitudinal associations

Longitudinal studies of the relationship between CRP and lung function have provided inconsistent outcomes. A 9 year longitudinal study of randomly selected adults aged 18-70 years indicated an inverse cross-sectional relationship between CRP and lung function but concluded that serum CRP was unrelated to subsequent lung function decline in that population (Fogarty et al. 2007). Shaaban et al assessed longitudinal relationships between CRP and FEV_1 over 8.5 years in 531 subjects aged 37+/- 7 years and reported that increases in CRP levels over time were associated with a steeper FEV_1 decline (Shaaban et al. 2006). This study, however, did not adjust for social class or occupation. Higashimoto et al reported that CRP is a good predictor of rapid decline on FEV_1 in COPD patients (Higashimoto et al. 2009). Higher levels of CRP in young adults have also been associated with subsequent decline in lung function (Rasmussen et al. 2009). In this chapter I set out to further examine the relationship between changes in CRP and pulmonary function decline over time.

5.1.4 Hypotheses

a) Plasma CRP levels are inversely associated with lung function (FEV1 and FVC). Small increases in CRP, within the 'normal' range, will be associated with accelerated lung function decline.

b) Plasma CRP levels are related to smoking history, BMI and WHR.

5.1.5 Aims

a) Examine the cross-sectional relationship between plasma CRP levels and lung function.

b) Examine the relationship between 10 year change in plasma CRP and 10 year change in lung function.

c) Examine the relationship between plasma CRP levels, smoking habit, BMI and WHR

5.2 Methods

5.2.1 Participants

2745 men were recruited into the Belfast cohort of the PRIME study as described in chapter 1.1.

5.2.2 Data Collection

Each subject completed a questionnaire on demographics, socio-economic factors and dietary habits. Anthropometric measurements included height, weight, and waist and hip circumferences. Only the Belfast cohort had lung function measurements by spirometry. 2010 of the original cohort were re-screened 10 years later. This involved a further questionnaire, anthropometric measurements, spirometry and venous blood sample. Full details are described in chapter 2.0.

Plasma levels of ultra sensitive C-Reactive Protein (CRP) were measured on an automated random access Clinical Chemistry Analyser Ilab 600 (Instrumentation Laboratories) using the Quantex Biokit CRP ultrasensitive assay (see chapter 2.7).

5.2.3 Data analysis

Data analysis was carried out using SPSS (Version 15). CRP values were not normally distributed (skewed to the right), therefore the values were log-transformed for parametric testing. The values were not log-transformed for non-parametric tests or for comparing categories. Men with CRP values >10mg/L were excluded to avoid bias from intercurrent infection. Cross-sectional associations between CRP and FEV_1 were analysed by linear regression using absolute values of FEV_1 in ml as the dependent variable and CRP as the main predictor with adjustment for height, age, smoking history, educational group and material conditions. Longitudinal associations between annual change in CRP in mg/L/yr and annual change in FEV_1 ml/yr were analysed by linear regression using absolute values of change in FEV_1 in ml per yr as the dependent variable and change in CRP in mg/L/yr as the main predictor with adjustment for baseline log_{10}CRP, FEV_1, height, age, smoking history, educational group and material conditions.

5.3 Results

5.3.1 Cross-sectional analysis of plasma CRP levels and lung function

5.3.1.1 Baseline cross-sectional analysis

2745 men were recruited into the Belfast cohort of the PRIME study from 1991 to 1993. When ATS/ERS criteria were applied to the spirometry traces, 1779 were valid. Of these subjects, 339 did not have an available plasma sample in storage. 52 men had a CRP-US of >10mg/l and were excluded. The baseline cross-sectional analysis was carried out on the 1388 subjects (50.6% of original cohort) who had both a valid spirometry trace and an available stored plasma sample.

There were no significant differences in body mass index, height, weight, social class or smoking status between those included and those excluded from the baseline cross-sectional study. The included group were slightly younger.

The 1388 men included in the study had a mean age of 54.5 (SD 2.9) years at baseline. 32.5% had never smoked, 30.9% were current smokers and 36.6% were former smokers. Mean pack-year history was 24.7 (SD 27.6) years. Mean FEV_1 was 3.12 (SD 0.68) L, mean %predicted FEV_1 was 91.5% (SD 17.4), mean FVC was 4.09 (SD 0.82) and mean %predicted FVC was 95.9 (SD 16.3). Mean FEV_1/FVC ratio was 0.77 (SD 0.9). Mean plasma CRP level was 1.94 mg/L (SD 1.6).

Figure 5.1 Distribution of C-Reactive Protein levels at baseline in the 1388 men with a valid spirometry trace and stored plasma sample (52 men with levels >10mg/L excluded)

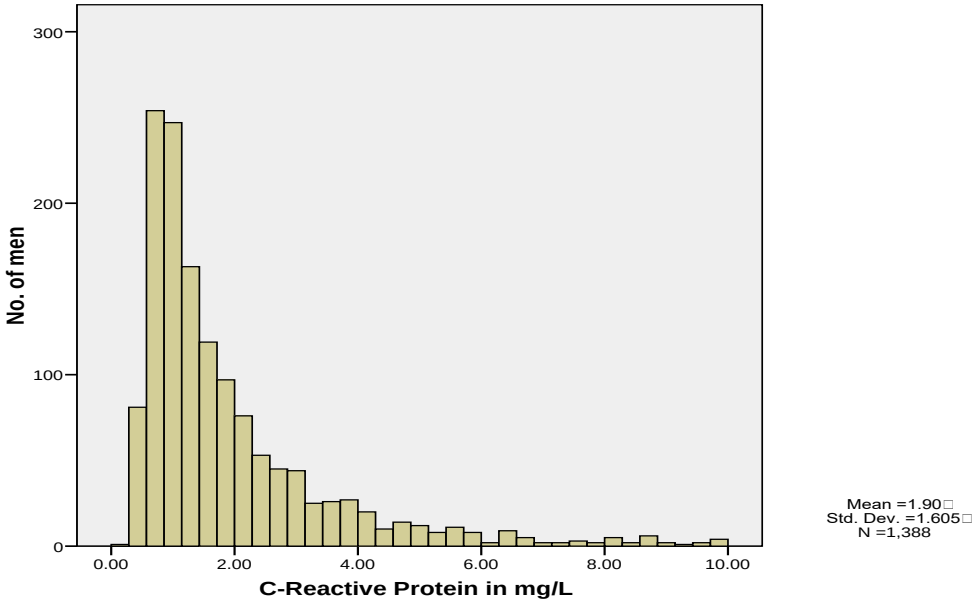
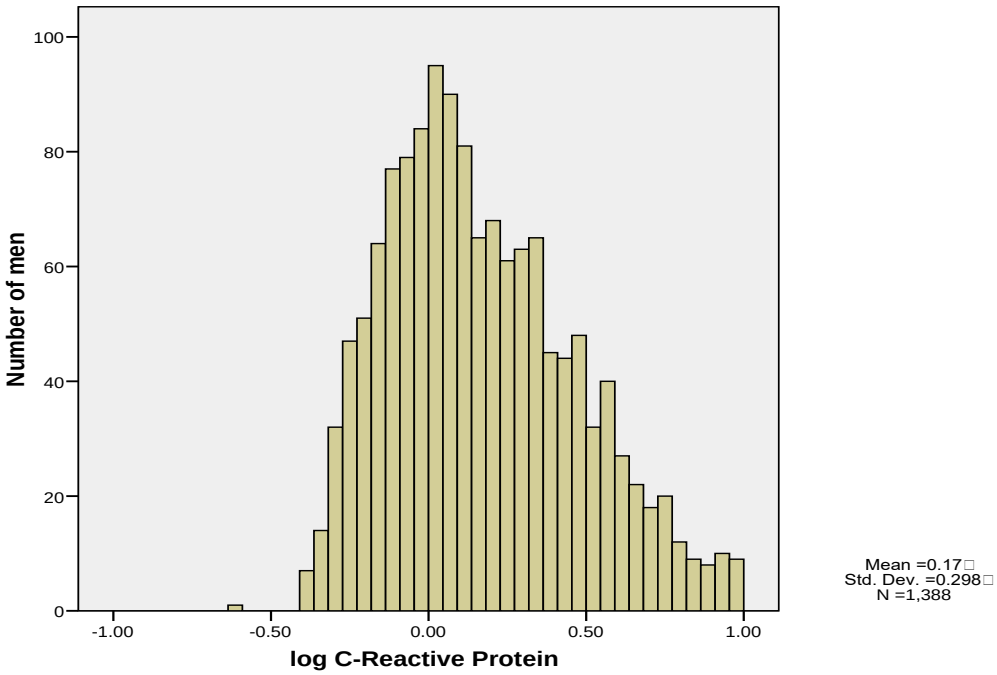


Figure 5.2 Distribution of log C-Reactive Protein levels at baseline in the 1388 men with a valid spirometry trace and stored plasma sample (52 men with levels >10mg/L excluded)



In the baseline cross-sectional analysis there was a strong negative relationship between CRP and FEV_1. FEV_1 was on average 598ml lower per tenfold increase in CRP (95% CI 483, 712, p<0.0001). For example, on average, a man in this study with a plasma C-Reactive Protein level of 5.0 mg/L would be expected to have an FEV_1 598ml lower than a man with a C-Reactive Protein level of 0.5mg/L. (Note that when the 560 men with 'WIDE' spirometry were included i.e. the two largest FEV_1 or FVC values greater than 150ml of each other, the results were similar: FEV_1 was on average 494 ml lower per tenfold increase in CRP (95% CI 417, 573, p<0.0001).)

After adjustment for baseline smoking group, BMI, age, height, educational group, material conditions and waist-hip ratio, FEV_1 was on average 289 ml lower per tenfold increase in CRP (95%CI 184, 393, p<0.0001).

FVC was on average 739ml lower per tenfold increase in CRP (95% CI -878, -599, p<0.0001). After adjustment for covariates, FVC on average 389 ml lower per tenfold increase in CRP (95%CI 262, 516, p<0.0001).

When the CRP measurements were split into fourths, similar results were obtained. The fourth of men with the highest CRP values had on average an FEV_1 231 ml lower than the fourth of men with the lowest CRP measurements (see figure 5.3).

Figure 5.3 Relationship between CRP in fourths and baseline FEV_1

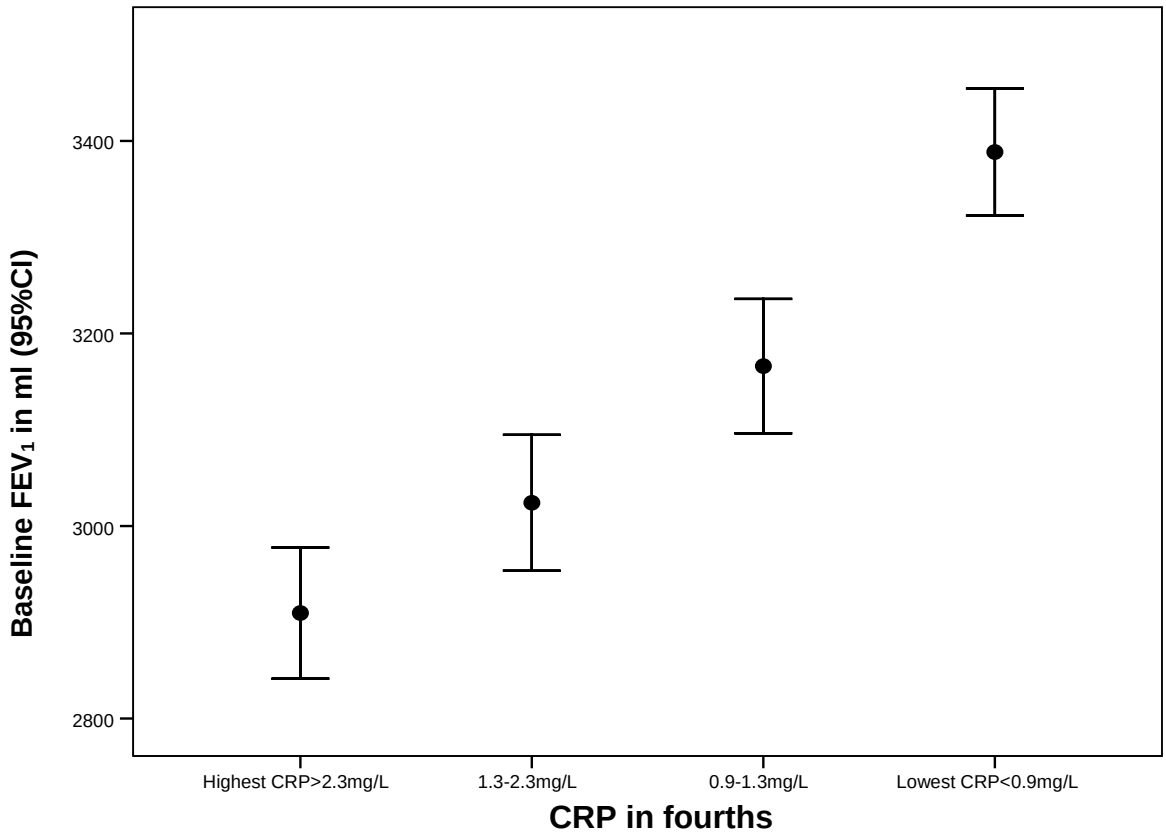


Table 5.1 Relationship between CRP, FEV_1 and FVC at baseline

Outcome	Characteristic	Crude		Adjusted*		
		Mean lung function outcome in ml Mean (SD)	p-value (trend across cats)	Difference in mean outcome (95% CI)	p-value (trend across cats)	R^2
Baseline FEV1 in ml	**CRP-US by category**					
	Lowest <0.9mg/l, n=347	3389 (624)		0 (ref. cat)		
	0.9-1.3mg/l, n=347	3164 (670)	<0.0001	-127 (-211,-43)	<0.0001	0.35
	1.3-2.3mg/l, n=348	3024 (666)		-175 (-261, -89)		
	Highest >2.3mg/l, n=346	2907 (656)	(<0.0001)	-231 (-320, -141)	(<0.0001)	
	Log_{10}CRP	-598 (-712,-483) †	<0.0001	-289(-393,-184) †	<0.0001	0.35
Baseline FVC in ml	**CRP-US by category**					
	Lowest <0.9mg/l, n=328	4415 (769)	<0.0001	0 (ref.cat.)	<0.0001	0.36
	0.9-1.4mg/l, n=312	4155 (784)		-136 (-237, -36)		
	1.4-2.5mg/l, n=316	3956 (813)	(<0.0001)	-235 (-339, -132)	(<0.0001)	
	>2.5mg/l, n=299	3815 (792)		-311 (-418, -203)		
	Log_{10}CRP	-739 (-878,-599) †	<0.0001	-389(-516, -262) †	<0.0001	0.36

*Adjusted for age, height, smoking history, body mass index, waist-hip ratio, educational group and material conditions at baseline.
† Increase in lung function outcome per unit ten-fold increase in CRP (based upon log_{10}CRP)

5.3.1.2 10 year follow-up cross-sectional analysis

Of the 2745 men originally included in the study, 232 died before rescreening and an additional 503 were not rescreened because they refused, were ill or were not able to be contacted.

Of the 2010 rescreened, 1229 had valid follow-up spirometry and CRP measurements. Cross-sectional analysis of these men confirmed the inverse relationships found in the baseline study. FEV_1 was on average 648 ml lower per tenfold increase in CRP (95% CI 529, 767, p<0.0001). After adjustment for smoking group, BMI, age, height, educational group, material conditions and waist-hip ratio, FEV_1 was on average 394 ml lower per tenfold increase in CRP (95% CI 284, 505, p<0.0001, R^2 0.34).

FVC was on average 742 ml lower per tenfold increase in CRP (95% CI 606, 877, p<0.0001). After adjustment for co-variates, FVC was on average 432 ml lower per tenfold increase in CRP (95%CI 312, 553, p<0.001, R^2 0.39).

5.3.2 Longitudinal analysis of relationship between CRP and lung function decline over 10 years

696 of the men had a valid spirometry trace and plasma sample at both timepoints. 49 men had a follow-up CRP of >10mg/L and were excluded. Longitudinal analysis was carried out on this cohort of 673 men.

Mean decline in FEV_1 over the 10 year period was 453.6ml (SD 336). Mean annual decline in FEV1 (+/- SD) was 47.2 +/- 35.0 ml. Mean annual decline in FVC was 53.8 (SD 48) ml. Mean change in CRP concentration over the 10 years of follow-up was 0.39 +/- 1.7 mg/L. Annual change in CRP was normally distributed and therefore did not need to be log-transformed for analysis. Mean follow-up time was 9.6 years (+/-0.75).

There was an association between annual change in CRP and annual decline in FEV_1 in the adjusted model (see table 5.2). Specifically, there was a reduction in FEV_1 of 14 ml/per year over the 10 years for each mg/l per year increase in CRP over the 10 years. After adjustment for baseline FEV_1, log_{10}CRP, BMI, WHR, age, height, educational group and material conditions and for smoking history at follow-up, annual decline in FEV_1 was accelerated on average by 26.8 ml per mg/l/yr change in CRP (95%CI 12, 42, p<0.0001).

The subjects were split into fourths according to change in CRP. After adjustment for covariates, subjects in the group with the largest annual increase in CRP had an annual FEV_1 decline which was on average 11mls greater than those with a reduction in CRP over time.

Table 5.2: Relationship between annual change in CRP and annual change in lung function

Outcome	Characteristic	Crude		Adjusted*		
		Mean change in lung function outcome in ml per year Mean (SD)	p-value (trend across cats)	Difference in mean change in outcome in ml per year (95% CI)	p-value (trend across cats)	R^2
Change in FEV1 per year in ml	**Change in CRP per year by category**					
	***Reduction** in CRP >-0.01mg/L/yr , n=168*	-44.8 (30)	0.095	0 (Ref. cat.)	0.008	0.16
	***No change** -0.01 to0.03mg/L/yr, n=168*	-46.2 (35)		-1.7 (-10, 7)		
	***Small increase**,0.02 to 0.09mg/L/yr n=169*	-44.7 (38)	(0.06)	-2.3 (-10, 6)	(0.002)	
	***Greatest increase**, >0.09mg/L/yr. n=168*	-53.0 (36)		-11.4 (-19,-4)		
	Change in CRP per year (mg/l/yr)	-14 (-28.6, 0.6) †	0.06	-26.8 (-42,-12) †	0.001	0.16
Change in FVC per year in ml	**Change in CRP per year by category**					
	***Reduction** in CRP >-0.01mg/L/yr , n=168*	-52.2 (41)	0.02	0 (Ref.cat.)	0.001	0.27
	***No change** -0.01 to0.03mg/L/yr, n=168*	-53.9 (49)		-3.2 (-14, 7)		
	***Small increase**,0.02 to 0.09mg/L/yr n=169*	-46.6 (53)	(0.15)	-1.3 (-12, 9)	(0.001)	
	***Greatest increase**, >0.09mg/L/yr. n=168*	-62.7 (47)		-16.6 (-26, -7)		
	Change in CRP per year (mg/l/yr)	-19.4 (-39, 0.6) †	0.06	-40 (-59, -21) †	<0.0001	0.27

* Adjusted for baseline FEV1 or FVC, logCRP, BMI, WHR, age, height, educational group and material conditions and for smoking history at follow-up

† Increase in lung function outcome per unit increase in CRP per year

The association between annual change in FVC and annual change in CRP was similar. After adjustment for age, height, WHR, BMI, FVC, $\log_{10}$CRP, smoking history, material conditions at baseline and smoking history at follow-up, FVC decline per year was on average 40 ml greater per annual mg/L change in CRP (95% CI 21 to 59, p<0.0001).

There was no significant association between baseline plasma $\log_{10}$CRP levels and annual decline in FEV_1 (p=0.7) or FVC (p=0.8). There was also no significant

association between the mean of the baseline and follow-up log_{10}CRP measurements and 10 year decline in FEV_1 or FVC (p=0.3).

Baseline log_{10}CRP levels were related to 10 year FEV_1. 10 year FEV_1 was on average 587ml lower per tenfold increase in baseline CRP (95%CI 424 to 750, p<0.0001) but this relationship became non-significant after adjustment for covariates.

5.3.3 Smoking and plasma CRP levels

At baseline, current smokers had a higher baseline CRP (p<0.0001), lower body mass index (BMI), lower FEV_1 and FVC and a lower FEV_1/FVC ratio than former and never smokers (see table 5.3).

Table 5.3 Baseline lung function, anthropometric data and CRP by smoking status

Smoking status	Never n=451 Mean (SD)	Former n=508 Mean (SD)	Current n=429 Mean (SD)	ANOVA p value
Age in years	54.4(2.9)	54.7 (3.0)	54.4 (2.9)	0.14
Best FEV_1(L)	3.32(0.6)	3.12 (0.7)	2.88 (0.7)	<0.0001
%predicted FEV_1	97.2(16.4)	91.6 (17.2)	85.5 (16.6)	<0.0001
Best FVC in Litres	4.22 (0.8)	4.10 (0.8)	3.92 (0.8)	<0.0001
%predicted FVC	98.7 (16.2)	95.9 (15.9)	92.9 (16.3)	<0.0001
BMI (kg/m^2)	26.3 (3.3)	27.0 (3.6)	25.6 (3.5)	<0.0001
FEV1/FVC Ratio	0.79 (0.8)	0.76 (0.97)	0.74 (0.98)	<0.0001
Waist-hip ratio	0.93 (0.05)	0.95 (0.06)	0.94 (0.06)	0.001
	Median (IQ range)	Median (IQ range)	Median (IQ range)	Kruskal-Wallis p value
CRP (mg/L)	1.08 (0.8-1.8)	1.32 (0.9-2.4)	1.72 (1.1-2.9)	<0.0001

When results were stratified according to smoking status, the inverse cross-sectional relationship between CRP and lung function was found to be significant in each of the smoking groups independently (never smokers, former smokers and current smokers) (see table 5.4).

Table 5.4. Cross-sectional relationship between CRP and lung function stratified by smoking status

Outcome	Characteristic	Crude		Adjusted*		
		Mean lung function outcome in ml Mean (SD)	p-value (trend across cats)	Difference in mean outcome (95% CI)	p-value (trend across cats)	R^2
Baseline FEV1 in ml	**Never smoker**					
	Log_{10}CRP	-615 (-820,-411) †	<0.0001	-398(-589,-207) †	<0.0001	0.33
	Former smoker					
	Log_{10}CRP	-455 (-649,-261) †	<0.0001	-195(-375,-15) †	0.03	0.29
	Current smoker					
	Log_{10}CRP	-404 (-608,-199) †	<0.0001	-289(-465,-113) †	0.001	0.35
Baseline FVC in ml	**Never smoker**					
	Log_{10}CRP	-802(-1064,-540) †	<0.0001	-474(-712,-236) †	<0.0001	0.37
	Former smoker					
	Log_{10}CRP	-679 (-910,-449) †	<0.0001	-303(-510,-95) †	0.004	0.32
	Current smoker					
	Log_{10}CRP	-576 (-833,-319) †	<0.0001	-402(-623,-181) †	<0.0001	0.36

*Adjusted for age, height, smoking history, body mass index, waist-hip ratio, educational group and material conditions at baseline.
† Increase in lung function outcome per unit ten-fold increase in CRP (based upon log_{10}CRP)

5.3.4 Obstructive spirometry and CRP

19.5 % of the men had obstructive spirometry (FEV_1/FVC ratio <0.7) at baseline. These men tended to be older with lower BMI measurements. CRP was higher in the men with obstructive spirometry (p=0.048). See table 5.5.

Table 5.5 Baseline lung function, anthropometric data and CRP by FEV_1/FVC ratio

	Obstructive spirometry n=270 Mean (SD)	Non-obstructive spirometry n=1118 Mean (SD)	p value from t test
Age (years)	55.1 (2.9)	54.4 (2.9)	<0.0001
Best FEV_1 (L)	2.58 (0.7)	3.25 (0.6)	<0.0001
%predicted FEV_1	75.6 (17.7)	95.4 (14.9)	<0.0001
Best FVC (L)	4.08 (0.9)	4.07 (0.8)	0.84
%predicted FVC	96.0 (18.1)	95.9 (15.8)	0.89
BMI (kg/m^2)	25.4 (3.8)	26.5 (3.5)	<0.0001
FEV_1/FVC Ratio	0.62 (0.1)	0.8 (0.1)	<0.0001
Waist-hip ratio	0.94 (0.06)	0.94 (0.06)	0.26
	Median (IQ range)	Median (IQ range)	
CRP (mg/L)	1.36 (0.94-2.44)	1.30 (0.86-2.28)	0.048*
Smoking history			
Never	18%	36%	
Former	34%	37%	<0.0001**
Current	48%	27%	

* from mann-whitney U –test. **from chi-squared test

The inverse relationship between CRP and lung function was significant in the non-obstructive spirometry group and in the obstructive spirometry group (see table 5.6).

Table 5.6. Cross-sectional relationship between CRP and lung function stratified by FEV_1 :FVC ratio

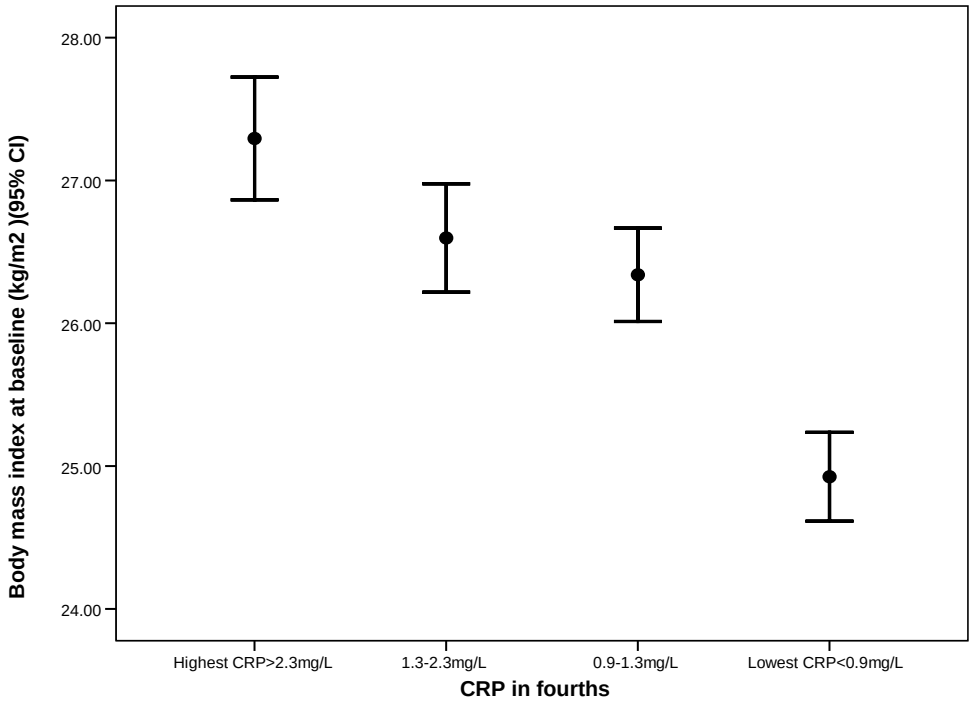
Outcome	Characteristic	Crude		Adjusted*		
		Mean lung function outcome in ml Mean (SD)	p-value (trend across cats)	Difference in mean outcome (95% CI)	p-value (trend across cats)	R^2
Baseline FEV1 in ml	**Obstructive spirometry**					
	Log_{10}CRP	-394 (-649,-139) †	0.003	-266(-498,-34) †	0.025	0.30
	Non-obstructive spirometry					
	Log_{10}CRP	-592 (-708,-476) †	<0.0001	-284(-387,-181) †	<0.0001	0.41
Baseline FVC in ml	**Obstructive spirometry**					
	Log_{10}CRP	-700(-1047,-353) †	<0.0001	-462(-763,-162) †	0.003	0.38
	Non-obstructive spirometry					
	Log_{10}CRP	-752 (-904,-600) †	<0.0001	-388(-527,-249) †	<0.0001	0.37

*Adjusted for age, height, smoking history, body mass index, waist-hip ratio, educational group and material conditions at baseline.
† Increase in lung function outcome per unit ten-fold increase in CRP (based upon log_{10}CRP)

5.3.5 CRP, body mass index (BMI) and waist-hip ratio (WHR)

CRP levels were positively correlated with both WHR (r=0.25 , p=0.01) and BMI (r=0.21, p=0.01).

Figure 5.4 Relationship between baseline mean BMI and CRP



There was a significant positive relationship between baseline BMI and CRP in this population in both the crude and adjusted models (see table 5.7).

Table 5.7 Cross-sectional relationship between CRP and BMI

Characteristic	Crude		Adjusted*		
	Mean BMI in kg/m^2 Mean (SD)	**p-value (trend across cats)**	**Difference in mean BMI kg/m^2(95% CI)**	**p-value (trend across cats)**	**R^2**
CRP-US by category					
Lowest <0.9mg/l, n=347	27.3 (4.1)		0 (ref. cat)		
0.9-1.3mg/l, n=347	26.6 (3.6)	<0.0001	1.5 (1.0, 2.0)	<0.0001	0.11
1.3-2.3mg/l, n=348	26.3 (3.1)		1.9 (1.4, 2.4)		
Highest >2.3mg/l, n=346	24.9 (2.9)	(<0.0001)	2.6 (2.1, 3.1)	(<0.0001)	
Log_{10}CRP	2.49 (1.87,3.1) †	<0.0001	2.8(2.2,3.4) †	<0.0001	0.09

*Adjusted for age, height, smoking history, educational group and material conditions at baseline.
† Increase in BMI per unit ten-fold increase in CRP (based upon log_{10}CRP)

There was no significant relationship between change in CRP and change in BMI over 10 years.

There was a significant positive relationship between baseline WHR and baseline plasma CRP level in this population in both the crude and adjusted models (see table 5.8). There was no significant relationship between change in CRP and change in WHR over 10 years.

Table 5.8. Cross-sectional relationship between CRP and WHR

Characteristic	Crude		Adjusted*		
	Mean WHR Mean (SD)	**p-value (trend across cats)**	**Difference in mean WHR (95% CI)**	**p-value (trend across cats)**	**R^2**
CRP-US by category					
Lowest <0.9mg/l, n=347	0.92 (0.05)		0 (ref. cat)		
0.9-1.3mg/l, n=347	0.94 (0.05)	<0.0001	0.019 (0.01, 0.03)	<0.0001	0.1
1.3-2.3mg/l, n=348	0.95 (0.05)		0.025(0.02, 0.03)		
Highest >2.3mg/l, n=346	0.96 (0.06)	(<0.0001)	0.036 (0.03, 0.04)	(<0.0001)	
$Log_{10}CRP$	0.047(0.04, 0.06)†	<0.0001	0.041 (0.03, 0.05) †	<0.0001	0.09

*Adjusted for age, height, smoking history, educational group and material conditions at baseline.
† Increase in WHR per unit ten-fold increase in CRP (based upon $log_{10}CRP$)

5.3.6 Baseline CRP and Death during follow-up

Of the 2160 men who had a valid plasma CRP level at baseline, 1976 were alive at 10 years. In this group the mean baseline CRP was 2.6mg/L (SD 5.8). 184 of the men died over the subsequent 10 years of follow-up. This group had a baseline CRP of 3.9 mg/L (SD 6.6). The difference between the groups was significant (p=0.005). After adjustment for baseline smoking history, age, body mass index, material conditions and alcohol intake, the difference between the groups remained significant (p=0.047).

5.4 Discussion

In this population-based study conducted in middle-aged men from Northern Ireland, lung function and plasma CRP concentrations were inversely related in both cross-sectional and longitudinal analyses. The presence of systemic inflammation was demonstrated, even in subjects with modest decreases in pulmonary function.

These associations were independent of potential confounding factors and were also observed when the analysis was stratified according to smoking status, even in never smokers.

There was an association between annual change in CRP and annual decline in FEV_1 and FVC in the adjusted model in this population. There was no significant association between baseline serum $\log_{10}$CRP levels and subsequent annual decline in FEV_1 (p=0.7) or FVC (p=0.8). This may reflect the low levels of variability in CRP responses in healthy middle-aged subjects (Shaaban et al. 2006). Also, analyses based on a single baseline measurement and the within-person variability of CRP will tend to underestimate the effect of CRP on subsequent pulmonary function decline (regression dilution bias) (Lewington et al. 2003).

5.4.1 Strengths of study

This study drew data from a large ethnically homogenous general population sample with high-quality, standardized measurements. The participants in the PRIME study were recruited from the workplace and are essentially a 'healthy cohort'. A highly sensitive assay was used for measuring CRP. Levels of inflammation which may play a role in cardiovascular risk may be associated with CRP levels not detected by standard CRP assays (Libby, Ridker & Maseri 2002).

Lung function was measured using the same equipment for all study members and the blood samples were assayed in the same laboratory. Spirometry traces which did not achieve the standard set by the ATS/ERS criteria were excluded (Miller et al. 2007).

The associations between CRP and BMI and smoking habits were consistent with previously published results (Shaaban et al. 2006, Lazarus, Sparrow & Weiss 1997). The distribution of plasma CRP levels and lung function results were similar to that described for similar age participants in other studies (Jiang et al. 2008).

The PRIME study team have published a nested case-control study which showed that after adjustment for traditional risk factors, myocardial infarction, but not angina was significantly associated with CRP, interleukin-6 and fibrinogen. This was felt to reflect the underlying inflammatory reaction located in the atherosclerotic plaque (Luc et al. 2003b). A further nested case-control study reported that plasma levels of CRP are useful as a risk marker for future coronary events if soluble forms of the intercellular adhesion molecule-1 (ICAM-1) are also high (Luc et al. 2003a).

5.4.2 Limitations of study

The PRIME study only contains data from middle-aged men in Northern Ireland so our findings cannot be generalised to include other groups, for example, women. CRP levels are reported to be consistently higher in women than men in some studies (Hersh et al. 2006, Hancox et al. 2007). Cross-sectional data is limited in that valid inferences regarding causal pathways cannot be drawn.

Spirometry was measured on two occasions, 10 years apart. It would be preferable to have had annual measurements of both spirometry and plasma CRP levels. Spirometry is dependent on the voluntary effort exerted in performing the manoeuvre and was a potential source of bias. However, reproducibility criteria were met by all of the spirometry traces included in the analysis and the strong independent relationship between pulmonary function and CRP suggests that such measurement error would lead to an underestimation of the true effect. We did not have reliable data for co-morbidities or medication. It would have been helpful to know if any of the men had a history of asthma or were on bronchodilators.

Plasma CRP is a measure of systemic inflammation rather than a direct measure of lung inflammation. CRP has a short half life in plasma (Vigushin, Pepys & Hawkins 1993) and it is therefore a transient marker of inflammation with substantial physiologic fluctuations (Muller, Tamm 2006). Unmeasured underlying conditions generating both high serum CRP concentrations and impaired pulmonary function via other mechanisms may also result in residual confounding. The inclusion of subjects with CRP concentrations>10mg/l had little or no effect on the reported associations.

This study was affected by survival bias; however, this would more likely lead to an underestimation of effect as the men who died had lower baseline FEV_1 levels than those who survived to follow-up.

5.4.3 Smoking and CRP

Current smokers had the highest plasma CRP levels in this population. This confirms findings from previous studies which have shown that cigarette smoking by itself leads to systemic inflammation (Hersh et al. 2006, Bazzano et al. 2003). Smoking causes systemic oxidative stress followed by low-grade inflammation and endothelial dysfunction. Elevated plasma levels of CRP in smokers are due in part to the direct chemical exposure of the lung to cigarette smoke which induces tissue damage (Yanbaeva et al. 2007). Other inflammatory cytokines such as TNF-α and IL-6 are elevated in smokers and these will stimulate further CRP production via the inflammatory cascade. Gan et al suggest an additive effect of active smoking and reduced FEV_1 on markers of systemic inflammation (Gan, Man & Sin 2005). The effects of smoking on inflammatory markers such as CRP can persist for many years (Lowe et al. 2001), however most of the adverse health effects of smoking are reversible.

5.4.4 CRP and Obstructive lung disease

We analysed participants with and without obstructive spirometry as we did not have robust data on co-morbidities such as COPD and asthma. Men with obstructive spirometry at baseline had significantly higher plasma CRP levels than those with non-obstructive spirometry. However, the inverse relationship between plasma CRP and FEV_1 was evident in both groups. This concurs with the findings of Yende et al who reported that participants with obstructive lung disease had higher CRP levels than those with normal spirometry. Plasma CRP levels have previously been found to be raised in COPD patients without clinically relevant IHD and independent of cigarette smoking (de Torres et al. 2006, Pinto-Plata et al. 2006). Broekhiuzen et al found that irrespective of FEV_1, COPD patients with a raised plasma level of CRP had more impaired energy metabolism, increased disability as defined by impaired exercise capacity, and

more distress due to respiratory symptoms than patients with normal CRP levels (Broekhuizen et al. 2006). CRP is a strong and independent predictor of future COPD outcomes in individuals with airway obstruction (Dahl et al. 2007). A study in patients with mild to moderate COPD showed an accelerated decline in lung function in those with higher baseline CRP levels (Man/Connett 2006).

5.4.5 CRP, BMI and WHR

In this population, plasma CRP levels were positively correlated with both WHR ($r=0.25$, $p=0.01$) and BMI ($r=0.21$, $p=0.01$) in the cross-sectional analysis.
In a recent study, obese (>30kg.m^2) COPD patients were 3.3 times more likely to have highly elevated CRP levels compared to those with normal weight and those with a low BMI (<21 kg/m^2) were 2 times more likely to have highly elevated CRP levels compared to normal weight peers (Breyer et al. 2009). Obesity is one of the five factors involved in the metabolic syndrome which is also associated with elevated CRP levels (Gonzalez et al. 2006). Inflammation and CRP have recently been linked to impaired insulin sensitivity and the development of dysglycaemic conditions such as incident type 2 diabetes (Gonzalez et al. 2006, Ndumele, Pradhan & Ridker 2006). A state of low-grade systemic inflammation has been found in normal BMI subjects who show subclinical insulin resistance (Bo et al. 2005).

5.4.6 CRP and Death during follow-up

We found that those who died during the study had had higher baseline CRP levels than those who survived to 10 year follow-up. We did not carry out a formal mortality analysis, however. CRP measurements have previously been shown to provide incremental prognostic information beyond that achieved by traditional markers of prognosis in patients with mild COPD (Man et al. 2006). It is also interesting that a study of cardiovascular risk stratification reported that middle-aged men with CRP levels >3.0mg/L were 4.1 to 5 times more likely to die of cardiovascular disease than men with CRP levels <1mg/L during the follow-up period of the study (Laaksonen et al. 2005).

5.4.7 Genetic influences

Genetic factors are likely to affect both plasma CRP levels and lung function (Hancox et al. 2007). Variation in CRP levels has been shown to have a significant genetic component in families from the general population (Pankow et al. 2001). It may be that individuals with an 'inherited' inflammatory genetic profile have a higher risk of developing conditions such as COPD if they are exposed to environmental factors such as smoking and pollution (Pinto-Plata et al. 2006).

5.4.8 Cause or consequence?

These results raise an important question. Are raised serum CRP levels the cause of, or a consequence of, reduced lung function?

Several possible mechanisms for the inverse association between systemic inflammation and reduced pulmonary function have been suggested in the literature. It may be that reduced lung function is responsible for increased systemic inflammation. Inflammatory mediators including CRP are released following damage to lung tissue. The lungs are a primary defence organ against

the myriad infectious agents, noxious gases and particulates which are inhaled during gas exchange and are therefore dependent on tightly regulated immunologic and inflammatory processes. Subjects with lower FEV_1 may have had higher exposure to tobacco smoke or environmental insults which lead to a subtle decline in lung function and, in parallel, induce a low-grade inflammatory response (Aronson et al. 2006, van Eeden et al. 2001). Pulmonary epithelial cells have been shown to express CRP (Gould, Weiser 2001), other inflammatory mediators and cytokines (Hancock et al. 1998) including IL-6 (Gould, Weiser 2001, Van Snick 1990) in response to these inhaled insults. IL-6 production is increased in inflammatory lung conditions associated with reduced FEV_1 such as COPD. This IL-6 circulates in the bloodstream and thereby stimulates the production of CRP and other inflammatory mediators in the liver which can in turn lead to further activation of pulmonary inflammation. In our population, baseline plasma CRP levels did not predict changes in lung function over the following 10 years but there were associations between decline in FEV_1 and rising CRP levels. This adds weight to the suggestion that systemic inflammation may be a consequence of a decline in lung function rather than the cause of the decline (Hancox et al. 2007).

High levels of CRP in the peripheral circulation may be a cause rather than a consequence of poor lung function. Systemic inflammation may damage lung tissue, leading to reduced lung function (reverse causation (Shaaban et al. 2006)). The cytokine IL-6 is the main stimulator of CRP production in the liver. It may lead to the activation and adhesion of inflammatory cells to the pulmonary epithelium, leading to changes in endothelial function and increases in pulmonary vascular filtration and ultimately an acceleration of the age-related decline in pulmonary function (Jiang et al. 2008, Shaaban et al. 2006). Against this theory,

however, is the finding that the inverse relationship between plasma CRP levels and lung function was seen in both smokers and non-smokers.

5.4.9 Conclusion

This analysis provides further evidence that plasma CRP levels are inversely associated with lung function in cross-sectional studies even within the normal range. This study also demonstrates an inverse longitudinal relationship between change in serum CRP and change in lung function in this population of middle-aged men over the 10 year study period. These findings suggest that subclinical inflammation may be important in the pathogenesis of accelerated lung function decline even in apparently healthy middle-aged men.

There are potential implications for clinical practice. Perhaps we should consider routinely measuring CRP levels in conjunction with lung function measurement as an indicator of prognosis even in stable respiratory patients. There are also implications for potential drug therapies. Would specifically reducing CRP, improve pulmonary function or slow FEV_1 decline and thereby improve survival? There have been recent reports of plasma CRP reduction following treatment with statin therapy (Shyamsundar et al. 2009). Simvastatin has antiinflammatory effects in the pulmonary and systemic compartment in humans exposed to inhaled lipopolysaccharide. Further research in this area is ongoing.

6.0 Antioxidants and Lung Function

6.0 ANTIOXIDANTS AND LUNG FUNCTION

6.1 Introduction

6.1.1 Background

The oxidant burden to the lungs from pollutants like ozone, cigarette smoke and nitrogen dioxide and the pulmonary antioxidant defences need to be finely balanced to prevent potential acceleration of the rate of decline of pulmonary function. Dietary intake, which is the major source of antioxidants, may be related to modulation of the antioxidant/oxidant balance within the lung, thereby influencing lung function decline.

This study examines plasma levels of vitamins A and E (retinol, γ-tocopherol, α-tocopherol), and the carotenoids (lutein, zeaxanthin, betacryptoxanthin, α-carotene, ß-carotene and lycopene) and their relationship with lung function both in cross-section and longitudinally in the PRIME cohort.

6.1.2 Hypotheses

a) Plasma levels of antioxidants and lung function are positively correlated (cross-sectional).

b) 10 year change in lung function and 10 year change in antioxidant levels are positively correlated.

c) Self-reported fruit and vegetable intake is positively correlated with lung function and plasma levels of antioxidants.

6.1.3 Aims

In this population of middle-aged men from Northern Ireland, the aims of this study are to:

a) Examine the relationship between plasma levels of antioxidants and lung function (cross-sectional and longitudinal).

b) Examine the relationship between fruit and vegetable intake and lung function (cross-sectional and longitudinal).

c) Examine the relationship between fruit and vegetable intake and plasma levels of antioxidants (cross-sectional).

6.2 Methods

6.2.1 Participants

2745 healthy men were recruited into the Belfast cohort of the Prospective Epidemiological Study of Myocardial Infarction (PRIME) study at baseline as described in chapter 2.0.

6.2.2 Data Collection

Each subject completed a questionnaire on demographics, socio-economic factors and dietary habits. Venous blood samples were collected after a 12 hour fast and centrifuged within 4 hours. The samples were then frozen at -80°C. Anthropometric measurements included height, weight, and waist and hip circumferences. The Belfast cohort also had lung function measurements by spirometry. 2010 of the original cohort were re-screened 10 years later. This involved a further questionnaire, anthropometric measurements, spirometry and venous blood sample. Further details are in chapter 2.0.

Plasma levels of retinol, γ-tocopherol, α-tocopherol, lutein, zeaxanthin, ß-cryptoxanthin, α-carotene, ß-carotene and lycopene were measured by HPLC (High-performance liquid chromatography) analysis (see chapter 2.6). The baseline samples had been in storage for an average of 15 years before being thawed and analysed while the follow-up samples had been in storage for 4 years. At baseline plasma samples for antioxidant analysis were only made available for our study for men who had paired spirometry data. This was due to paucity of total stored plasma from baseline. Plasma was available from all of the men at follow-up who had a blood sample taken. Analysis of all samples was completed within 3 years.

6.2.3 Standardisation of spirometry

Each individual spirometry trace was reviewed for validity using ATS/ERS criteria as described in chapter 2.4

6.2.4 Dietary assessment

At baseline only, dietary information was elicited by the use of a short food frequency questionnaire (see appendices). Frequency of fruit and vegetable intake was obtained for four categories of fruit and vegetables ('citrus fruit', 'other fruit', 'raw vegetables' and 'baked vegetables'). Subjects were asked, through a personal interview at their home to indicate their usual frequency of consumption of a standard portion of fruit, fruit juice and vegetables for the last weeks using the following scale: more than once per day (number per day); daily; three to four times per week; twice per week; once per week; twice per month; once per month; never. Frequency of total 'fruit', 'vegetables' and 'fruit and vegetables' intake scores were calculated as the sum of number of servings per day of fruit and vegetables and fruit juice. A maximum of one portion of juice per person per day was included in the score. Potatoes were not included in the dietary scores because: (a) their vitamin and fibre composition is different from most other leafy vegetables and (b) their carbohydrate content is mostly of high glycaemic index (Dauchet et al. 2004b).

The questionnaire was not validated against another dietary assessment method, however, a correlation analysis between the frequency of fruit and/or vegetable intake and plasma vitamins was previously performed in 25 men to assess the ability of the questionnaire to discriminate large versus small consumers of fruits and vegetables (Dauchet et al. 2004b).

6.2.5 Data analysis

Data analysis was carried out using SPSS (Version 15). Because of the non-normal distribution of plasma levels of zeaxanthin, ß-cryptoxanthin, α-carotene, ß-carotene and lycopene, analyses were performed with log-transformed values. Cross-sectional associations between the various antioxidants and FEV_1 or FVC were analysed by linear regression using absolute values of FEV_1 or FVC in ml as the dependent variable and the individual antioxidants as the main predictors with adjustment for height, age, smoking history, educational group, material conditions and alcohol intake. Longitudinal associations between baseline plasma antioxidant levels and 10 year change in FEV_1 or FVC in ml were analysed by linear regression using absolute values of change in FEV_1 or FVC in ml over 10 years as the dependent variable and change in plasma antioxidant level in μmol/L/10 yr as the main predictor. Adjustment was made for baseline, FEV_1 or FVC, height, age, smoking history, educational group and material conditions. Non-parametric correlations were performed using Spearman's rank correlation. Non-linear associations were investigated by the use of categories.

6.3 Results

6.3.1 Plasma biomarker data

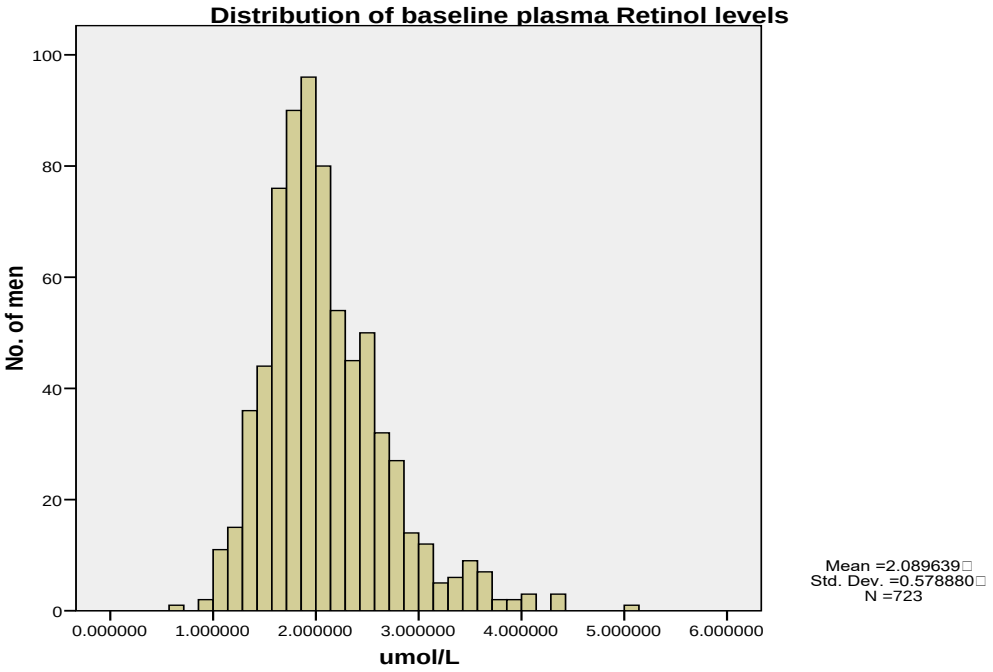
6.3.1.1. Mean plasma levels of antioxidants at baseline and follow-up

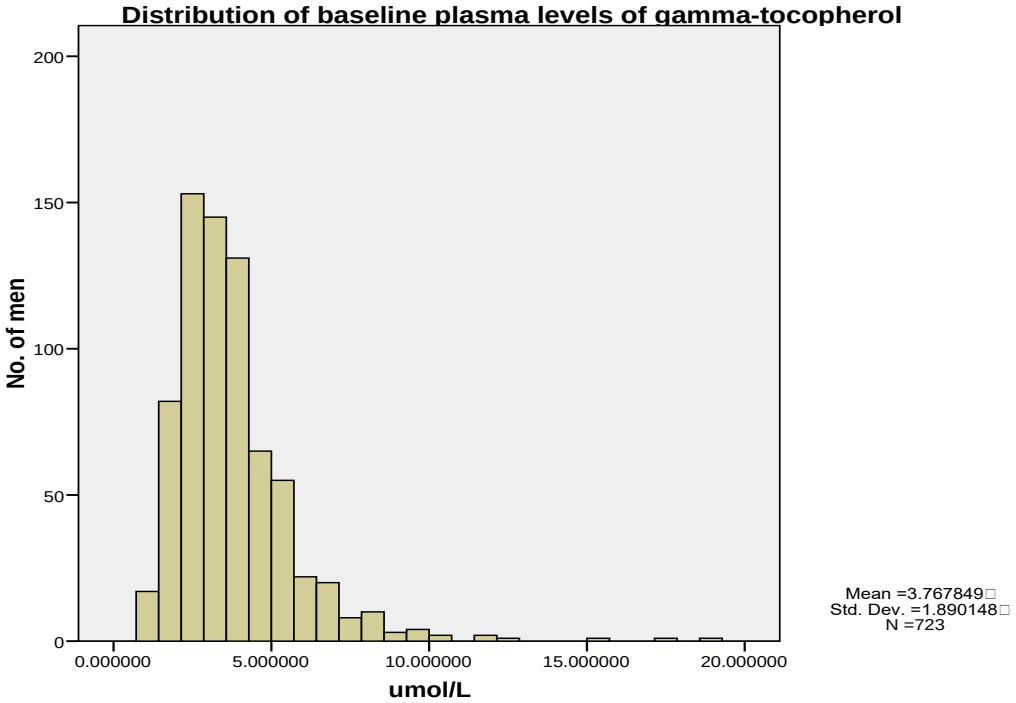
At baseline, plasma samples for antioxidant analysis were only made available for men who had paired spirometry data. This was due to the paucity of stored plasma at baseline. 932 men had paired spirometry data, 186 of these had no available plasma sample and some samples were affected by machine failure (see table 6.1 for numbers of valid results for each individual antioxidant). Plasma was available from 1858 of the 2010 men screened at follow-up but machine failure affected 39 of the retinol and lutein results and up to 172 of the other antioxidant results (see table 6.1). Analysis of all samples was completed within 3 years. Table 6.1 shows the mean (SD) plasma levels of antioxidants at baseline and 10 year follow-up in the PRIME cohort.

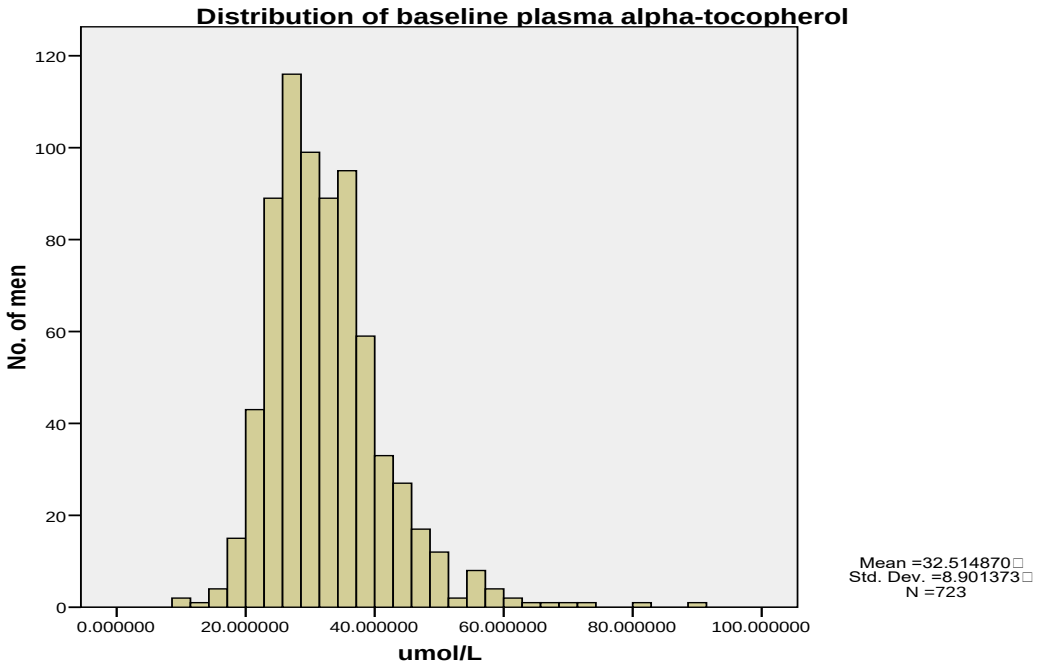
Table 6.1: Mean (SD) plasma levels of antioxidants at baseline and 10 year follow-up

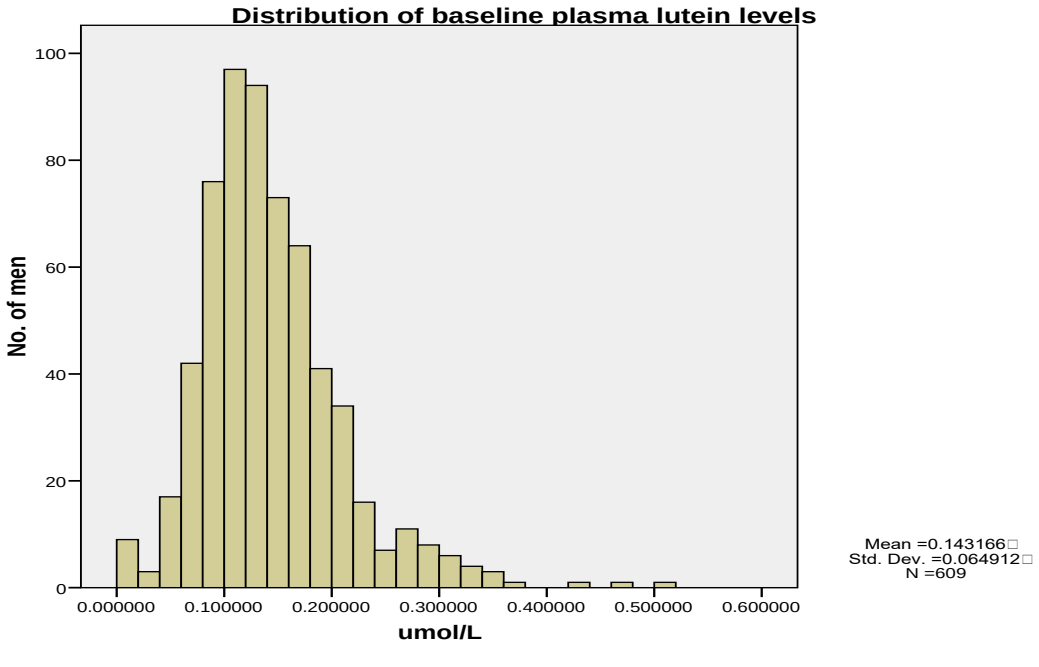
	Baseline Mean (SD) μmol/L	10 year follow-up Mean (SD) μmol/L	Distribution of results	Paired samples t test p
Retinol	2.09(0.58) n=721	2.15 (0.64) n=1829	Normal	**0.006**
γ-tocopherol	3.77(1.89) n=721	2.76 (1.10) n=1827	Normal	**<0.0001**
α-tocopherol	32.51 (8.9) n=721	31.08 (8.9) n=1829	Normal	0.1
Lutein	0.153(0.246) n=610	0.18(0.08) n=1825	Normal	**<0.0001**
Zeaxanthin	0.025(0.021) n=602	0.04(0.02) n=1821	Skewed to right	**<0.0001**
ß-cryptoxanthin	0.0645(0.053) n=561	0.075(0.06) n=1825	Skewed to right	**<0.0001**
α-carotene	0.059 (0.062) n=499	0.10(0.11) n=1823	Skewed to right	**<0.0001**
ß-carotene	0.285(0.241) n=498	0.36(0.32) n=1821	Skewed to right	**<0.0001**
Lycopene	0.317 (0.374) n=480	0.54(0.63) n=1786	Skewed to right	**<0.0001**

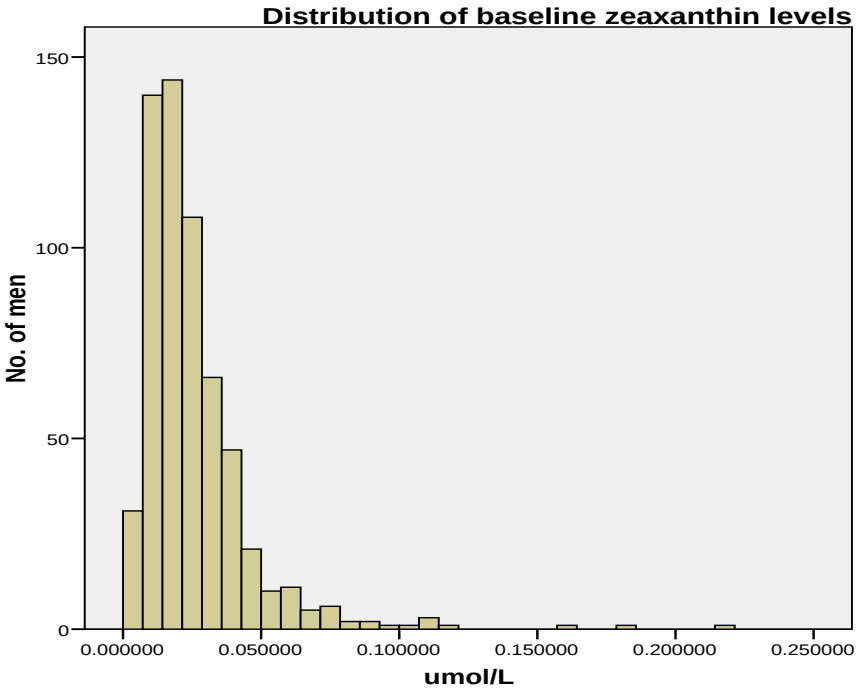
Figure 6.1 Distribution of levels of baseline plasma antioxidants

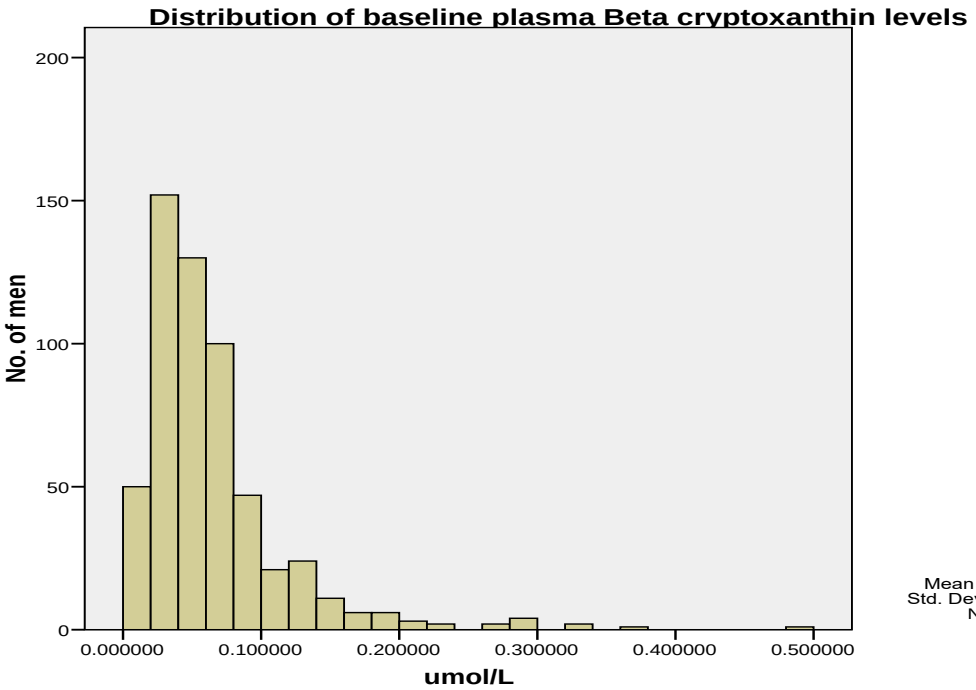


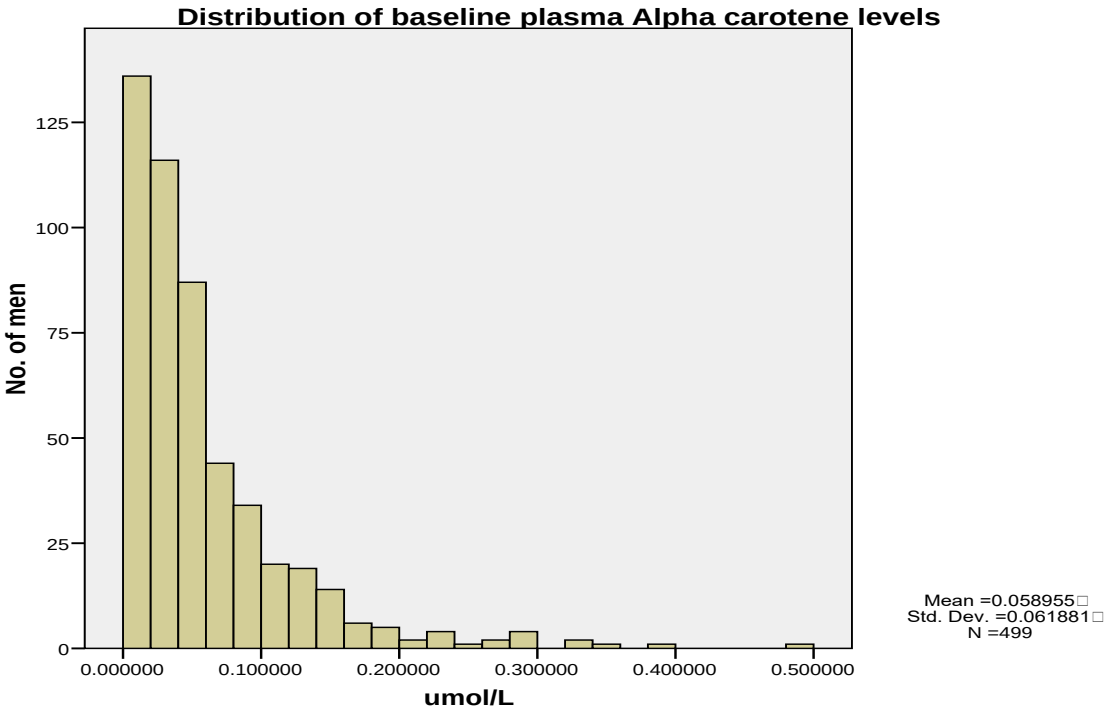


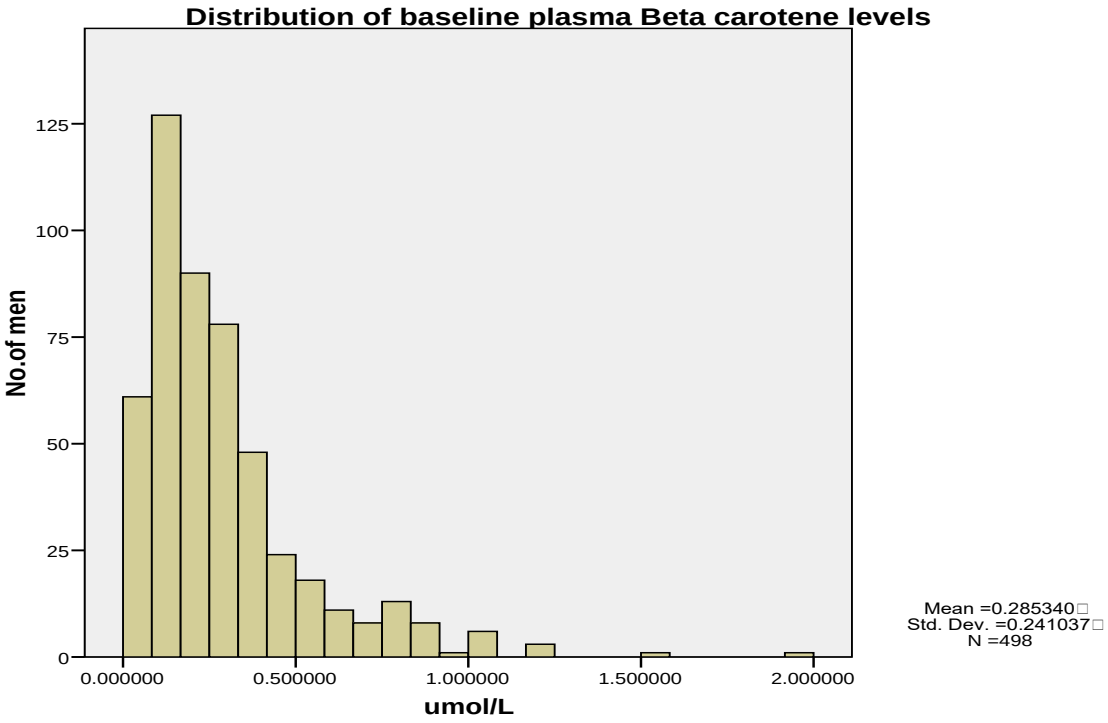

Distribution of baseline plasma alpha-tocopherol
No. of men
umol/L
0.000000
20.000000
40.000000
60.000000
80.000000
100.000000
0
20
40
60
80
100
120
Mean =32.514870
Std. Dev. =8.901373
N =723

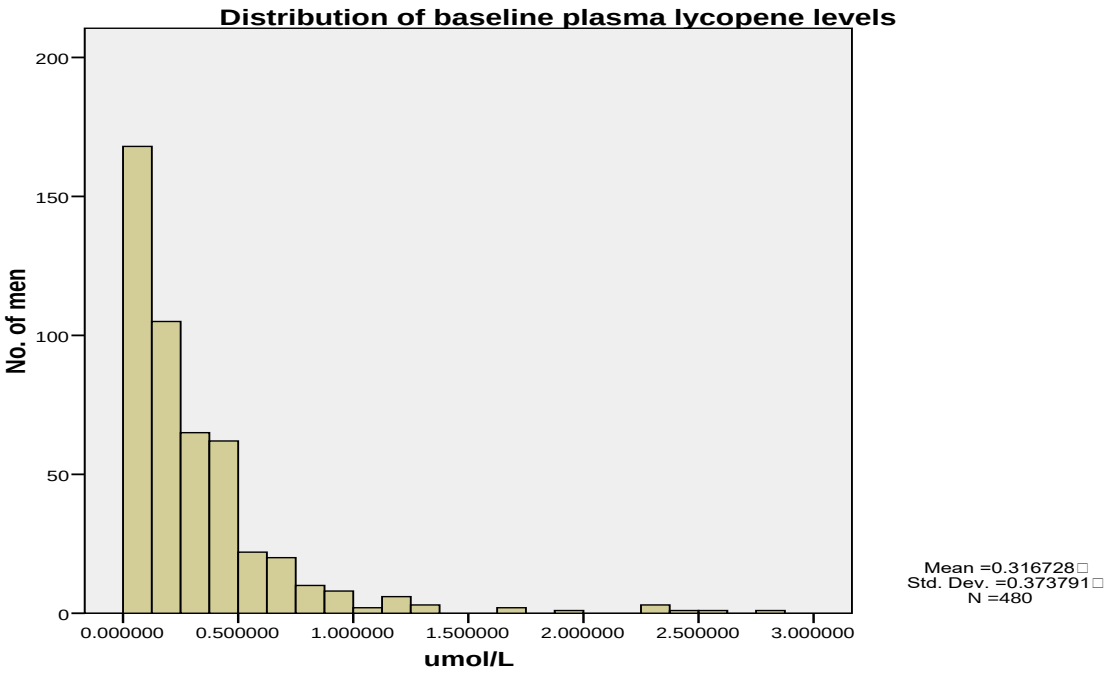

Distribution of baseline plasma lutein levels
No. of men
umol/L
0.000000
0.100000
0.200000
0.300000
0.400000
0.500000
0.600000
0
20
40
60
80
100
Mean =0.143166
Std. Dev. =0.064912
N =609


Distribution of baseline zeaxanthin levels
No. of men
150
100
50
0
0.000000
0.050000
0.100000
0.150000
0.200000
0.250000
umol/L
Mean =0.025497
Std. Dev. =0.020559
N =602


Distribution of baseline plasma Beta cryptoxanthin levels
No. of men
200
150
100
50
0
0.000000
0.100000
0.200000
0.300000
0.400000
0.500000
umol/L
Mean =0.064463
Std. Dev. =0.053392
N =562


Distribution of baseline plasma Alpha carotene levels
No. of men
125
100
75
50
25
0
0.000000
0.100000
0.200000
0.300000
0.400000
0.500000
umol/L
Mean =0.058955
Std. Dev. =0.061881
N =499


Distribution of baseline plasma Beta carotene levels
No.of men
125
100
75
50
25
0
0.000000
0.500000
1.000000
1.500000
2.000000
umol/L
Mean =0.285340
Std. Dev. =0.241037
N =498


Distribution of baseline plasma lycopene levels
No. of men
200
150
100
50
0
0.000000
0.500000
1.000000
1.500000
2.000000
2.500000
3.000000
umol/L
Mean =0.316728
Std. Dev. =0.373791
N =480

6.3.1.2 Relationship between baseline FEV_1 and plasma antioxidant levels

At baseline, retinol, α-tocopherol, zeaxanthin , β-cryptoxanthin, α-carotene, β-carotene and lycopene were all positively correlated with FEV_1. After adjustment for educational group, material conditions, age, height, body mass index, cholesterol, alcohol intake (g/week) and smoking history (pack years) the relationships, all became non-significant with the exception of retinol.

Table 6.2: Relationship between baseline FEV_1 and plasma antioxidant levels

	Crude		Adjusted*		
Antioxidant µmol/L	Increase in FEV_1 (ml) per µmol/L increase plasma level of antioxidant (95%CI)	p	Increase in FEV_1 (ml) per µmol/L increase serum level of antioxidant (95%CI)	p	R^2
Retinol	103.8 (24, 184)	**<0.0001**	114.9 (43, 187)	**0.002**	0.36
γ-tocopherol	-20.9 (-46, 4)	0.095	-7.8 (-29, 14)	0.473	0.35
α-tocopherol	6.6 (1, 12)	**0.013**	3.9 (-0.5, 8)	0.08	0.35
Lutein	698.8 (-66, 1463)	0.73	36.5 (-609, 6820	0.91	0.38
Zeaxanthin (log)	242.1 (76, 409) †	**0.004**	8.2 (-132, 148)	0.9	0.38
ß-cryptoxanthin (log)	361.1 (195, 528) †	**<0.0001**	98.2 (-46, 243)	0.18	0.38
α-carotene (log)	235.7 (107, 364) †	**<0.0001**	67.5 (-42, 177)	0.23	0.36
ß-carotene (log)	302.8 (152, 454) †	**<0.0001**	96.5 (-37, 229)	0.18	0.36
lycopene (log)	192.8 (78, 307) †	**0.001**	82.1 (-14, 178)	0.09	0.37

**Adjusted for educational group, material conditions, age, height, body mass index, cholesterol, alcohol intake (g/week) and smoking history (pack years)*
†Per tenfold increase in plasma level of antioxidant

6.3.1.3 Relationship between baseline FVC and plasma antioxidant levels

Retinol, γ-tocopherol, ß-cryptoxanthin, α-carotene, β-carotene and lycopene were all positively correlated with baseline FVC. After adjustment for educational group, material conditions, age, height, body mass index, cholesterol, alcohol intake (g/week) and smoking history (pack years) the relationships all became non-significant, with the exception of retinol.

Table 6.3. Relationship between baseline FVC and plasma antioxidant levels

	Crude		Adjusted*		
Antioxidant in	Increase in FVC (ml) per μmol/L increase in plasma level of antioxidant (95% CI)	p	Increase in FVC (ml) per μmol/L increase in serum level of antioxidant (95%CI)	p	R^2
Retinol	117.2 (20.5, 214)	**0.018**	129 (42, 216)	**0.004**	0.35
γ-tocopherol	-38 (-68, -8.6)	**0.012**	-18.8 (-45, 7)	0.16	0.35
α-tocopherol	3.9 (-2.4, 10.2)	0.23	1.5 (-5, 8)	0.6	0.34
Lutein	720.4 (-213, 1654)	0.13	-27.6 (-828, 772)	0.95	0.35
Zeaxanthin (log)	186 (-17.4, 389) †	0.07	-90 (-263, 83)	0.31	0.36
ß-cryptoxanthin (log)	362.6 (159, 567) †	**0.001**	52 (-129, 233)	0.57	0.36
α-carotene (log)	208 (50, 366) †	**0.01**	7.21 (-128, 142)	0.92	0.35
ß-carotene (log)	302 (116, 487) †	**0.001**	13.5 (-151, 178)	0.87	0.35
lycopene (log)	168 (27, 309) †	**0.02**	33.3 (-205, 98)	0.49	0.34

**Adjusted for educational group, material conditions, age, height, body mass index, cholesterol, alcohol intake (g/week) and smoking history (pack years)*
† Per tenfold increase in serum level of antioxidant

6.3.1.4 Relationship between baseline FEV_1/FVC ratio and plasma antioxidant levels

355 of the 1779 men with valid baseline spirometry had FEV_1/FVC ratios of <0.7. Men with FEV_1/FVC ratios less than <0.7 were on average 0.6 years older, had a BMI 1.1kg/m^2 lower, had smoked for 10.5 more pack years and drank 30.6g more alcohol per week than men with FEV_1/FVC ratios of >0.7.

Table 6.4. Relationship between baseline FEV_1/FVC ratio and plasma antioxidant levels

	FEV_1/FVC<0.7 μmol/L mean (SD)	FEV_1/FVC ≥0.7 μmol/L mean (SD)	p (t-test)
Retinol	2.08 (0.65) n=177	2.09 (0.56) n=605	0.86
γ-tocopherol	3.61 (1.85) n=117	3.8 (1.9) n=605	0.32
α-tocopherol	30.7 (7.8) n=117	32.9 (9.1) n=605	**0.016**
Lutein	0.13 (0.05) n=88	0.14 (0.07) n=520	0.095
Zeaxanthin	0.022 (0.015) n=86	0.026 (0.02) n=515	0.06
ß-cryptoxanthin	0.06 (0.04) n=77	0.07 (0.06) n=484	0.11
α-carotene	0.051 (0.06) n=69	0.06 (0.06) n=429	0.24
ß-carotene	0.26 (0.21) n=70	0.29 (0.25) n=427	0.4
lycopene	0.3 (0.47) n=64	0.32 (0.36) n=416	0.08

6.3.1.5 Relationship between 10 year plasma antioxidant levels and 10 year FEV_1 and FVC.

In the 10 year cross-sectional analysis there was a positive linear relationship between plasma levels of lutein, zeaxanthin, ß-cryptoxanthin, α-carotene, ß-carotene and 10 year FEV_1. After adjustment for educational group, material conditions, age, height, body mass index, cholesterol and smoking history (pack years), the relationships remained significant (with the exception of lutein). There was a significant negative relationship with plasma levels of γ-tocopherol which remained borderline significant after adjustment for confounding variables.

In the 10 year cross-sectional analysis there was a positive linear relationship between plasma levels of lutein, zeaxanthin, ß-cryptoxanthin, α-carotene, ß-carotene and 10 year FVC. After adjustment for educational group, material conditions, age, height, body mass index, cholesterol and smoking history (pack years), the relationships remained significant (with the exception of lutein). There was a significant negative relationship with plasma levels of γ-tocopherol which remained significant after adjustment for confounding variables. Similar results are obtained modelling the antioxidant levels in quartiles as categorical variables (see table 6.7).

Table 6.5 Relationship between plasma antioxidant levels and 10 year FEV_1 (linear relationships)

	Crude		Adjusted*		
Antioxidant	Increase in FEV_1 (ml) per µmol/L increase in plasma level of antioxidant (95%CI)	p	Increase in FEV_1 (ml) per µmol/L increase in plasma level of antioxidant (95%CI)	p	R^2
Retinol	24.3 (-29, 78)	0.37	18.6 (-28, 65)	0.43	0.3
γ-tocopherol	-49 (-80, -18)	**0.002**	-27.4 (-55, 0.2)	**0.052**	0.3
α-tocopherol	2.09 (-1.7, 5.8)	0.27	-0.14 (-4, 3)	0.94	0.3
Lutein	804.4 (367, 1242)	**<0.0001**	263.5 (-131, 658)	0.19	0.3
Zeaxanthin (log)	558.7 (400, 717) †	**<0.0001**	319 (176, 462)	**<0.0001**	0.31
ß-cryptoxanthin (log)	501.4 (398, 605) †	**<0.0001**	285.4 (191, 379)	**<0.0001**	0.32
α-carotene (log)	247.8 (153, 342) †	**<0.0001**	117.9 (34, 202)	**0.006**	0.30
ß-carotene (log)	274 (166, 382) †	**<0.0001**	109 (12.7, 205)	**0.026**	0.3
lycopene (log)	74 (-9.7, 158) †	0.083	33.3 (-38.4, 105)	0.37	0.3

**Adjusted for educational group, material conditions, age, height, body mass index, cholesterol and smoking history (pack years)*
†*Per tenfold increase in serum level of antioxidant*

Table 6.6 Relationship between plasma antioxidant levels and 10 year FVC (Cross-sectional linear relationships)

	Unadjusted		Adjusted*		
Antioxidant	Increase in FVC (ml) per µmol/L increase in plasma level of antioxidant (95%CI)	p	Increase in FVC (ml) per µmol/L increase serum level of antioxidant (95%CI)	p	R^2
Retinol	45.7 (-14.5, 106)	0.14	45.2 (-6, 96)	0.08	0.36
γ-tocopherol	-61.4 (-96, -26)	**0.001**	-33.7 (-63.5, -4)	**0.027**	0.36
α-tocopherol	1.0 (-3.3, 5.2)	0.65	-1.2 (-5.1, 2.6)	0.54	0.36
Lutein	571 (391, 751)	**<0.0001**	391 (-36.7, 819)	0.07	0.36
Zeaxanthin (log)	570.8 (391, 751)	**<0.0001**	299 (144, 455)	**<0.0001**	0.36
ß-cryptoxanthin (log)	485.7 (368, 604)	**<0.0001**	234.5 (132, 337)	**<0.0001**	0.37
α-carotene (log)	262.4 (155, 370)	**<0.0001**	123.7 (32.8, 215)	**0.008**	0.36
ß-carotene (log)	308.3 (186, 430)	**<0.0001**	113.3 (9, 218)	**0.033**	0.36
lycopene (log)	81.3 (-13, 176)	0.09	44.2 (-33.5, 1220	0.27	0.35

**Adjusted for educational group, material conditions, age, height, body mass index, cholesterol and smoking history (pack years)*
†*Per tenfold increase in serum level of antioxidant*

Table 6.7 Relationship between 10 year antioxidant levels by quartile and 10 year FEV_1

10 year plasma antioxidant level by quartile	Crude		Adjusted*		
	Mean 10 yr FEV1 in ml Mean (SD)	p-value	Difference in mean 10 yr FVC mld (95% CI)	p-value (trend across cats)	r^2
Retinol					
Lowest levels <1.73 μmol/L n=306	2723 (634)	0.33 (0.1)	0 (Ref. cat)	0.52 (0.1)	0.30
1.73-2.07 μmol/L n=312	2782 (609)		37 (-46, 120)		
2.07- 2.5 μmol/L, n=314	2805 (636)		41 (-43, 125)		
Highest levels >2.5 μmol/L n=308	2800 (596)		66 (-21, 153)		
γ-tocopherol					
Lowest levels <2.07 μmol/L n=310	2679 (595)	**0.003**	0 (Ref. cat)	**0.046** (0.005)	0.31
2.07-2.59 μmol/L n=304	2772 (630)		-79 (-161, 4)		
2.59- 3.30 μmol/L, n=316	2796 (618)		-82 (-165, 2)		
Highest levels >3.3 μmol/L n=309	2863 (622)		-120 (-206, -34)		
α-tocopherol					
Lowest levels <26.0 μmol/L n=310	2726 (649)	0.096	0 (Ref. cat)	0.43 (0.13)	0.3
26.0-30.6 μmol/L n=310	2751 (603)		4 (-80, 89)		
30.6-35.6 μmol/L, n=310	2791 (605)		54 (-34, 142)		
Highest levels >35.6 μmol/L n=310	2843 (615)		61 (-34, 155		
Lutein					
Lowest levels <0.13 μmol/L n=307	2645 (586)	**<0.0001**	0 (Ref. cat)	0.13 (0.13)	0.3
0.13-0.17 μmol/L n=313	2812 (591)		83 (0, 166)		
0.17-0.22 μmol/L, n=310	2794(640)		34 (-50, 119)		
Highest levels >0.22 μmol/L n=308	2863 (638)		88 (1, 175)		
ß-cryptoxanthin					
Lowest BCX levels <0.035 μmol/L n=308	2531 (595)	**<0.0001**	0 (Ref.cat)	**<0.0001** (<0.0001)	0.33
0.035-0.06 μmol/L n=309	2748 (610)		122 (40, 203)		
0.06-0.1μmol/L, n=310	2854 (570)		172 (89, 256)		
Highest BCX levels >0.1 μmol/L n=308	2982 (614)		267 (182, 352)		
Zeaxanthin					
Lowest levels <0.024 μmol/L n=309	2593 (619)	**<0.0001**	0 (Ref.cat)	**<0.0001** (<0.0001)	0.32
0.024-0.03 μmol/L n=309	2769 (624)		89 (6, 172)		
0.03-0.05 μmol/L, n=311	2839 (591)		109 (25, 193)		
Highest levels >0.05 μmol/L n=308	2915 (629)		186 (101, 271)		
α-carotene					
Lowest levels <0.04 μmol/L n=305	2613 (602)	**<0.0001**	0 (Ref. cat)	**0.002** (<0.0001)	0.31
0.04-0.07 μmol/L n=315	2756 (607)		78 (-5, 161)		
0.07-0.12 μmol/L, n=307	2861 (600)		146 (62, 229)		
Highest levels >0.12 μmol/L n=309	2880 (634)		139 (53, 225)		

ß-carotene					
Lowest levels <0.18 µmol/L n=310	2642 (553)		0 (Ref Cat.)		
0.18-0.28 µmol/L n=309	2744 (614)	<**0.0001**	45 (-37, 128)	0.11 (0.02)	0.31
0.28-0.44µmol/L, n=308	2831 (629)		91 (7, 175)		
Highest levels >0.44 µmol/L n=308	2890 (648)		93 (7, 179)		
lycopene					
Lowest levels <0.19 µmol/L n=301	2675 (620)		0 (Ref. cat)		
0.19-0.38 µmol/L n=302	2819 (597)	0.014	94 (10, 179)	0.09 (0.18)	0.3
0.38-0.64 µmol/L, n=301	2808 (624)		94 (10, 179)		
Highest levels >0.64 µmol/L n=301	2796 (607)		61.4 (-23, 146)		

10 year plasma antioxidant level by quartile	Crude		Adjusted*		
	Mean 10 yr FVC in ml Mean (SD)	p-value	Difference in mean 10 yr FVC ml (95% CI)	p-value (trend across cats)	R^2
ß-cryptoxanthin					
Lowest plasma levels <0.035 μmol/L n=308	3445 (642)	**<0.0001**	0 (Ref.cat.)	**<0.0001**	0.37
0.035-0.06 μmol/L n=309	3630 (721)	(<0.0001)	80.6 (-9, 170)	(<0.0001)	
0.06-0.1μmol/L, n=310	3761 (666)		145 (54, 236)		
Highest plasma levels >0.1 μmol/L n=308	3882 (696)		219 (126, 312)		
Zeaxanthin					
Lowest plasma levels <0.024 μmol/L n=309	3484 (662)	**<0.0001**	0 (Ref.cat.)	**0.005** (0.001)	0.37
0.024-0.033 μmol/L n=309	3676 (697)		96 (6, 186)		
0.033-0.05 μmol/L, n=311	3744 (676)		101 (10, 193)		
Highest plasma levels >0.05 μmol/L n=308	3814 (725)		171 (78, 264)		
α-carotene					
Lowest levels <0.04 μmol/L n=305	3508 (677)	**<0.0001**	0 (Ref.cat)	**0.007** (0.001)	0.36
0.04-0.07 μmol/L n=315	3651 (688)		83 (-7, 173)		
0.07-0.12 μmol/L, n=307	3766 (676)		144 (53, 235)		
Highest levels >0.12 μmol/L n=309	3787 (726)		143 (49, 237)		
ß-carotene					
Lowest levels <0.18 μmol/L n=310	3527 (630)	<0.0001	0 (Ref. cat.)	0.07 (0.009)	0.36
0.18-0.28 μmol/L n=309	3623 (693)		30 (-59, 120)		
0.28-0.44μmol/L, n=308	3735 (712)		93 (1, 184)		
Highest levels >0.44 μmol/L n=308	3825 (725)		110 (17, 203)		

Table 6.8 Relationship between 10 year antioxidant levels by quartile and 10 year FVC

6.3.1.6 Longitudinal relationships between plasma antioxidant levels and lung function

6.3.1.6.1 Retinol

721 men had paired valid spirometry results and a baseline plasma retinol level (211 didn't have a baseline retinol level). Mean change in FEV_1 over 10 years in this group was -458.4 (320) ml and mean change in FVC over 10 years was -530.6 (453) ml. Mean retinol level at baseline was 2.09 (0.58) μmol/L. The mean change in plasma retinol level over 10 years was 0.07 (0.7) μmol/L.

There was no significant relationship between baseline retinol levels (as a continuous or categorical variable) and change in FEV_1 or change in FVC over 10 years in either the crude or adjusted models.

Baseline Retinol levels were positively related to 10 year FEV_1 and FVC. FEV_1 at 10 years was on average 89 ml greater per μmol/L increase retinol level at baseline (95%CI 12, 167 p=0.024). After adjustment for age, BMI, height and smoking history (pack years) at 10 years and educational group, material conditions and cholesterol at baseline, FEV_1 at 10 years was on average 110 ml greater per μmol/L increase retinol level at baseline (95%CI 41,180, p=0.002, R^2=0.32). FVC at 10 years was on average 109 ml greater per μmol/L increase retinol level at baseline (95%CI 21, 197 p=0.015). After adjustment for age, BMI, height and smoking history (pack years) at 10 years and educational group, material conditions and cholesterol at baseline, FVC at 10 years was on average 140 ml greater per μmol/L increase retinol level at baseline (95%CI 65,216, p<0.0001, R^2=0.37).

There was no significant relationship between change in plasma retinol level and change in FEV_1 or FVC over 10 years. Change in retinol level over 10 years was not related to 10 year FEV_1 or FVC.

6.3.1.6.2 γ-tocopherol

721 men had paired valid spirometry results and a baseline plasma γ-tocopherol level (211 didn't have a baseline γ-tocopherol level). Mean change in FEV_1 over 10 years in this group was -458.4 (320) ml and mean change in FVC over 10 years was -530.6 (453) ml. Mean γ-tocopherol level at baseline was 3.77 (1.89) μmol/L. The mean change in plasma γ-tocopherol level over 10 years was -0.91 (1.8) μmol/L.

There was no significant relationship between baseline γ-tocopherol levels (as a continuous or categorical variable) and change in FEV_1 or change in FVC over 10 years in either the crude or adjusted models.

Baseline γ-tocopherol levels were not related to 10 year FEV_1 in either the crude or adjusted model (as a continuous or categorical variable). Baseline γ-tocopherol levels were inversely related to 10 year FVC. FVC at 10 years was on average 34 ml lower per μmol/L increase γ-tocopherol level at baseline (95%CI -61, -8 p=0.012). After adjustment for age, BMI, height and smoking history (pack years) at 10 years and educational group, material conditions and cholesterol at baseline, the relationship was no longer statistically significant. (FVC at 10 years was on average 12 ml lower per μmol/L increase in γ-tocopherol level at baseline (95%CI -35,12, p=0.33, R^2=0.36).

There was no significant relationship between change in plasma γ-tocopherol level and change in FEV_1 over 10 years. There was an inverse relationship between change in plasma γ-tocopherol level and change in FVC over 10 years. Change in FVC over 10 years was on average 19 ml lower per μmol/L increase γ-tocopherol level over 10 years (95%CI -37, -0.5 p=0.044). After adjustment for educational group, material conditions, smoking history, body mass index, age, height, cholesterol, baseline γ-tocopherol level and baseline FVC this relationship was no longer significant (Change in FVC over 10 years was on average 25 ml lower per μmol/L increase in γ-tocopherol level over 10 years (95%CI -54, 3, p=0.08)).

Change in γ-tocopherol level over 10 years was not related to 10 year FEV_1 or FVC.

6.3.1.6.3 α-tocopherol

721 men had paired valid spirometry results and a baseline plasma α-tocopherol level (211 didn't have a baseline α-tocopherol level). Mean change in FEV_1 over 10 years in this group was -458.4 (320) ml and mean change in FVC over 10 years was -530.6 (453) ml. Mean α-tocopherol level at baseline was 32.5 (8.9) μmol/L. The mean change in plasma α-tocopherol level over 10 years was -0.64 (10) μmol/L.

There was no significant relationship between baseline α-tocopherol levels (as a continuous or categorical variable) and change in FEV_1 or change in FVC over 10 years in either the crude or adjusted models.

Baseline α-tocopherol levels were not related to 10 year FEV_1 or FVC in either the crude or adjusted model (as a continuous or categorical variable).

There was no significant relationship between change in plasma α-tocopherol level over 10 years and change in FEV_1 or FVC over 10 years in either crude or adjusted models.

Change in α-tocopherol level over 10 years was not related to 10 year FEV_1 or FVC.

6.3.1.6.4 γ-tocopherol:α-tocopherol ratio

721 men had paired valid spirometry results and paired plasma α-tocopherol and γ-tocopherol levels. Baseline γ-tocopherol:α-tocopherol ratio was 0.118 (0.05) and 10 year ratio was 0.092 (0.03).

There was no significant relationship between baseline γ-tocopherol:α-tocopherol ratio (as a continuous or categorical variable) and change in FEV_1 or change in FVC over 10 years in either the crude or adjusted models.

Baseline γ-tocopherol:α-tocopherol ratio and 10 year FEV_1 ($r = -0.122$, $p=0.001$) and FVC ($r =-0.15$, $p<0.0001$) were negatively correlated. The significance of the relationship was lost after adjusting for confounding variables (BMI, age, height, cholesterol, smoking history, educational group and material conditions).

There was no significant relationship between change in γ-tocopherol:α-tocopherol ratio over 10 years and change in FEV_1 or FVC over 10 years in either crude or adjusted models. Change in γ-tocopherol:α-tocopherol ratio over 10 years was not related to 10 year FEV_1 or FVC.

6.3.1.6.5 Lutein

607 men had paired valid spirometry results and a baseline plasma lutein level.. Mean lutein level at baseline was 0.14 (0.07) μmol/L. The mean change in plasma lutein level over 10 years was 0.04 (0.08) μmol/L.

There was no significant relationship between baseline lutein levels (as a continuous or categorical variable) and change in FEV_1 or change in FVC over 10 years in either the crude or adjusted models.

Baseline lutein levels were not related to 10 year FEV_1 or FVC in either the crude or adjusted model (as a continuous or categorical variable).

There was no significant relationship between change in plasma lutein level over 10 years and change in FEV_1 or FVC over 10 years in either crude or adjusted models.

Change in lutein level over 10 years was positively correlated with 10 year FEV_1 ($r=0.115$, $p=0.006$) and FVC ($r=0.106$, $p=0.012$). This relationship became non-significant after adjustment for body mass index, height, age, smoking history, cholesterol, educational group and material conditions.

6.3.1.6.6 Zeaxanthin

600 men had paired valid spirometry results and a baseline plasma zeaxanthin level. Mean zeaxanthin level at baseline was 0.026 (0.02) μmol/L. The mean change in plasma zeaxanthin level over 10 years was 0.012 (0.025) μmol/L.

There was no significant relationship between baseline zeaxanthin levels and change in FEV_1 over 10 years. There was a positive correlation between baseline zeaxanthin levels and change in FVC over 10 years (r=0.106, p=0.009). This relationship persisted after adjustment for age, height, BMI, cholesterol, smoking history, educational group and smoking history (p=0.004).

Baseline zeaxanthin (log) levels were positively correlated with 10 year FEV_1 (r=0.134, p=0.001) and FVC (r=0.149, p<0.0001). After adjustment for age, BMI, height and smoking history (pack years) at 10 years and educational group, material conditions and cholesterol at baseline, the relationships became non-significant.

There was no significant relationship between change in plasma zeaxanthin level and change in FEV_1 over 10 years. There was a negative correlation between change in zeaxanthin over 10 years and change in FVC over 10 years (r=-0.12, p=0.004) which remained after adjustment for confounding variables (p=0.003).

Change in zeaxanthin level over 10 years was not related to 10 year FEV_1 or FVC.

6.3.1.6.7 ß-cryptoxanthin

560 men had paired valid spirometry results and a baseline plasma ß-cryptoxanthin level. Mean ß-cryptoxanthin level at baseline was 0.065 (0.05) μmol/L. The mean change in plasma ß-cryptoxanthin level over 10 years was 0.015 (0.06) μmol/L.

There was no significant relationship between baseline ß-cryptoxanthin levels and change in FEV_1 or FVC over 10 years.

Baseline ß-cryptoxanthin (log) levels were positively correlated with 10 year FEV_1 ($r=0.21$, $p<0.0001$) and FVC ($r=0.2$, $p<0.0001$). After adjustment for age, BMI, height and smoking history (pack years) at 10 years and educational group, material conditions and cholesterol at baseline, the relationship with FEV1 was maintained ($p=0.026$), however, the continuous relationship with FVC became non-significant ($p=0.74$). When modelled as categorical variables, quartiles of baseline ß-cryptoxanthin had a positive relationship with 10 year FEV_1 and FVC.

Table 6.9: Relationship between baseline quartiles of ß-cryptoxanthin and 10 year lung function

	Crude			Adjusted*		
Outcome	Baseline antioxidant level by quartile	Mean 10 year lung function outcome ml Mean (SD)	p	Difference in mean 10 year lung function outcome ml (95%CI)	p	R^2
10 year FEV_1	Lowest levels of ß-cryptoxanthin <0.03μmol/L n=140	2646 (570)		0 (Ref.cat.)		
	0.03-0.05μmol/L n=136	2697 (589)	<0.0001	-42 (-162, 78)	0.032	0.34
	0.05-0.08μmol/L n=144	2854 (630)		110(-9, 228)		
	Highest levels of ß-cryptoxanthin >0.08μmol/L n=140	2975 (582)		104 (-22, 230)		
10 year FVC	Lowest levels of ß-cryptoxanthin <0.03μmol/L n=140	3516 (643)		0 (Ref. cat.)		
	0.03-0.05μmol/L n=136	3546 (674)	<0.0001	-68 (-202, 65)	0.012	0.39
	0.05-0.08μmol/L n=144	3747 (736)		125 (-7, 257)		
	Highest levels of ß-cryptoxanthin >0.08μmol/L n=140	3897 (694)		115 (-24, 255)		

* Adjusted for age, BMI, height and smoking history (pack years) at 10 years and educational group, material conditions and cholesterol at baseline

There was no significant relationship between change in plasma ß-cryptoxanthin level and change in FEV_1 or FVC over 10 years.

Change in ß-cryptoxanthin level over 10 years was positively correlated with 10 year FEV_1 (r=0.123, p=0.004) and FVC (r=0.128, p=0.005). After adjustment for height, age, cholesterol, educational group, material conditions, smoking history and BMI, the relationships were maintained (p=0.049 and 0.04).

6.3.1.6.8 α-carotene

497 men had paired valid spirometry results and a baseline plasma α-carotene level. Mean α-carotene level at baseline was 0.06 (0.06) μmol/L. The mean change in plasma α-carotene level over 10 years was 0.04 (0.12) μmol/L.

There was no significant relationship between baseline α-carotene levels (as a continuous or categorical variable) and change in FEV_1 over 10 years .There was a positive relationship with change in FVC over 10 years in the adjusted model (continuous and categorical) (p=0.046, 0.014).

Baseline α-carotene levels were positively correlated with 10 year FEV_1 (r=0.18, p<0.0001) and FVC (r=0.18, p<0.0001). After adjustment for age, BMI, height and smoking history (pack years) at 10 years and educational group, material conditions and cholesterol at baseline, the relationships became non-significant.

There was no significant relationship between change in α-carotene level and change in FEV_1 or FVC over 10 years. Change in α-carotene level over 10 years was not related to 10 year FEV_1 or FVC.

6.3.1.6.9 ß-carotene

461 men had paired valid spirometry results and a baseline plasma ß-carotene level. Mean ß-carotene level at baseline was 0.29 (0.24) μmol/L. The mean change in plasma ß-carotene level over 10 years was 0.08 (0.35) μmol/L.

There was no significant relationship between baseline ß-carotene levels (as a continuous or categorical variable) and change in FEV_1 or FVC over 10 years.

There was no relationship between baseline ß-carotene levels and 10 year FEV_1 or FVC in the crude or adjusted models.

There was no relationship between change in ß-carotene over 10 years and change in FEV1 or FVC over 10 years. Change in ß-carotene level over 10 years was not related to 10 year FEV_1 or FVC.

6.3.1.6.10 Lycopene

430 men had paired valid spirometry results and paired plasma lycopene levels. Mean lycopene level at baseline was 0.317 (0.37) μmol/L. The mean change in plasma lycopene level over 10 years was 0.26 (0.81) μmol/L.

There was no significant relationship between baseline lycopene levels (as a continuous or categorical variable) and change in FEV_1 or FVC over 10 years.

Baseline lycopene levels were positively correlated with and 10 year FEV_1 (r=0.15, p=0.001) or FVC (r=0.15, p=0.001) in the crude model but the relationships became non-significant in the adjusted models.

There was no relationship between change in lycopene over 10 years and change in FEV1 or FVC over 10 years. Change in lycopene level over 10 years was not related to 10 year FEV_1 or FVC.

6.3.2 Food frequency questionnaire data

Food frequency questionnaire data was available from all 2745 men in the Belfast cohort at baseline.

6.3.2.1 Mean intake of fruit and vegetables

Mean daily intake of fruit and vegetables (including a maximum of one portion of fruit juice per day) was 2.3 portions (SD 1.33). 42.3% of fruits consumed were citrus fruits and 28.4% of vegetables consumed were raw (see table 6.10).

Table 6.10: Mean daily fruit, fruit juice and vegetable intake in 2745 men at baseline

	Mean (SD) n=2745
Total daily portions of fruit and vegetables *	2.30 (1.33)
Total daily portions of vegetables	0.95 (0.5)
Total daily portions of fruit	0.98 (0.97)
Total daily portions of fruit juice	0.39 (0.49)

*Including a maximum of one portion per day of fruit juice

6.3.2.2 Intake of fruit and vegetables by smoking status

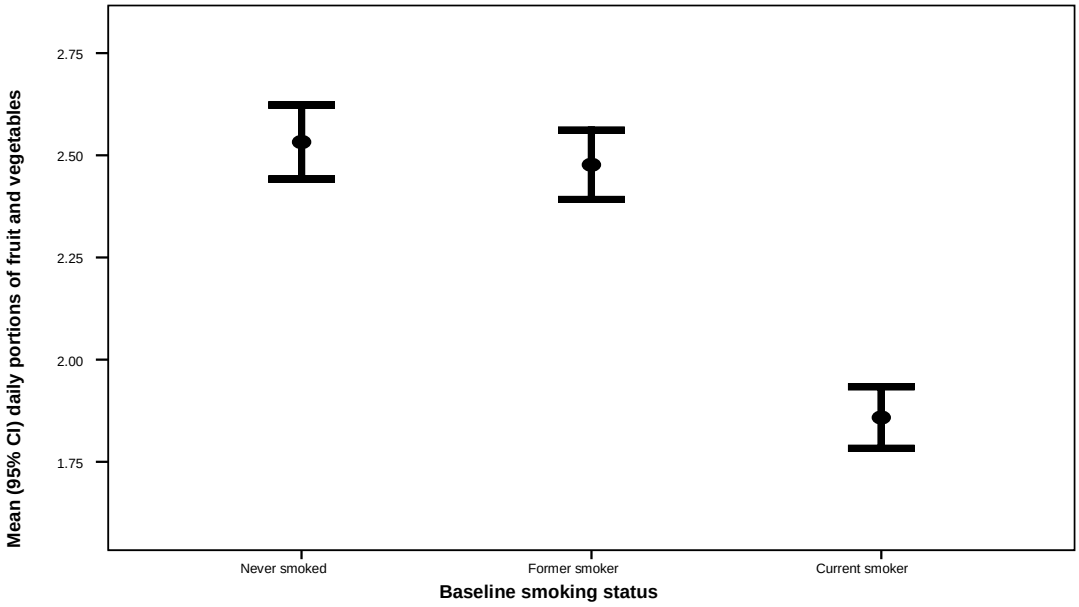
Current smokers ate less fruit, drank less fruit juice and ate less vegetables than former and never smokers (see table 6.11 and figure 6.2).

Table 6.11: Mean daily fruit, fruit juice and vegetable intake stratified by smoking status

	Current smokers n=871 Mean (SD)	Former smokers n=970 Mean (SD)	Never smokers n=904 Mean (SD)	P value (ANOVA)
Total daily portions of fruit and vegetables *	1.85(1.13)	2.48(1.34)	2.53(1.4)	<0.0001
Total daily portions of vegetables	0.88(0.48)	1.00(0.48)	0.97 (0.51)	<0.0001
Total daily portions of fruit	0.67(0.76)	1.08(1.04)	1.14(1.02)	<0.0001
Total daily portions of fruit juice	0.33(0.46)	0.41(0.48)	0.44(0.52)	<0.0001

*Including a maximum of one portion per day of fruit juice

Figure 6.2: Mean total daily fruit, fruit juice and vegetable intake by smoking status at baseline



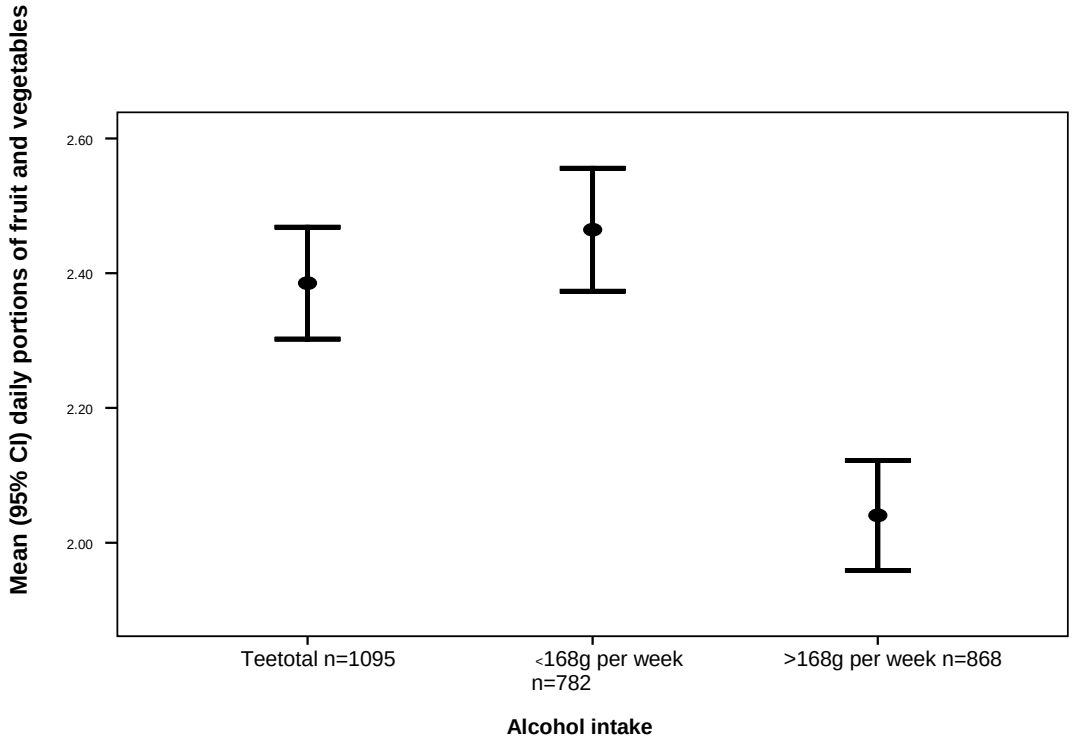
6.3.2.3 Intake of fruit and vegetables by alcohol intake

Men who drank >168g of alcohol (>21 units) per week ate less fruit and vegetables and drank less fruit juice on average than those men who drank < 168g of alcohol per week or who were tee-total (see table 6.12 and figure 6.3).

Table 6.12. Mean total daily fruit, fruit juice and vegetable intake by alcohol intake at baseline

	Teetotal n=1095 Mean (SD)	168g per week or less n=782 Mean (SD)	>168g per week n=868 Mean (SD)	P value (ANOVA)
Total daily portions of fruit and vegetables *	2.39 (1.4)	2.46 (1.3)	2.04 (1.23)	<0.0001
Total daily portions of vegetables	0.95 (0.49)	1.03 (0.49)	0.89 (0.5)	<0.0001
Total daily portions of fruit	1.05 (1.04)	1.04 (0.95)	0.82 (0.89)	<0.0001
Total daily portions of fruit juice	0.42 (0.53)	0.42 (0.48)	0.34 (0.44)	<0.0001

Figure 6.3: Mean total daily fruit, fruit juice and vegetable intake by alcohol intake at baseline



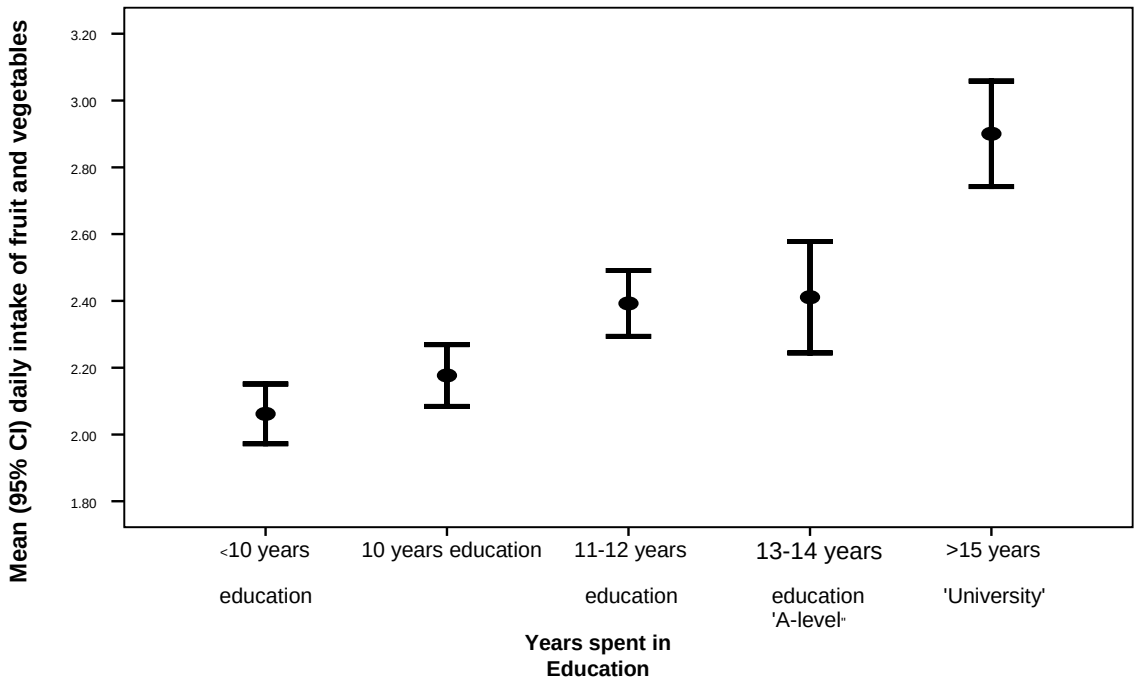
6.3.2.4 Intake of fruit and vegetables by years spent in education

There was a positive relationship between mean fruit, vegetable and fruit juice intake and number of years spent in education (see table 6.13 and figure 6.4).

Table 6.13: Mean total daily fruit, fruit juice and vegetable intake by years spent in education

	Years spent in education – Mean (SD)				
	<10 yr n=795	10 yr n=752	11-12 yr n=595	13-14 yr n=282	>15 yr n=321
Total daily portions of fruit and vegetables *	2.06(1.28)	2.17(1.29)	2.39(1.23)	2.41(1.42)	2.9(1.44)

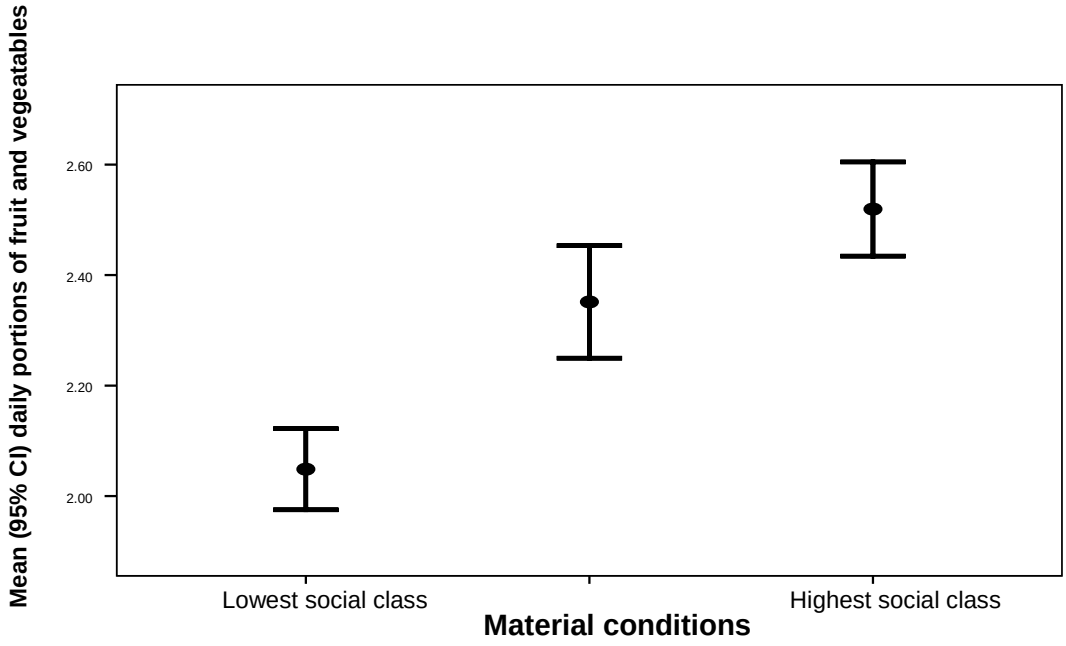
Figure 6.4 Mean daily fruit, fruit juice and vegetable intake by years spent in education at baseline



6.3.2.5 Intake of fruit and vegetables by educational group

Men with better material conditions at baseline had on average a higher intake of fruit, vegetables and fruit juice (see figure 6.5).

Figure 6.5. Mean fruit, vegetable and fruit juice intake by material conditions



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6.3.2.6 Intake of fruit and vegetables by BMI and WHR

There was a positive relationship between mean fruit, vegetable and fruit juice intake and BMI (r=0.07, p=0.01). For every unit increase in fruit, vegetable and fruit juice intake, BMI was on average 0.16 kg/m^2 higher (95% CI 0.06, 0.03; p=0.001, R^2=0.04) after adjustment for age, smoking history, alcohol intake, educational group and material conditions. There was no relationship with waist-hip ratio (p=0.6). These comparisons were not adjusted for total energy intake as this information was not available.

6.3.2.7 Relationship between fruit, fruit juice and vegetable intake and lung function (Cross-sectional)

1779 men had a valid baseline spirometry result at recruitment.

FEV_1 was on average 74.9 ml greater per portion increase in fruit, vegetable and fruit juice intake ($p<0.0001$). When adjusted for age, height, BMI, material conditions, educational group, smoking history (pack years at baseline) and alcohol intake (g/week at baseline), this was attenuated to 22.4 ml, $p=0.035$ (see table 6.14).

FVC was on average 80.1 ml greater per portion increase in fruit, vegetable and fruit juice intake ($p<0.0001$). When adjusted for age, height, BMI, material conditions, educational group, smoking history (pack years at baseline) and alcohol intake (g/week at baseline), this was attenuated to 25.9ml, $p=0.04$ (see table 6.14).

Table 6.14 Relationship between lung function and total fruit, fruit juice and vegetable intake

Outcome	Characteristic	Crude		Adjusted*		
		Mean lung function outcome in mls Mean (SD)	p-value (trend across cats)	Difference in mean outcome (95% CI)	p-value (trend across cats)	R^2
Baseline FEV1 in mls	**Daily Fruit/vegetables and fruit juice intake by category**					
	<1.3 portions/day, n=431	2955 (617)		0 (ref. cat)		
	1.3-2.0 portions/day, n=465	3129 (688)	<0.0001	45.3 (-30, 121)	<0.12	0.31
	2.0-3.0 portions/day , n=433	3173 (706)	(<0.0001)	64.6 (-11, 140)	(0.02)	
	> 3.0 portions/day, n=442	3255 (651)		93.6 (16,171)		
	Daily Fruit/veg and fruit juice intake (portion)	74.9 (51, 99) †	<0.0001	22.4 (1.5, 43) †	0.035	0.31
Baseline FVC in mls	**Daily Fruit/vegetables and fruit juice intake by category**					
	<1.3 portions/day, n=431	3950 (816)	<0.0001	0 (ref.cat.)	<0.15	0.33
	1.3-2.0 portions/day, n=465	4104 (810)	(<0.0001)	8 (-82, 98)	(<0.03)	
	2.0-3.0 portions/day , n=433	4167 (864)		63 (-26, 152))		
	> 3.0 portions/day, n=442	4263 (797)		91.7 (-0.8, 184)		
	Daily Fruit/veg and fruit juice intake (portion)	80.1 (52, 109) †	<0.0001	25.9 (1, 51) †	0.041	0.33

*Adjusted for age, height, smoking history (pack years), body mass index, alcohol intake (g/wk), educational group and material conditions at baseline.
† Increase in lung function outcome per portion increase in fruit/vegetable and fruit juice intake

6.3.2.8 Relationship between lung function and fruit and fruit juice intake (Cross-sectional)

FEV_1 was on average 76 ml greater per portion increase in fruit and fruit juice intake (95%CI 48, 104 p<0.0001). When adjusted for age, height, BMI, material conditions, educational group, smoking history (pack years at baseline) and alcohol intake (g/week at baseline), this was attenuated to 42 ml (95%CI 15, 69, p=0.002).

FVC was on average 72 ml greater per portion increase in fruit and fruit juice intake (95%CI 38, 106, p<0.0001). When adjusted for age, height, BMI, material conditions, educational group, smoking history (pack years at baseline) and alcohol intake (g/week at baseline), this was attenuated to 25 ml (95%CI -5, 54, p=0.1).

When the results were stratified according to smoking status it was clear that the positive association between lung function and fruit and fruit juice consumption in this group was mainly seen in the current smokers. See table 6.15.

Table 6.15 Relationship between fruit and fruit juice intake and lung function, stratified by smoking status

		Crude		*Adjusted*		
Outcome	Smoking status	Mean increase in lung function outcome per portion increase in daily fruit and fruit juice consumption Mean (95%CI)	p	Mean increase in lung function outcome per portion increase in daily fruit and fruit juice consumption Mean (95%CI)	p	R^2
FEV1	Never n=571	24 (-21, 70)	0.29	-1 (-41, 40)	0.97	0.26
	Former n=628	57 (10, 105)	0.02	30 (-12, 73)	0.16	0.28
	Current n=580	73 (18, 128)	0.01	52 (4, 99)	**0.04**	0.29
FVC	Never n=571	40 (-18, 97)	0.17	7 (-41, 55)	0.77	0.33
	Former n=628	54 (-3, 111)	0.06	24 (-25, 73)	0.34	0.32
	Current n=580	76 (7, 145)	0.03	58 (0, 116)	**0.05**	0.32

* Adjusted for baseline age, height, educational group, material conditions, body mass index and alcohol intake (g/week)

6.3.2.9 Relationship between lung function and vegetable intake (cross-sectional)

FEV_1 was on average 143 ml greater per portion increase in vegetable intake (95%CI 80, 206 p<0.0001). When adjusted for age, height, BMI, material conditions, educational group, smoking history (pack years at baseline) and alcohol intake (g/week at baseline), this relationship became non-significant (26 ml, 95%CI -29, 82, p=0.35).

FVC was on average 201 ml greater per portion increase in vegetable intake (95%CI 125, 277 p<0.0001). When adjusted for age, height, BMI, material conditions, educational group, smoking history (pack years at baseline) and alcohol intake (g/week at baseline), this was relationship also became non-significant (60 ml, 95%CI -5, 125, p=0.07). The crude relationship was mainly seen in the never smokers (data not shown).

6.3.2.10 Lung function decline and fruit, fruit juice and vegetable intake (longitudinal)

932 men had valid spirometry traces at baseline and 10 year follow-up.

There was no significant relationship between 10 year lung function decline and fruit, fruit juice and vegetable intake at baseline (p=0.3). There was a trend however. The quartile with the highest fruit, fruit juice and vegetable intake had a slower decline in lung function at 45.2 ml/y (SD 43.9) than those who were in the lowest quartile of fruit, fruit juice and vegetable consumption (42.3 ml/yr, SD 47.0).

6.3.2.11 Baseline fruit, fruit juice and vegetable consumption and 10 year lung function (longitudinal)

FEV_1 at 10 years was on average 62 ml greater per portion increase in fruit/fruit juice/vegetable intake at baseline (95%CI 32, 91 p<0.0001). When adjusted for age, height, BMI, and smoking history at follow-up and material conditions, educational group and alcohol intake (g/week at baseline), this relationship became non-significant (23 ml, 95%CI -4, 49, p=0.09).

FVC at 10 years was on average 63 ml greater per portion increase in fruit and fruit juice intake (95%CI 29, 97 p<0.0001). When adjusted for age, height, BMI, and smoking history at follow-up and material conditions, educational group and alcohol intake (g/week at baseline), this relationship became statistically non-significant (19 ml, 95%CI -10, 48, p=0.2).

6.3.2.12 Baseline fruit, fruit juice and vegetable consumption and C-reactive protein (CRP)

There was an inverse relationship between baseline fruit, fruit juice and vegetable consumption and C-reactive protein (see table 6.16). However, this relationship was not significant when adjustment was made for co-variates.

Table 6.16 Relationship between fruit, fruit juice and vegetable consumption and plasma CRP

Outcome	Characteristic	Crude		Adjusted*		
		Mean lung CRP in mg/L Mean (SD)	**p-value (trend across cats)**	**Difference in mean outcome (95% CI)**	**p-value (trend across cats)**	**R^2**
Baseline CRP in mg/L	**Daily Fruit/vegetables and fruit juice intake by category**					
	<1.3 portions/day, n=431	3.05 (5.5)		0 (ref. cat)		
	1.3-2.0 portions/day, n=465	2.55 (3.8)	0.37	-0.42 (-1.4, 0.5)	0.36	0.02
	2.0-3.0 portions/day , n=433	2.36 (4.6)	(0.83)	-0.48 (-1.4, 0.5)	(0.7)	
	> 3.0 portions/day, n=442	3.03 (10.1)		0.3 (-0.7, 1.3)		
	Daily Fruit/veg and fruit juice intake (portion)	-0.03 (-0.04, -0.01) †	**0.001**	-0.01 (-0.03,0.004) †	0.16	0.13

*Adjusted for age, height, smoking history (pack years), body mass index, alcohol intake (g/wk), educational group and material conditions at baseline.
† Increase in CRP outcome per portion TENFOLD increase in fruit/vegetable and fruit juice intake

6.3.3 Correlations between FFQ and plasma levels

6.3.3.1 Relationship between fruit, fruit juice and vegetable intake and serum levels of antioxidants

α-tocopherol (r=0.11), lutein (r=0.18), ß-cryptoxanthin (r=0.37), and α-carotene (r=0.12) were positively associated with self-reported fruit, fruit juice and vegetable intake, in both the adjusted and unadjusted models.

Zeaxanthin (r=0.13) was positively associated with self-reported fruit, fruit juice and vegetable intake in the unadjusted model only.

γ-tocopherol was negatively associated with self-reported fruit, fruit juice and vegetable intake in the adjusted model.

Retinol, ß-carotene and lycopene were not significantly related to self-reported fruit, fruit juice and vegetable intake.

Table 6.17: Relationship between fruit, fruit juice and vegetable intake and serum levels of antioxidants

	Crude		Adjusted*		
Antioxidant	Increase in serum level of antioxidant per unit increase in fruit, fruit juice and vegetable intake µmol/L (95%CI)	P	Increase in serum level of antioxidant per unit increase in fruit, fruit juice and vegetable intake µmol/L (95%CI)	p	R^2
Retinol	0.004(-0.03,0.04)	0.8	0.005 (-0.03, 0.04)	0.75	0.2
γ-tocopherol	-0.1 (-0.2, 0.01)	0.08	-0.11 (-0.22, -0.003)	**0.045**	0.13
α-tocopherol	0.7 (0.3, 1.2)	**0.003**	0.51 (0.1, 0.9)	**0.02**	0.275
Lutein	0.009(0.005, 0.1)	**<0.001**	0.007 (0.003, 0.01)	**<0.001**	0.12
Zeaxanthin (log)	0.03 (0.01, 0.05)	**0.002**	0.02 (-0.003, 0.03)	0.1	0.11
ß-cryptoxanthin (log)	0.09 (0.07, 0.1)	**<0.001**	0.07 (0.06, 0.09)	**<0.001**	0.26
α-carotene (log)	0.04 (0.009, 0.06)	**0.01**	0.03 (0.001, 0.06)	**0.046**	0.07
ß-carotene (log)	0.02 (-0.002, 0.05)	0.07	0.017 (-0.006, 0.04)	0.14	0.15
lycopene (log)	0.03 (-0.003, 0.06)	0.08	0.02 (-0.01, 0.05)	0.3	0.07

*Adjusted for smoking history, material conditions, educational group, BMI, age, cholesterol and alcohol intake

Table 6.18 Relationship between fruit, vegetable and fruit juice intake and plasma levels of antioxidants

	Mean daily portions of fruit, vegetables and fruit juice			
Plasma antioxidant levels in μmol/L	***<1.36 portions per day***	***1.36-2.0 portions per day***	***2-3 portions per day***	***>4 portions per day***
	Mean (SD)	***Mean (SD)***	***Mean (SD)***	***Mean (SD)***
Retinol	2.12 (0.61)	2.06 (0.58)	2.11 (0.56)	2.07 (0.58)
γ-tocopherol	3.84 (2.07)	4.05 (2.28)	3.57 (1.51)	3.68 (1.71)
α-tocopherol	30.7 (8.9)	33.0 (8.98)	32.4 (8.47)	33.7 (9.1)
Lutein	0.13 (0.05)	0.14 (0.07)	0.13 (0.06)	0.16 (0.07)
	Median (IQ range)	***Median (IQ range)***	***Median (IQ range)***	***Median (IQ range)***
Zeaxanthin	0.018 (0.012-0.03)	0.02 (0.012-0.03)	0.019 (0.012-0.032)	0.023 (0.017-0.035)
ß-cryptoxanthin	0.037 (0.024-0.06)	0.046 (0.03-0.06)	0.05 (0.033-0.075)	0.074 (0.04 – 0.11)
α-carotene	0.025 (0.013-0.065)	0.045 (0.023-0.07)	0.04 (0.02-0.07)	0.045 (0.02 – 0.08)
ß-carotene	0.19 (0.09-0.32)	0.257 (0.12-0.36)	0.22 (0.14-0.34)	0.23 (0.13-0.37)
lycopene	0.16 (0.07-0.4)	0.194 (0.1-0.4)	0.23 (0.07-0.44)	0.21 (0.09-0.42)

6.3.3.2 ß-cryptoxanthin

561 men had available baseline serum ß-cryptoxanthin levels. For this study, baseline serum was only available for the men who had valid baseline and follow-up spirometry measurements (932 men). 371 men did not have a valid serum ß-cryptoxanthin measurement due to lack of available plasma (202 men) or machine failure (169 men). There was a positive relationship between self-reported fruit, fruit juice and vegetable intake and plasma ß-cryptoxanthin levels (r=0.37). Plasma ß-cryptoxanthin levels were 0.007μmol/L higher per additional portion of fruit/fruit juice/vegetables eaten daily.

Table 6.19: Relationship between self-reported fruit, fruit juice and vegetable intake and plasma ß-cryptoxanthin levels

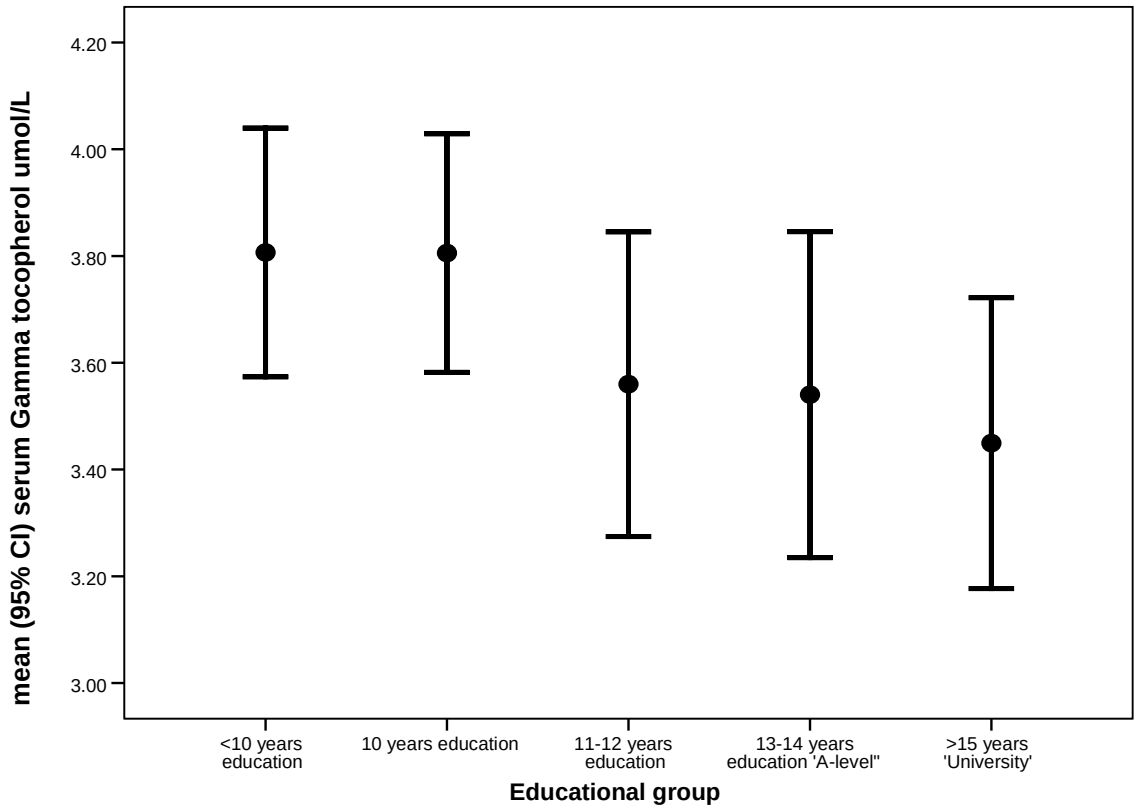
Outcome	Characteristic	Crude		Adjusted*		
		Plasma ß-cryptoxanthin level μmol/l Mean (SD)	**P**	**Difference in mean outcome (95% CI)**	**p-value (trend across cats)**	**R^2**
Baseline plasma ß-cryptoxanthin level μmol/l	**Fruit/veg intake by category**					
	<1.5 portions, n=140	0.049 (0.05)		0 (ref. cat)		
	1.5-2.3 portions, n=150	0.052 (0.03)	<0.0001	0.002 (-0.01,0.014)	<0.0001	0.19
	2.3-3.0 portions, n=133	0.065 (0.04)	(<0.0001)	0.016 (0.004, 0.027)	(<0.0001)	
	>3 portions, n=138	0.091 (0.07)		0.036 (0.024, 0.048)		
	Fruit/fruit juice/veg intake	0.0086 (0.007, 0.01) †	<0.0001	0.0074 (0.006, 0.009) †	<0.0001	0.24

*Adjusted for age, smoking history, body mass index, cholesterol, educational group and material conditions at baseline.
† Increase in ß-cryptoxanthin per unit increase in fruit, fruit juice and vegetable intake

6.3.3.3 γ-tocopherol

γ-tocopherol appears to be a marker of poor nutrition in this population. It was negatively associated with self-reported fruit, fruit juice and vegetable intake in the adjusted model. Men who spent less time in education had higher levels of γ-tocopherol.

Figure 6.6 Relationship of plasma γ-tocopherol and educational group



6.3.3.4 Relationship between vegetable intake and serum levels of antioxidants

α-tocopherol (r=0.1), lutein (r=0.18), ß-cryptoxanthin (r=0.17), and α-carotene (r=0.12) and ß-carotene (r=0.1) were positively associated with self-reported vegetable intake.

Retinol, γ-tocopherol, zeaxanthin and lycopene were not significantly related to self-reported vegetable intake. Daily vegetable intake was normally distributed.

Table 6.20: Relationship between vegetable intake and serum levels of antioxidants

	Unadjusted		Adjusted*		
Antioxidant	Increase in serum level of antioxidant per unit increase in vegetable intake. μmol/l	p	Increase in serum level of antioxidant per unit increase in vegetable intake. μmol/l	p	R^2
Retinol	0.03 (-0.06, 0.1)	0.5	0.012 (-0.07, 0.09)	0.8	0.19
γ-tocopherol	-0.19 (-0.46, 0.09)	0.19	-0.17 (-0.44, 0.1)	0.2	0.13
α-tocopherol	1.69 (0.4, 3)	**0.01**	1.17 (0.03, 2.3)	**0.045**	0.27
Lutein	0.023 (0.01, 0.03)	**<0.001**	0.02 (0.01, 0.03)	**<0.001**	0.12
Zeaxanthin (log)	0.05 (-0.002, 0.09)	0.06	0.02 (-0.03, 0.07)	0.43	0.1
ß-cryptoxanthin (log)	0.1 (0.05, 0.15)	**<0.001**	0.073 (0.03, 0.12)	**0.003**	0.18
α-carotene (log)	0.1 (0.03, 0.17)	**0.009**	0.09 (0.02, 0.2)	**0.015**	0.07
ß-carotene (log)	0.07 (0.004, 0.132)	**0.036**	0.07 (0.004, 0.13)	**0.038**	0.15
lycopene (log)	0.08 (-0.003, 0.17)	0.06	0.07 (-0.01, 0.2)	0.1	0.07

*Adjusted for smoking history, material conditions, educational group, BMI, age, cholesterol and alcohol intake

6.3.3.5 Relationship between fruit and fruit juice intake and serum levels of antioxidants

Serum levels of ß-cryptoxanthin (r=0.3), α-tocopherol (r=0.07) and α- carotene (r=0.11) were positively associated with self-reported fruit and fruit juice intake. Serum levels of retinol, γ-tocopherol, lutein, zeaxanthin, ß-carotene and lycopene were not significantly related to self-reported fruit and fruit juice intake. The distribution of fruit and fruit juice intake skewed to right therefore it was log transformed for analysis.

Table 6.21: Relationship between fruit and fruit juice intake and serum levels of antioxidants

	Unadjusted		Adjusted*		
Antioxidant	Increase in serum level of antioxidant per tenfold increase in fruit and fruit juice. μmol/l (95%CI)	p	Increase in serum level of antioxidant per tenfold increase in fruit and fruit juice. μmol/l (95%CI)	p	R^2
Retinol	-0.014 (-0.12, 0.09)	0.8	-0.02 (-0.12, 0.08)	0.73	0.19
γ-tocopherol	-0.05 (-0.38, 0.3)	0.76	-0.098 (-0.4, 0.23)	0.56	0.13
α-tocopherol	2.2 (0.6, 3.7)	**0.06**	1.8 (0.4, 3.16)	**0.01**	0.27
Lutein	0.008 (0.003, 0.012)	**0.016**	0.012 (0.00, 0.024)	0.06	0.11
Zeaxanthin (log)	0.05 (-0.01, 0.1)	0.12	0.017 (-0.04, 0.08)	0.5	0.11
ß-cryptoxanthin (log)	0.21 (0.16, 0.27)	**<0.001**	0.177 (0.121, 0.23)	**<0.001**	0.26
α-carotene (log)	0.1 (-0.02, 0.2)	**0.018**	0.09 (-0.001, 0.18)	**0.05**	0.07
ß-carotene (log)	0.04 (-0.04, 0.1)	0.31	0.02 (-0.06, 0.1)	0.6	0.14
lycopene (log)	0.08 (-0.03, 0.2)	0.14	0.06 (-0.05, 0.16)	0.3	0.07

*Adjusted for smoking history, material conditions, educational group, BMI, age, cholesterol and alcohol intake

ß-cryptoxanthin was the only antioxidant to have a positive relationship with citrus fruit intake (P<0.001). Per portion increase in citrus fruit intake, serum ß-cryptoxanthin levels were on average 0.019 μmol/l higher (95%CI 0.015, 0.024, p<0.001, r^2=0.26)

6.3.3.6 Relationship between plasma antioxidant levels and smoking history at baseline

Serum levels of retinol, lutein, zeaxanthin, ß-cryptoxanthin, α-carotene, ß-carotene and lycopene were highest in never smokers and lowest in current smokers in this population at baseline. There were no significant differences in serum levels of γ-tocopherol and α-tocopherol between the smoking groups at baseline.

Table 6.22 Relationship between plasma antioxidant levels and smoking history at baseline

μmol/L	Never smoker mean (SD) μmol/L	Former smoker mean (SD) μmol/L	Current smoker mean (SD) μmol/L	p
Retinol	2.00(0.5) n=257	2.18 (0.6) n=295	2.06 (0.6) n=170	**0.001**
γ-tocopherol	3.77(1.8) n=256	3.85 (2.1) n=296	3.64 (1.7) n=170	0.52
α-tocopherol	32.7 (8.9) n=256	33 (9) n=295	31.4 (8.6) n=171	0.19
Lutein	0.15 (0.07) n=217	0.14 (0.07) n=244	0.13 (0.06) n=147	**0.02**
Zeaxanthin	0.029 (0.024) n=215	0.026 (0.02) n=245	0.020 (0.015) n=141	**0.001**
ß-cryptoxanthin	0.08 (0.06) n=201	0.062 (0.05) n=228	0.044 (0.03) n=132	**<0.0001**
α-carotene	0.07 () n=182	0.054 () n=202	0.05 () n=114	**0.006**
ß-carotene	0.35 (0.27) n=183	0.26 (0.24) n=200	0.23 (0.17) n=114	**<0.0001**
Lycopene	0.40 () n=176	0.29 () n=196	0.24 () n=480	**0.001**

6.3.4 Summary

Table 6.23. Summary table of relationships between baseline levels of antioxidants measured and lung function as measured by FEV_1

Baseline Plasma levels	Baseline FEV_1 (Unadjusted)	Baseline FEV_1 (Unadjusted)	10 year FEV_1	Change in FEV_1 over 10 year study period
Retinol	**Yes**	**Yes**	**Yes**	No
γ-tocopherol	No	No	No	No
α-tocopherol	**Yes**	No	No	No
Lutein	No	No	No	No
Zeaxanthin	**Yes**	No	No	No
ß-cryptoxanthin	**Yes**	No	**Yes**	No
α-carotene	**Yes**	No	No	No
ß-carotene	**Yes**	No	No	No
Lycopene	**Yes**	No	No	No

Yes indicates a significant positive relationship unless otherwise stated

Table 6.24 Summary table of relationships between 10 year follow-up levels of antioxidants and lung function as measured by FEV_1

10 year Plasma levels of antioxidants	10 year FEV_1 (Unadjusted)	10 year FEV_1 (Unadjusted)	Change in FEV_1 over previous 10 years
Retinol	No	No	No
γ-tocopherol	**Yes (inverse)**	**Yes (inverse)**	No
α-tocopherol	No	No	No
Lutein	**Yes**	No	No
Zeaxanthin	**Yes**	**Yes**	No
ß-cryptoxanthin	**Yes**	**Yes**	**Yes**
α-carotene	**Yes**	**Yes**	No
ß-carotene	**Yes**	**Yes**	No
Lycopene	No	No	No

**Yes indicates a significant positive relationship unless otherwise stated*

6.4 Discussion

This study adds to the evidence base of the role of diet in lung health. This area has been widely examined and it is uncertain if the accumulated findings represent causal links (Cassano 2009).

6.4.1 Plasma antioxidant levels and lung function

Mean 10 year plasma antioxidant levels were higher for all of the antioxidants except γ- and α-tocopherol. This may have been due to the fact that some of the men gave up smoking over the 10 year study period or possibly that the men's diet changed as they moved into retirement. It may also be possible that the antioxidant levels in the baseline samples degraded over time. This is unlikely as the samples were stored appropriately at -80°C (Drammeh et al. 2008, Comstock, Alberg & Helzlsouer 1993, Ocke et al. 1995).

6.4.1.1 Cross-sectional associations

At baseline, retinol, α-tocopherol, zeaxanthin , β-cryptoxanthin, α-carotene, β-carotene and lycopene were all positively correlated with FEV_1. Retinol, β-cryptoxanthin, α-carotene, β-carotene and lycopene were also positively correlated with FVC. γ-tocopherol was negatively associated with FVC. After adjustment for educational group, material conditions, age, height, body mass index, cholesterol, alcohol intake (g/week) and smoking history (pack years) the relationships all became non-significant with the exception of retinol which remained positively related to FEV_1 and FVC.

Serum levels of lutein, zeaxanthin, ß-cryptoxanthin, α-carotene, ß-carotene and lycopene were highest in never smokers and lowest in current smokers in this population at baseline. There were no significant differences in serum levels of γ-tocopherol and α-tocopherol between the smoking groups at baseline.
In the 10 year follow-up cross-sectional analysis there was a positive linear relationship between plasma levels of lutein, zeaxanthin, ß-cryptoxanthin, α-carotene, ß-carotene both 10 year FEV_1 and 10 year FVC. After adjustment for educational group, material conditions, age, height, body mass index, cholesterol and smoking history (pack years), the relationships remained significant (with the exception of lutein). There was a significant negative relationship with plasma levels of γ-tocopherol which remained borderline significant after adjustment for confounding variables.

It is interesting that there are more cross-sectional associations in the study at 10 years than at baseline. This may be because the numbers of men included in the ten year study were greater as we did not have available stored blood from some of the men at baseline.

Cross-sectional associations are difficult to interpret. It is unclear if associations represent causal and potentially modifiable effects on lung health, or whether they arise form the effects of unrecognised confounding e.g. correlation with other causally-related nutrients or non-dietary exposures (McKeever et al. 2002, Allen, Britton & Leonardi-Bee 2009). All observational studies are at risk of selection, response bias and recall bias.

It has been suggested that some diseases arising in adulthood had their origins in early life so studies of adult diet may mispresent the magnitude of the true causal association (Cassano 2009). Consumption of apples and fish during pregnancy may have a protective effect against the development of childhood asthma and allergic disease (Willers et al. 2007).

Grievink et al reported that serum levels of the carotenoids lycopene, α-carotene and β-carotene were positively associated with lung function in an elderly sample of the Dutch population (aged 65-85y) (Grievink et al. 2000). Schunemann et al found that the strongest associations were for ß-cryptoxanthin, lutein and zeaxanthin (Schunemann et al. 2001). McKeever et al found that FEV_1 was independently and directly related to levels of vitamin A, ß-cryptoxanthin, lutein/zeaxanthin, lycopene, vitamin C and vitamin E (McKeever et al. 2008). Of nutrients with linear associations with FEV_1 the strongest effect per standard deviation change was evident for vitamin A. Of variables with non-linear associations, the strongest category effect was seen with ß-cryptoxanthin.

Ochs Balcom et al studied 218 patients with chronic airflow limitation and found cross-sectional positive associations between percent predicted FEV_1 and serum levels of β-cryptoxanthin, lutein/zeaxanthin and retinol, and dietary β-carotene, β-cryptoxanthin, lutein/zeaxanthin , vitamin C and lycopene. Serum β-

cryptoxanthin, lutein/zeaxanthin, lycopene and dietary β-cryptoxanthin, β-carotene, Vitamin C and lutein/zeaxanthin were positively associated with percent predicted FVC (Ochs-Balcom et al. 2006). Relatively low dietary intakes of vitamins A and C are associated with statistically significant increased odds of asthma and wheeze. Vitamin E intake does not appear to be related to asthma status (Allen, Britton & Leonardi-Bee 2009). In a population-based FFQ study, lutein and zeaxanthin were found to have the strongest association with pulmonary function (Schunemann et al. 2002).

6.4.1.2 Longitudinal associations

Only a few of the antioxidants tested showed significant longitudinal correlations with lung function. Baseline plasma retinol levels were positively related to 10 year FEV_1 and FVC. Baseline plasma zeaxanthin levels were related to change in FVC over 10 years. Baseline plasma β-cryptoxanthin levels were positively correlated with 10 year FEV_1. Also, change in β-cryptoxanthin over 10 years positively correlated with 10 year FEV_1.

6.4.2 Self-reported fruit, vegetable and fruit juice intake and lung function

Biochemical measurement of plasma antioxidant levels is more objective than dietary intake estimates by food frequency questionnaire (Redlich et al. 1996). However, plasma levels often reflect only recent intake and are influenced by other metabolic or excretory influences (Allen, Britton & Leonardi-Bee 2009). Measurement of plasma levels of antioxidants may not necessary reflect antioxidant levels within the lung (Britton 1995), however, levels of carotenoids in lung tissue have correlated more strongly to blood levels of carotenoids than to dietary intake of carotenoids measured by questionnaire (Grievink et al. 2000, Redlich et al. 1996).

The food frequency questionnaire which was used at baseline in the PRIME study to estimate dietary intake focussed on fruit, fruit juice and vegetable intake (see appendices). These foods are rich sources of the antioxidant vitamins and carotenoids which were measured in the stored plasma samples at baseline and 10 year follow-up. The middle-aged men in this study had an average intake of only 2.3 portions per day which is less than half of the current recommended amount (Department of Health 2005). Despite this low intake, relationships between fruit and vegetable intake and lung function were apparent.

6.4.2.1 Cross-sectional associations

At baseline, FEV_1 was on average 22.4 ml greater per portion increase in fruit, fruit juice and vegetable intake after adjustment for confounding variables. The results were similar for baseline FVC. When fruit and fruit juice intake were considered separately, the positive relationship between FEV_1 at baseline and fruit/fruit juice intake was maintained but was most marked among the current smokers. Antioxidants along with vitamin C may play a role in protecting the lungs from damage by tobacco smoke, ozone and nitrogen dioxide (Watson et al. 2002, Butland, Strachan & Anderson 1999). It is possible be that we require a threshold level of antioxidants and anything above this level does not add any extra benefit.

When vegetable intake was considered separately, there was a positive relationship with lung function but this became non-significant after adjustment for confounding variables. This may be related to the fact that the average daily vegetable intake was less than one portion per day and therefore, variation in vegetable intake between individual men may not be large enough to see any effects of increased vegetable intake.

6.4.2.2 Longitudinal associations

Baseline fruit, fruit juice and vegetable intake did not significantly relate to lung function decline. There was a trend, however. The quartile with the highest fruit, fruit juice and vegetable intake had a slower decline in lung function than those who were in the lowest quartile of fruit, fruit juice and vegetable consumption. There was also a crude relationship between baseline fruit, fruit juice and vegetable consumption and 10 year lung function.

Other studies have shown a relationship between dietary intake and lung function decline. Tabak et al reported an inverse relationship between apple consumption

and lung function decline (Tabak et al. 1998). It is possible that fresh fruit is acting as a marker for another lifestyle intervention or food or behaviour which can positively affect lung function. It may be that other antioxidants (eg flavonoids) found in hard fruits such as apples are important (Butland, Fehily & Elwood 2000, Miedema et al. 1993).

6.4.2.3 Correlation with plasma antioxidants levels

There was a positive correlation between plasma antioxidant levels and dietary intake. α-tocopherol (r=0.11), lutein (r=0.18), ß-cryptoxanthin (r=0.37), and α-carotene (r=0.12) were positively associated with self-reported fruit, fruit juice and vegetable intake. Plasma ß-cryptoxanthin was the best biomarker of fruit, fruit juice and vegetable intake (r=0.37). Plasma ß-cryptoxanthin levels were 0.007μmol/L higher per additional portion of fruit, fruit juice and vegetables eaten daily. These findings concur with many previous studies (Al-Delaimy et al. 2005, Dixon et al. 2006).

It is interesting to note that γ-tocopherol was negatively associated with self-reported fruit, fruit juice and vegetable intake in the adjusted model. Plasma γ-tocopherol levels were also inversely related to time spent in education. There is disagreement about the health significance of γ-tocopherol. It possesses metabolic attributes not shared by the α-isomer so it may be differently related to risk indices and factors (Bates, Mishra & Prentice 2004). In this study γ-tocopherol does seem to be a biomarker of poor nutrition. This has been suggested by other studies (Bates, Mishra & Prentice 2004).

α-tocopherol (r=0.1), lutein (r=0.18), ß-cryptoxanthin (r=0.17), and α-carotene (r=0.12) and ß-carotene (r=0.1) were positively associated with self-reported

vegetable intake. Serum levels of ß-cryptoxanthin (r=0.3), α-tocopherol (r=0.07) and α- carotene (r=0.11) were positively associated with self-reported fruit and fruit juice intake.

6.4.2.4 Smoking and fruit consumption

In this population, smokers had a statistically lower intake of fruit, vegetables and fruit juice than former smokers and never smokers. 50.0% of the men in the lowest quartile of fruit consumption were current smokers, 31.3% were former smokers and 18.7% had never smoked. This effect was independent of educational group, age, alcohol intake and material conditions.

It is unclear why smokers eat less fruit and vegetables. It is possible that smokers generally have an unhealthier lifestyle and are more likely to ignore healthy eating advice or who adopt a fatalistic attitude to health (Maynard et al. 2006, Tang et al. 1997). Financial constraints may also have a role – perhaps cigarettes are purchased instead of fresh food. Smoking may alter the way that fruit and vegetables taste to smokers or increase the appetite for foods other than fruit and vegetables (Maynard et al. 2006, de Jong et al. 1999).

Men who drank >168g alcohol per week also had a lower intake of fruit, fruit juice and vegetables. Years spent in education was also independently proportional to total daily intake of fruit and vegetables. It has been suggested that below average fruit and vegetable intake in lower socioeconomic groups is secondary to poverty, poor nutrition knowledge and social and environmental barriers (Estaquio et al. 2008).

6.4.3 Limitations of study

This study was limited by the fact that of the 2745 men who were recruited into the study, paired plasma antioxidant levels are only available for 721 men (480 for lycopene). This was due to the scarcity of baseline plasma available and the fact that many of the spirometry measurements were not valid. We were only permitted to use the stored baseline plasma from men with valid paired spirometry.

Only men were included in the PRIME study so we cannot comment on relationships between lung function and antioxidants in women. We did not adjust for vitamin supplement use as the questionnaire data was not robust enough. Also supplements in the early 1990s are unlikely to have contained carotenoids, which are our main focus. The high rate of non-responders and missing data leaves the possibility for selection bias. Therefore findings can only be generalised with caution.

The food frequency questionnaire was not repeated at 10 year follow-up. This would have been useful to assess changes in diet experienced by the men as they retired from work. The majority of the cohort was married or co-habiting. It is possible that the dietary patterns of many of these men were influenced by the shopping and cooking choices of their partners (Schafer et al. 1999). The type of food available in the workplace would also be relevant e.g. canteen, packed lunch. This information was not ascertained in the questionnaire. It is unfortunate that we do not have information on type of fruit eaten or portion sizes.

6.4.4 Antioxidant supplementation

There have been several studies involving antioxidant supplementation. A meta-analysis of antioxidant supplement trials has suggested that supplementation may increase mortality (Bardia et al. 2008). The MRC/BHF Heart Protection Study involved an intervention of 5 years of vitamin C, E and ß-carotene supplementation. There was no difference in lung function between control and intervention groups. Regular dietary supplementation with vitamin C or magnesium adds no clinical benefit to current standard therapy of asthma in primary care patients (Fogarty et al. 2003). It is possible that supplements are only beneficial when the oxidative burden is high and the initial antioxidant status is low. With these results in mind, it is hard to justify further supplement trials in their current form. Several ‘whole food’ intervention trials are underway at present. This may prove more beneficial than isolated nutrient replacement.

6.4.5 Conclusion

This work confirms that, in terms of respiratory function, a diet rich in fruit, vegetables and fruit juice is associated with higher values of FEV_1 and FVC. Plasma levels of retinol, lutein, zeaxanthin, ß-cryptoxanthin, α-carotene and ß-carotene were positively correlated with lung function at either baseline or follow-up. There were also some longitudinal associations between baseline retinol, zeaxanthin, β-cryptoxanthin and 10 year lung function. Self-reported fruit and vegetable intake was positively correlated with both lung function and plasma levels of antioxidants.

These findings add weight to current government recommendations to include 5 portions of fruit and vegetables per day in an individual's daily diet. It is important that these habits be established early in life in order to impact long-term lung function and lung function decline.

Epidemiological studies are useful for identifying association between exposure and diseases but cannot reliably establish causation (Egger, Schneider & Davey Smith 1998). Further investigations are necessary to account for the observed associations using well-designed randomised-controlled trials of dietary intervention.

7.0 Summary, further work and closing thoughts

7.0 Summary, further work and closing thoughts

7.1 Summary of main findings

In this population of middle-aged Northern Irish men, lifestyle choices such as smoking and alcohol intake affected lung function. Smoking accelerated lung function decline. Heavy alcohol intake was related to poor lung function but moderate alcohol intake was associated with higher mean FEV_1 levels than abstinence.

Cross-sectional and longitudinal relationships between lung function, WHR, BMI and waist circumference have also been demonstrated. WHR, BMI and waist circumference were inversely associated with pulmonary function. FEV_1 decline was related to the change in distribution of weight as well as to weight gain. This study supports the use of WHR in addition to BMI in assessing potential lung function decline.

This thesis provides further evidence that plasma CRP levels are inversely associated with lung function in cross-sectional studies even within the normal range. There was an inverse longitudinal relationship between change in serum CRP and change in lung function in this population of middle-aged men over the 10 year study period. The presence of systemic inflammation was demonstrated, even in subjects with modest decreases in pulmonary function. Current smokers had the highest plasma CRP levels. Plasma CRP levels were positively correlated with both WHR ($r=0.25$, $p=0.01$) and BMI ($r=0.21$, $p=0.01$) in the cross-sectional analysis.

This thesis also confirms that a diet rich in fruit, vegetables and fruit juice is associated with higher values of FEV_1 and FVC. Plasma levels of retinol, lutein, zeaxanthin, ß-cryptoxanthin, α-carotene and ß-carotene were positively correlated with lung function at either baseline or follow-up. There were also some longitudinal associations between baseline retinol, zeaxanthin, β-cryptoxanthin and 10 year lung function. Self-reported fruit and vegetable intake was positively correlated with both lung function and plasma levels of antioxidants.

7.2 Limitations of these studies

The PRIME study only contains data from middle-aged men in Northern Ireland so our findings cannot be generalised to include other groups, for example, women. The sick and unemployed are unrepresented so there is likely a healthy worker effect. However, it must be noted that many of the men would have been exposed to various industrial gases and chemicals which may have affected their lung function. It is difficult to compare the data directly to the NI Census data from 1991 as the published census data is not fully categorised by age and sex. The proportions of married and single men can be ascertained and these are very similar to the PRIME figures (Northern Ireland Statistics and Research Agency).

There was a 52% response rate to the initial mailings so there may be some selection bias, however this response rate is similar to that obtained in the MONICA (MONitoring CArdiovascular disease) surveys (Evans et al. 2001). No information is available about the non-responders so it is difficult to quantify this. Caution must therefore be used in translating findings from this study to a different population.

Cross-sectional data is limited in that valid inferences regarding causal pathways cannot be drawn.

Spirometry was measured on two occasions, 10 years apart. It would be preferable to have had both annual spirometry measurements and plasma samples. Spirometry is dependent on the voluntary effort exerted in performing the manoeuvre and was a potential source of bias. However, reproducibility criteria were met by all of the spirometry traces included in the analysis. We did not have reliable data for co-morbidities or medication. It would have been helpful to know if any of the men had a history of asthma or were on bronchodilators.

Each individual spirometry trace (baseline and 10 year follow-up) was reviewed for validity using ATS/ERS criteria (Miller et al. 2005). It may be argued that these criteria were too strict and that too many lung function results were counted as invalid (35.2% at baseline). It may have been a better approach to allow traces in which there were 2 attempts with less than 10% variability or to include traces in which the two largest FEV_1 or FVC values were greater than 150 ml of each other.

I decided with my supervisors to apply the ATS/ERS Criteria to the PRIME spirometry traces at baseline and follow-up as I wanted to ensure that the data used in my analyses was accurate and standardized. I felt that this was important as I had not been present when the original data was being collected and it was clear that some of the spirometry traces were invalid.

Disadvantages of this decision were that this discounted 560 traces at baseline and 341 at 10 year follow-up which had poor repeatability (i.e. the two largest values of FEV_1 or FVC were greater than 150 ml of each other). This reduced the

number of men who could be included in the studies included in this thesis and may have excluded some men with reversible airways disease unnecessarily. The ATS/ERS criteria were published in 2005 which was after the PRIME study lung function data collection had been completed, however, previous guidelines actually had even stricter criteria (Anonymous1995).

This study was also limited by the fact that of the 2745 men who were recruited into the PRIME study, paired plasma antioxidant levels were only available for 721 men (480 for lycopene). This was due to the scarcity of baseline plasma available and the fact that many of the spirometry measurements were not valid. We were only permitted to use the stored baseline plasma from men with valid paired spirometry.

Multiple comparisons

Because the effects of many exposures were measured we need to take some caution in interpreting the results. This is due to the fact that even if there was no association between an exposure and an outcome we would expect one in twenty comparisons to be statistically significant at the 5% level (Kirkwood, Sterne 2004).

Analysis of lung function decline

As previously discussed, from the age of 25, lung function begins to decline gradually throughout adult life, the rate of decline differing among individuals (Jiang et al. 2008). Analysing baseline variables and their relationship to 10 year lung function is helpful in identifying potential predictors of lung function decline e.g. waist-hip ratio. Analysis of change in variables with respect to change in lung function can perhaps suggest possible causation e.g. C-reactive protein.

However, it must be remembered that observational studies cannot prove causation.

The effect of adjusting for confounding variables was most marked in the antioxidant studies. In particular, adjusting for material conditions and educational group significantly changed the associations seen in the crude analysis. It is possible that other unmeasured social class variables also affect lung function decline in this population.

7.3 Relevance of this study for patients, clinicians and populations

This study suggests that waist-hip ratio should be used in addition to body mass index in assessing potential lung function decline. WHR is a simple well-standardized, low cost measurement which should be easy to include in population studies (Harik-Khan, Wise & Fleg 2001) and in clinical assessments and may improve interpretation of longitudinal changes in lung function.

This analysis provides further evidence that plasma CRP levels are inversely associated with lung function in cross-sectional studies even within the normal range. Perhaps we should consider routinely measuring CRP levels in conjunction with lung function measurement as an indicator of prognosis even in stable respiratory patients. There are also implications for potential drug therapies. Would specifically reducing CRP, improve pulmonary function or slow FEV_1 decline and thereby improve survival?

These findings also add weight to current government recommendations to include 5 portions of fruit and vegetables per day in an individual's daily diet. It is

important that these habits be established early in life in order to impact long-term lung function and lung function decline.

7.4 Future Research

7.4.1 Genetic data

White cells for DNA extraction were collected from the cell pellets of citrated blood from each of the men at baseline. There are plans to extract the DNA for each individual man. This would be an excellent opportunity to study a huge range of genetic factors influencing lung function.

7.4.2 Vitamin C analysis

The samples which were stored for future Vitamin C analysis from the Belfast men were originally sent to France for storage and analysis and were unavailable to me at the time of writing. The analysis has only been carried out on a subset of the samples so far but there are plans to measure vitamin C levels at both baseline and 10 year follow-up for the cohort as a whole. I plan to be involved in the analysis of this data and in particular to study the relationships between vitamin C levels and lung function decline, dietary intake of fruit and vegetables and C-reactive protein.

7.4.3 C-Reactive Protein and antioxidants

I plan to carry out further statistical analysis on this dataset to look for any relationship between plasma antioxidant levels and subclinical inflammation as measured by C-Reactive Protein.

7.4.4 Mortality study

Of the baseline 2745 men in the PRIME study, 50 died from a smoking associated lung cancer and 182 died from other causes. It would be useful to

carry out a mortality study to investigate the predictive value of baseline FEV_1 in this group of men at baseline.

7.5 Closing thoughts

This body of work has been both challenging and rewarding to prepare. I was given the opportunity to learn new laboratory techniques and procedures which have given me an insight into the world of scientific research from the frustrations of machine failure, to the joys of finding that missing sample! I have benefitted from preparing and giving oral presentations on this work at local, national and international conferences and I have learned a great deal from attending epidemiology courses and lectures both about the complexity of epidemiological analysis and the amount I still have to learn!

I now have experience in working with a large dataset and a better understanding of statistical methods which is helpful when reading literature. I found it challenging to become involved in a project over 14 years from when it was first designed. It would have been excellent to have had full pulmonary function test including transfer factor, reversibility and lung volumes, respiratory medical histories, symptoms and medication lists for each participant but the study was originally designed to examine cardiovascular endpoints.

I am interested in getting involved in the design and execution of future local and national epidemiological studies.

Kathy M McClean

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