Dietary intake of naturally occurring plant sterols in relation to serum cholesterol and myocardial infarction

-Epidemiological studies from Sweden and the UK

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ABSTRACT

Cardiovascular diseases (CVDs) are the leading cause of death in the world. High serum level of cholesterol is one of the major risk factors for CVD development. Serum levels of cholesterol can be modified by diet. Generally, these dietary effects have been attributed to different fats and soluble fibres, but other nutrients like plant sterols may play an important role.

The aim of this doctoral thesis was to to investigate the dietary intake of naturally occurring plant sterols and their relation to serum levels of total and low density lipoprotein (LDL)- cholesterol and to the risk of contracting a first myocardial infarction (MI). These investigations were performed within the UK European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study and within the Northern Sweden Health and Disease Study (NSHDS). In both studies, dietary intake of naturally occurring plant sterols was estimated with food frequency questionnaires.

Reported intake of naturally occurring plant sterols was around 250 mg/day for men and 210 mg/day for women in northern Sweden. In the investigated UK population, the reported intake has previously been shown to be around 300 mg/day for both men and women. In the UK, bread and other cereals, vegetables and added fats were the three most important food sources of naturally occurring plant sterols, together contributing with more than 50% of the total intake. In Sweden, intake of vegetable oil was highly correlated to both absolute and energy-adjusted plant sterol intake. In Sweden, plant sterol intake was inversely related to serum levels of total cholesterol in both men and women, and to serum levels of LDL-cholesterol in women. Odds ratio for a first MI was 0.76 for men in the highest quarter of plant sterol intake compared to men in the lowest quarter, while no effect was seen for women.

The present epidemiological studies suggest that dietary intake of naturally occurring plant sterols reduce serum levels of cholesterol and reduce the risk of contracting a first MI. Advice to enhance intake of naturally occurring plant sterols may be incorporated in the nutritional treatment of hyperlipidaemia and into the prevention of CVDs. To firmly establish the effect of naturally occurring plant sterols on serum levels of total and LDL-cholesterol, intervention studies are however needed.

Keywords: plant sterols, dietary intake, food sources, serum cholesterol, CVD, myocardial infarction, nutrition, epidemiology

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SAMMANFATTNING

Hjärt-kärlsjukdomar är de ledande dödsorsakerna i världen. Höga serumnivåer av kolesterol är bland de viktigaste riskfaktorerna för att utveckla hjärtkärlsjukdomar, och dessa kolesterolnivåer kan modifieras med kosten. Fett och lösliga kostfibrer påverkar serumnivåer av kolesterol, men även andra ämnen i kosten kan inverka. Ett sådant ämne är växtsteroler.

Syftet med avhandlingen var att undersöka intaget av naturligt förekommande växtsteroler och identifiera viktiga kostkällor samt att undersöka om intag av växtsteroler är relaterat till serumnivåer av total och LDL-kolesterol och risk för en första hjärtinfarkt. Undersökningarna genomfördes på data som samlats in i en brittisk studie, European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk och en svensk studie, Northern Sweden Health and Disease study (NSHDS). Intaget av naturligt förekommande växtsteroler uppskattades matfrekvensformulär.

Rapporterat intag av naturligt förekommande växtsteroler var i norra Sverige omkring 250 mg/dag för män och 210 mg/dag för kvinnor. I den aktuella brittiska populationen har intaget tidigare visats vara omkring 300 mg/dag för både män och kvinnor. Bröd och cerealier, grönsaker och matfett var de tre största källorna till växtsterolintag i Storbritannien. I Sverige var samvariationen hög mellan intag av växtsteroler och vegetabilisk olja. Ett högre intag av växtsteroler var i Sverige relaterat till lägre serumnivåer av totalkolesterol hos både män och kvinnor och ett lägre LDL-kolesterol hos kvinnor. Hos män var ett högt intag av naturligt förekommande växtsteroler relaterat till lägre risk för en första hjärtinfarkt. Hos kvinnor sågs ingen effekt.

Resultaten från de aktuella epidemiologiska studierna tyder på att kostintag av naturligt förekommande växtsteroler sänker serumnivåer av kolesterol och reducerar risken för en första hjärtinfarkt. Naturligt förekommande växtsteroler kan användas i nutritionsbehandling av hyperlipidemi och i prevention av hjärt-kärlsjukdom. För att helt säkerställa effekten av naturligt förekommande växtsteroler på serumnivåer av total och LDL-kolesterol behövs dock interventionsstudier.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Klingberg S, Andersson H, Mulligan A, Bhaniani A, Welch A, Bingham S, Khaw K-T, Andersson S, Ellegård L. **Food sources of plant sterols in the EPIC-Norfolk population.** Eur J Clin Nutr 2008;62:695-703.

II. Klingberg S, Winkvist A, Hallmans G, Johansson I. **Evaluation of plant sterol intake estimated with the Northern Sweden Food Frequency Questionnaire.** Public Health Nutr 2012;Jul 2 [Epub ahead of print].

III. Klingberg S, Ellegård L, Johansson I, Hallmans G, Weinehall L , Andersson H, Winkvist A. **Inverse relation between dietary intake of naturally occurring plant sterols and serum cholesterol in northern Sweden.** Am J Clin Nutr 2008;87:993-1001.

IV. Klingberg S, Ellegård L, Johansson I, Jansson J-H, Hallmans G, Winkvist A. **Dietary intake of naturally occurring plant sterols and the risk of a first myocardial infarction: a nested case-referent study in Northern Sweden.** Manuscript.

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CONTENT

ABBREVIATIONS

BMI	Body mass index (kg/m^2)
Caco-2-cells	Human epithelial colorectal adenocarcinoma cells
CI	Confidence interval
CVDs	Cardiovascular diseases
E%	Percentage of energy intake
EPIC	European Prospective Investigation into Cancer and Nutrition
FIL	Food intake level
FFQ	Food frequency questionnaire
HDL	High density lipoprotein
IHD	Ischaemic heart disease
LDL	Low density lipoprotein
MI	Myocardial infarction
mmol/L	Millimol/liter
MONICA	Multinational Monitoring of Trends and Determinants in Cardiovascular Disease
NSHDS	Northern Sweden Health and Disease Study
OGGT	Oral glucose tolerance test
PAL	Physical activity level
VIP	Västerbotten Intervention Program
24-HDR	24-hour dietary recall

1 INTRODUCTION

Cardiovascular diseases (CVDs), including myocardial infarction (MI), are the major cause of death in the world. Atherosclerosis, caused by high serum levels of total and low density lipoprotein (LDL)-cholesterol, is an important component in the development of CVDs. Diet is one modifiable factor affecting serum levels of total and LDL-cholesterol [1]. Naturally occurring plant sterols are present in all vegetable foods [2]. They have the ability to reduce cholesterol absorption in humans [3, 4] and potentially also reduce serum levels of total and LDL-cholesterol [5, 6]. The aims of this thesis were to investigate the dietary intake of naturally occurring plant sterols and the relation to serum levels of total and LDL-cholesterol as well as to the risk of contracting a first MI. Figure 1 shows the basic concepts and hypotheses of the present thesis.

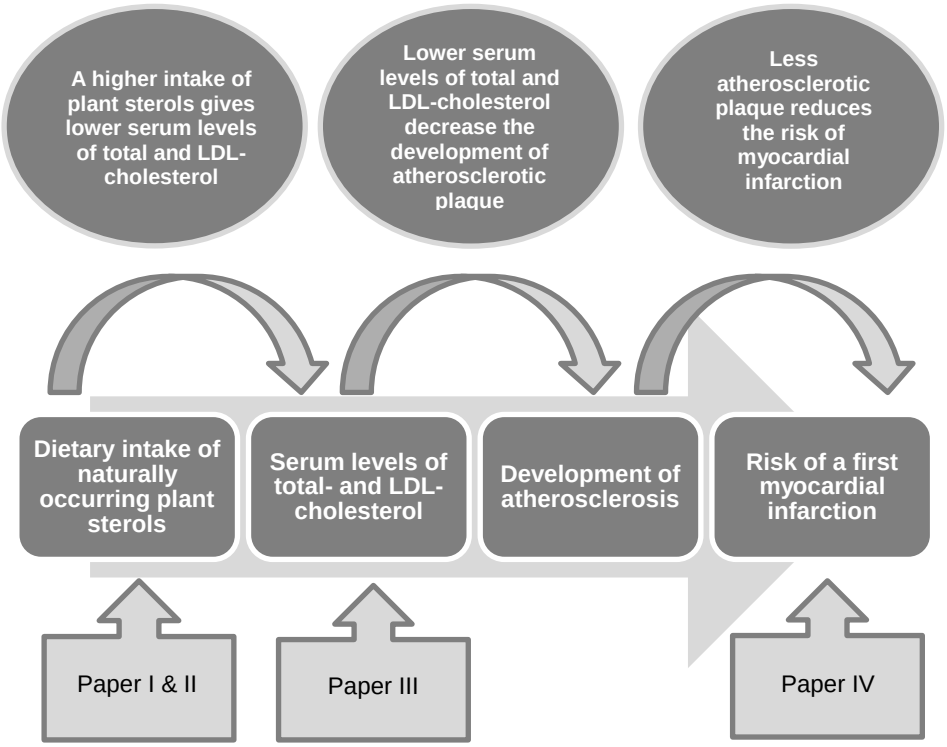

Figure 1. Presentation of the basic concepts of the present thesis.

2 BACKGROUND

2.1 Cardiovascular disease

2.1.1 Definition of cardiovascular disease

CVDs include diseases caused by atherosclerosis: ischaemic heart disease (IHD), cerebrovascular disease, hypertension and peripheral vascular disease, but also diseases with other causes: rheumatic heart disease, congenital heart disease, cardiomyopathies and cardiac arrhythmias [1].

IHD can further be divided into myocardial infarction (MI) and angina pectoris. Chest pain caused by angina pectoris is a condition where constrictions in the coronary arteries cause a reduced blood flow to the myocardium. Angina pectoris is further divided into stable and unstable angina pectoris. Stable angina pectoris appears especially when the oxygen requirement is increased, i.e. during physical activity, and levels off at rest. In contrast, unstable angina pectoris appears also at rest. An MI causes an irreversible damage to the myocardium, due to an interruption of the blood supply to a part of the heart, caused by a total occlusion of a coronary artery. The interrupted blood supply leads to oxygen deficiency in the heart cells affected by the occlusion, causing necrosis [7].

2.1.2 Descriptive epidemiology

In 2008, more than 17 million people all over the world died from CVDs. Figure 2 shows the distribution of major causes of death. CVDs represent more than 30% of all deaths; hence CVDs are the leading cause of death in the world. Even though the incidence of, and mortality from CVDs are decreasing in affluent societies, the incidence of CVDs is increasing globally [1].

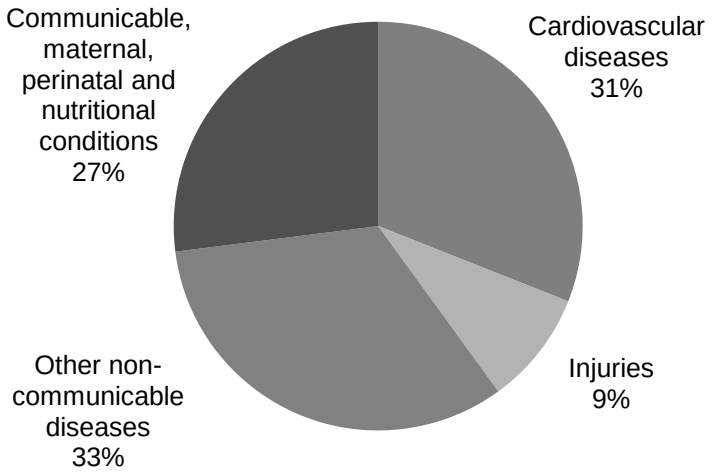

Figure 2. Major causes of death in the world [1].

In Europe, the mortality from CVDs is even higher, representing almost half of all deaths. IHD is the single most common cause of death in Europe, representing more than 1.9 million deaths each year. This means that more than one fifth of all deaths in Europe, in both men and women are caused by IHD. In northern and western parts of Europe, the incidence is decreasing, while it is increasing in most parts of central and eastern Europe [8].

In the UK, the age-standardized death rates from IHD have declined during the past decades. In men aged 55-74, mortality from IHD has decreased by around 50% between 1998 and 2008, and by around 30% in men aged 35-54. In women the corresponding numbers were around 55% in the older age group and around 30% in the younger age group. Still, around 88 000 deaths in 2008 were caused by IHD, representing one of five deaths in males and one of eight deaths in women [9].

The incidence of MI in Sweden is steady around 40 000 per year, even though the age-standardized incidence has decreased by approximately 25% during the last decade. During the same time, the age-standardized mortality from MI decreased by 39% in both men and women [10]. However, IHD still causes almost 19% of all deaths in males and 15% in women, of which MI is responsible for around half [11].

Differences in incidence of and mortality from MI are evident both between sexes and between age groups. Figure 3 shows the incidence of MI in Sweden in 2010. The incidence of MI is higher in men than in women, and the increasing incidence with increasing age is obvious.

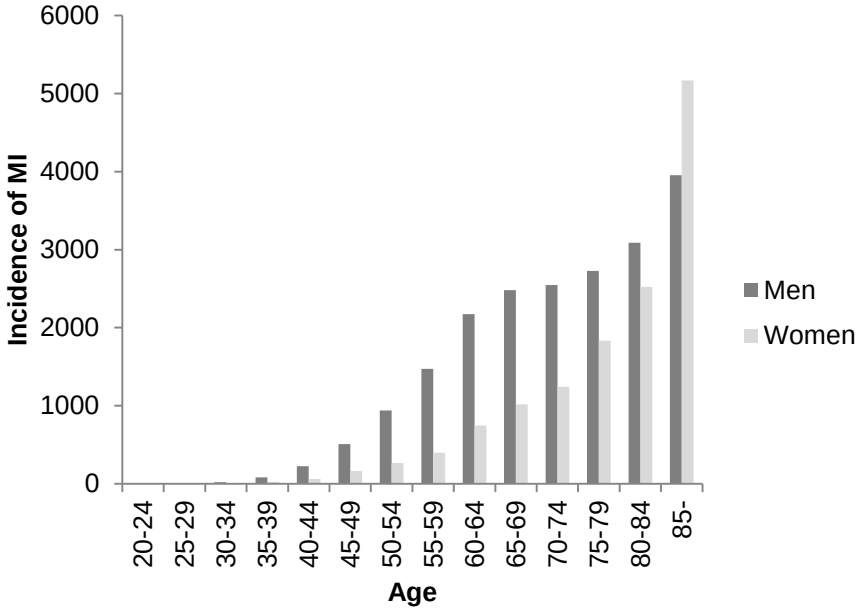

Figure 3. Incidence of MI in Sweden 2010, according to sex and age group [10].

2.1.3 Atherosclerosis

As described earlier, a large part of all CVDs are caused by the atherosclerotic process. Atherosclerosis is induced by several risk factors such as tobacco use, physical inactivity, unhealthy diet, harmful use of alcohol, hypertension, diabetes, hyperlipidaemia, overweight and obesity [1].

The atherosclerotic process is a complex inflammatory process in which the endothelial wall of the artery becomes vulnerable. This vulnerability leads to an increased permeability and adhesiveness of the endothelium. Leukocytes and monocytes are attracted to the site of inflammation and lipid-loaded foam-cells are formed when LDL-particles are enclosed by monocytes. As a response to the inflammatory process, smooth muscle cells from the deeper layer of the artery are relocated to the inflammatory area, and an intermediate

lesion is formed. As the inflammation progresses, macrophages and lymphocytes are accumulated, eventually leading to necrosis within the lesion. As a final step, the lesion is covered by a fibrous cap, resulting in a complicated lesion. This complicated lesion can become unstable and rupture, leading to thrombosis, thus reducing arterial blood flow, potentially causing an MI [12].

The atherosclerotic process starts already in childhood in most affluent populations and leads to a gradual thickening of the arteries. Still, it usually takes several decades before the atherosclerotic lesions cause any symptoms. Thus, atherosclerosis and IHD progress side by side, but can remain asymptomatic for many decades. Figure 4 illustrates the atherosclerotic process.

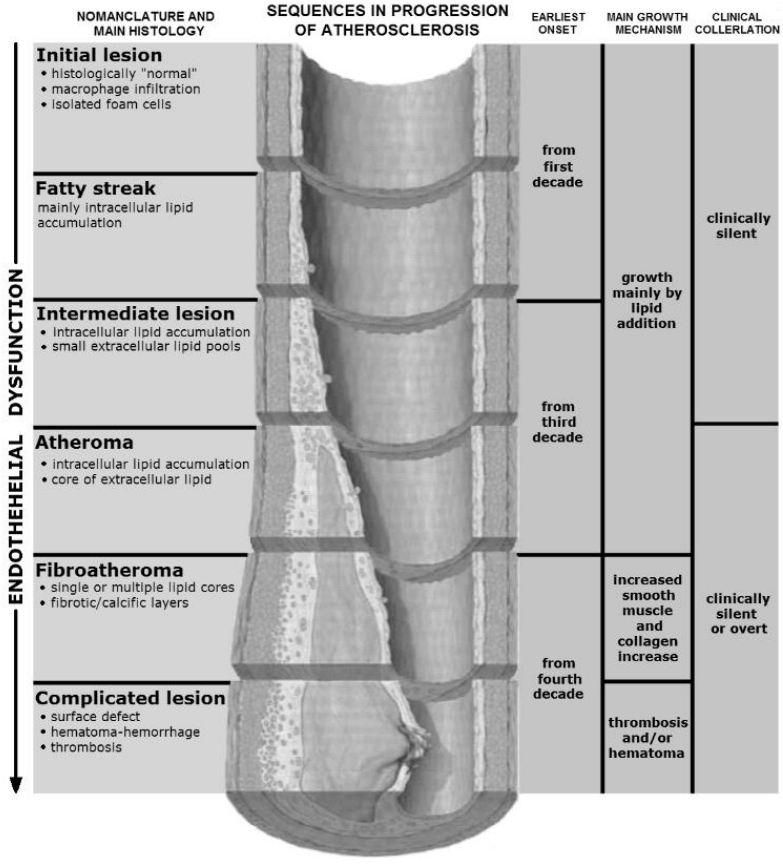

Figure 4. Illustration of the atherosclerotic process (© Grahams Child http://en.wikipedia.org/wiki/File:Endo_dysfunction_Athero.PNG / CC-BY-SA-3.0)

2.1.4 Risk factors for CVDs

In addition to the risk factors mentioned for atherosclerosis, risk factors for CVDs also include sex, age, heredity, psychological factors, poverty and educational status [1]. It has been estimated that nine modifiable risk factors account for more than 90% of the risk of contracting an MI, namely hyperlipidaemia, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors, consumption of fruits, vegetables and alcohol and physical activity [13].

The relationship between total and LDL-cholesterol and CVDs has been found in both epidemiological studies [14, 15] and clinical trials [16, 17]. Diet is one way of modifying serum levels of total and LDL-cholesterol. Evidently, a large part of all CVDs could be prevented.

2.1.5 Diet and CVDs

Food intake is complex and there are several nutrients that have the potency of increasing or decreasing the risk of CVDs, mainly through their effect on serum levels of cholesterol. Numerous papers have been published, investigating the effect of different fatty acids on serum lipids [18-22]. The results are conclusive. Saturated fatty acids raise serum levels of total and LDL-cholesterol. This is especially evident for myristic, lauric and palmitic fatty acids. The replacement of carbohydrates by polyunsaturated fat lowers serum levels of total and LDL-cholesterol, while the effect of substitution with monounsaturated fat is very moderate. Reduction in dietary cholesterol intake is related to a decrease in serum levels of total and LDL-cholesterol.

Some dietary fibres have also been shown to affect serum lipids. Water-soluble dietary fibres, i.e. gel-forming fibres, reduce serum levels of total and LDL-cholesterol [23-26], while water-insoluble fibres have no effect [25, 26].

When it comes to the relation between dietary factors and CVD endpoints, the picture is not as clear. Most studies have failed to show any significant effects of manipulation of dietary fat on CVD endpoints. The exception is on the effect of trans fatty acids and polyunsaturated fatty acids. Trans fatty acids have been shown to increase the risk of coronary heart disease events and mortality. Polyunsaturated fatty acids, long chain n-3 fatty acids and fish intake have been shown to reduce risk of coronary heart disease mortality [27].

Other dietary constituents have also been suggested to affect risk factors for CVDs. Examples of such constituents are soy proteins, flavonoids, folic acid and plant sterols [28].

2.2 Plant sterols

2.2.1 Chemical structure

Plant sterols are 28- or 29-carbon steroid alcohols found in vegetables [29, 30]. Plant sterols resemble cholesterol found in vertebrates, both in function and in structure. They are important for the cell membranes, and are assumed to control membrane fluidity and permeability [30]. The chemical structure differs from cholesterol by an additional side chain (Figure 5). Saturated plant sterols are called plant stanols and are less abundant than the unsaturated plant sterols. The term plant sterols usually includes both unsaturated plant sterols and saturated plant stanols [30, 31]. Plant sterols occur naturally mainly as free sterols but also as esterified steryls and steryl glycosides. There are more than 250 identified plant sterols, but most of them are very rare or occur in very low concentrations [30]. The most abundant plant sterols in nature are β-sitosterol, campesterol and stigmasterol [29, 31].

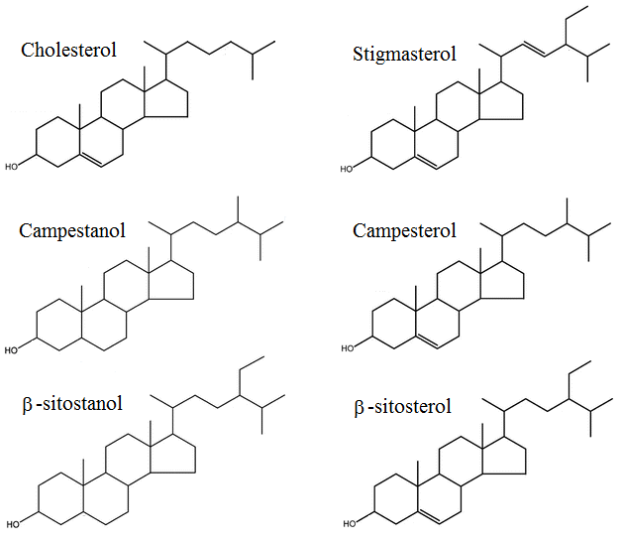

Figure 5. Chemical structure of cholesterol and five plant sterols and stanols.

2.2.2 History of plant sterols in the treatment of hyperlipidemia

In 1951, Peterson et al. reported that hypercholesterolemia in chicks could be prevented by adding soybean sterols to their cholesterol containing feed [32]. The year after, a second paper confirmed the previous results, and in addition, showed that inclusion of soybean sterols to a cholesterol containing feed in chicks decreased the incidence and severity of atherosclerosis [33]. In 1953, it was reported that sitosterol lowered serum levels of cholesterol also in humans [34]. The following years several trials, with varying results, were performed investigating the effect of administration of sitosterol on serum levels of cholesterol in humans [35-37].

In the mid 1950's the drug Cytellin was introduced. Cytellin consisted of crystallised sitosterols, with very low bioavailability. This made the daily doses very large, i.e. 6-18 g/d. In addition, the drug had an unpleasant taste and texture, reducing compliance. Nevertheless, Cytellin was used in hyperlipidaemia treatment until the 1980's.

In 1994, Miettinen and Vanhanen showed that a plant sterol intake of around 1 g/day, significantly decreased serum levels of total and LDL-cholesterol by 3.4% and 5.9%, respectively [38]. In this trial, the plant sterols were administered as plant sterol esters dissolved in rapeseed oil, which increased the bioavailability considerably. The year after, the results from a one-year randomized controlled trial on a mildly hyperlipidemic population were presented [39]. The administration of margarine, containing between 1.8 g and 2.6 g of sitostanol, decreased total and LDL-cholesterol by 10.2% and 14.1%, respectively, while the serum levels of cholesterol in the control group essentially did not change.

In the following years, this discovery lead to the market introduction of commercial food products enriched with plant sterols. The effect of these products is well documented. A meta-analysis of 41 trials showed that the intake of 2 g/d of stanols or sterols reduced serum levels of LDL-cholesterol by 10% [40]. In Sweden, these products are marketed under the names of Benecol® and Becel pro.active®. At the beginning only margarines were available, but nowadays there are also other products available, like yoghurts and milk drinks. These products are so-called functional foods, and are recommended to persons with hyperlipidaemia. The use of these functional foods could be as part of a nutritional treatment but has also been shown to have an additive effect to pharmacological treatment with statins [41].

2.2.3 Possible mechanisms of action

Plant sterols were, as mentioned above, discovered to lower serum levels of cholesterol already in the 1950's, but at that time, the mechanism of action was unknown. However, it was suggested that the effect was related to reduced intestinal cholesterol absorption [33]. Several mechanisms have been proposed, but yet to date the mechanisms are not fully understood. Studies of the effect of plant sterols on cholesterol absorption on the cellular level have been performed in animals or in cell-cultures, thus the accordance in vivo in humans are mostly unknown.

Cholesterol is absorbed through several steps: hydrolysis of cholesterol esters; micellar binding; entry into the enterocyte, i.e. the mucosal cell of the small intestine; re-esterification in the enterocyte; incorporation into chylomicrons and transport into the lymph [42]. Plant sterols may act on a number of these steps.

Plant sterols have been shown to reduce incorporation of cholesterol into bile salt micelles [43]. This is explained by the higher affinity to bile salt micelles of plant sterols compared to cholesterol [44].

The restricted solubility of cholesterol, through reduced micellar incorporation, is part of the explanation of how plant sterols inhibit cholesterol absorption, but additional mechanisms have been put forward. In 2000, Plat and colleagues showed that the reduced cholesterol absorption, after administration of 2.5 g of plant stanols, did not differ when these were administered once per day or divided over three times per day [45]. This made the authors hypothesize that plant sterols remain in the intestinal lumen or within the enterocyte, and that the effect could not only depend on the reduced incorporation of cholesterol into mixed micelles.

Initially cholesterol was thought to passively diffuse from the intestinal lumen into the enterocyte. Today it is known that different proteins are involved in the transport of cholesterol and plant sterols into and out of the enterocyte. The first cholesterol transporter protein, called Niemann-Pick C1 Like 1 Protein (NPC1L1), was discovered in 2004 [46]. NPC1L1 is located in the cell membrane of the enterocytes in the small intestine, and is essential for the uptake of cholesterol across the cell membrane of the intestinal enterocyte [46]. Additionally, plant sterols are transported over the cell membrane through NPC1L1 [47]. Sitosterol has been shown to down-regulate the expression of NPC1L1, which partly could explain the reduction in cholesterol absorption [48].

Secretion of cholesterol and plant sterols from within the enterocyte back to the intestinal lumen is mediated through two transporter molecules, ATP-binding cassette G5 and G8 (ABCG5 and ABCG8) [49, 50]. The presence of plant sterols has been shown to affect an intracellular cholesterol sensor called liver X receptor (LXR), causing an up-regulation of ABCG5 and ABCG8 in Caco-2-cells [51]. The up-regulation of ABCG5 and ABCG8 would result in a higher efflux of cholesterol and plant sterols from the enterocyte back into the intestinal lumen.

Within the enterocyte, cholesterol is re-esterified by acyl CoA: cholesterol acyltransferase-2 (ACAT-2) [52]. It has been suggested that plant sterols lower the secretion of cholesterol esters by inhibiting ACAT activity, as found in Caco-2-cells. This inhibition of ACAT activity is thought to depend on reduced transport of cholesterol from the cell membrane to the site of esterification within the enterocyte [53]. Reduction in the ACAT activity, results in a decrease in the amount of cholesterol esters incorporated into chylomicrons and finally absorbed.

Figure 6 illustrates these proposed mechanisms of plant sterols on the inhibition of cholesterol absorption.

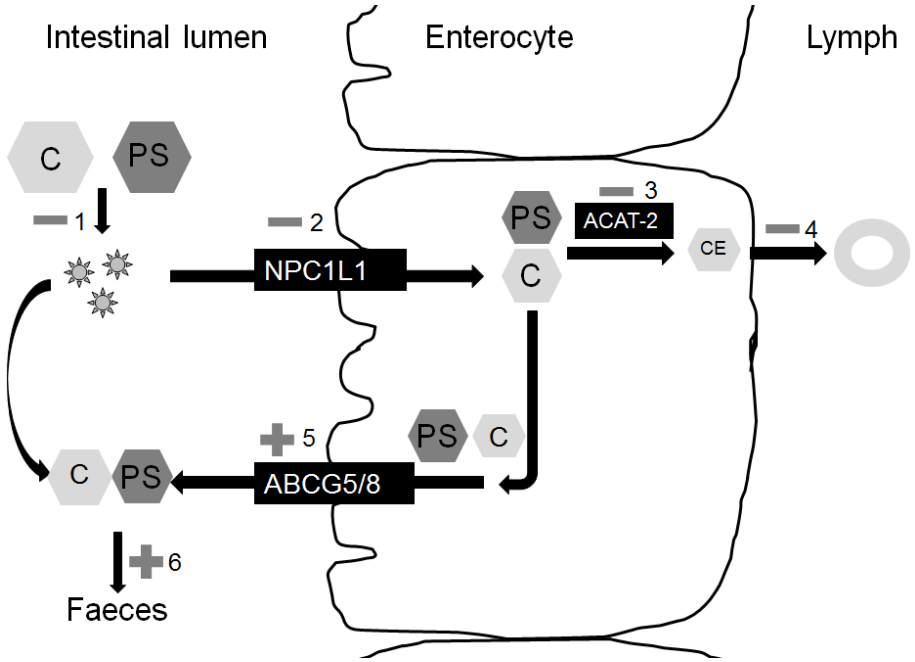

Figure 6. Illustration of the proposed mechanisms in which plant sterols may act on the inhibition of cholesterol absorption. Plant sterols and cholesterol from the diet, as well as bilary secreted cholesterol compete for space in bile salt micelles. Plant sterols have a higher affinity to bile salt micelles, which reduce the incorporation of cholesterol into these micelles (1). Plant sterols down regulate the expression of the transporter protein NPC1L1, resulting in a decreased uptake of cholesterol to the enterocyte (2). Within the enterocyte the esterification of cholesterol by ACAT-2 is reduced (3), and consequently less cholesterol esters are available for incorporation into chylomicrons (4). The expression of the transporter proteins, ABCG5 and ABCG8, are up-regulated, causing an increased efflux of cholesterol and plant sterols from the enterocyte back into the intestinal lumen (5). Taken together, this result in an increased excretion of cholesterol with faeces (6).

2.2.4 Why study naturally occurring plant sterols?

Research on the digestion, absorption and excretion of nutrients at the former Department of Clinical Nutrition at University of Gothenburg, leading to the present thesis, started more than 30 years ago. In 1981, Sandberg et al. published a paper investigating the effects of dietary fibre on nutrient excretion in ileostomy subjects [54]. The study of ileostomy subjects, made it possible to investigate the effects of different dietary components on nutrient absorption and excretion, without the bacterial degradation in the large bowel. Several studies on different dietary components and compositions have been performed through the years: wheat-bran and pectin [55]; low in fat, or high in polyunsaturated fat [56]; varying in fat and fiber [57]; high in monounsaturated fat [58]; oat-bran [59, 60]; soluble fiber from sugar beet [61]; varying in cholesterol [62]; inulin and oligofructose [63]. In 2000, Normén et al. reported that addition of plant sterol or plant stanol esters corresponding to 1.5 g/d to a basal diet in ileostomy subjects, inhibited cholesterol absorption with the same efficiency [64]. The same year, Ellegård et al. reported on an ileostomy study of the effect on cholesterol absorption and excretion of four diets with varying content of saturated fat, fibre, and plant sterols [65]. Besides effects of alterations in saturated fat and fiber content, an inverse relationship between excreted plant sterols and absorbed cholesterol was found. The plant sterol content of these diets varied between 273 and 469 mg, which are within the range of a normal diet. Hence, it was hypothesized that the effect on cholesterol absorption by these diets, could partly be explained by the content of naturally occurring plant sterols. Concurrently, other researchers hypothesized that naturally occurring plant sterols could play a role in the effect of different diets on cholesterol absorption and serum lipids [6, 31].

2.2.5 Plant sterols in food

Plant sterols are found in all vegetable foods at varying concentrations. The variation in plant sterol concentration is wide both between, and within food groups. The highest concentration of plant sterols is found in vegetable oils, with corn oil and wheat germ oil containing over 900 mg of plant sterols per 100 g oil. In contrast, palm oil and coconut oil contain less than 100 mg of plant sterols per 100 g oil [66]. Cereal whole-grains contain more plant sterols than refined cereal products. As an example, the plant sterol content of whole wheat flour is 70 mg per 100 g while refined wheat flour contain less than 30 mg per 100 g [67]. As for vegetables, cabbages have a high content of plant sterols while fruity vegetables contain much less. For instance, the

plant sterol content of broccoli and cauliflower is nearly 40 mg per 100 g while it is below 10 mg per 100 g of cucumber, tomato and pepper [68].

In addition, the occurrence of different plant sterols varies between foods and food groups. In oils, nuts, fruits and vegetables the three plant sterols campesterol, stigmasterol and β-sitosterol make up most of the plant sterol content [66, 68]. Campestanol and β-sitostanol are almost only found in cereals, even though they only contribute to a minor part of the total plant sterol content of these foods [67].

2.2.6 Dietary intake of plant sterols

Dietary intake of plant sterols has been sparsely studied, probably depending on the lack of analysed food items in general nutritional databases.

Dietary intake of naturally occurring plant sterols in European countries has been estimated to range from around 220 to 260 mg/d in women and from around 280 to 340 mg/d in men [69-72]. Estimated energy-adjusted plant sterol intake ranges from 30 to 35 mg/MJ, with women having higher intakes than men [70, 72]. In Japan, the estimated energy-adjusted intake is higher, with mean intakes of around 40 mg/MJ [73, 74]. Studies of vegetarian and vegan diets show that compared to a mixed diet, the plant sterol content of these diets is usually higher [75, 76].

The most important sources of naturally occurring plant sterols in the Netherlands are bread and cereals contributing with 37% of the total intake and vegetable fats and oils contributing with 26% [69]. Also in Belgium, bread and cereals and vegetable fats were the major sources of naturally occurring plant sterols representing 25% and 16% of the total plant sterol intake, respectively [72]. Additionally, based on national food consumption data, the two main sources in the Spanish diet is oils and fats and bread and cereals, representing 39% and 30% of the intake, respectively [71].

2.3 Nutritional epidemiology

The term epidemiology can be defined in different ways, one of them being quoted by the Australian epidemiologist John Last:

"Epidemiology is the study of the distribution and determinants of health related states or events in specified populations, and the application of this study to the control of health problems".

This definition gives a broad perspective to epidemiology, including both descriptive epidemiology and elucidation of the aetiology of diseases as well as providing information for the management of diseases through prevention, control and treatment.

Nutritional epidemiology aims to give scientific evidence to support the role of nutrition in the development, treatment and prevention of different health related states and diseases [77]. In the early days of nutritional epidemiology, focus was mainly at deficiency states, while modern nutritional epidemiology mainly focuses at diseases of the Western world, such as cancer and CVDs [78].

Nutritional epidemiology is associated with a number of limitations as well as possibilities, as exemplified below. The dietary and nutrient intakes are often estimated with low precision, and estimation of nutrient intake is prone to different measurement errors. In addition, nutrient intakes are often correlated, making it difficult to separate the effect of one nutrient from the effect of another. On the other hand, epidemiological studies give the possibility to study long term effects of dietary intake, which is necessary when studying diseases with a long induction period, i.e. period from exposure to disease. This is often difficult to achieve in experimental studies. Compared to interventions, where only a few levels of exposure can be studied, observational studies can study the whole spectrum of exposure, making it easier to generalize results from epidemiological studies.

3 AIMS

The overall aim of this thesis were to investigate the dietary intake of naturally occurring plant sterol in one UK and one Swedish population and to evaluate if intake is related to serum levels of cholesterol and risk of a first MI.

Specific aims

1. To describe the most important sources of naturally occurring plant sterols in a UK population.

2. To evaluate the ability of the Northern Sweden Health and Disease Study (NSHDS) 84-item food frequency questionnaire (FFQ) to estimate plant sterol intake, with ten repeated 24-hour dietary recalls (24-HDRs) as the reference method, and to investigate the reproducibility of the FFQ regarding estimation of plant sterol intake.

3. To investigate if a high dietary intake of naturally occurring plant sterols is related to lower serum levels of total and LDL-cholesterol in a Swedish population.

4. To investigate if a high dietary intake of naturally occurring plant sterols is related to a lower risk of contracting a first MI in a Swedish population.

4 SUBJECTS AND METHODS

4.1 Study populations

The European Prospective Investigation into Cancer and Nutrition (EPIC) is the world's largest study of diet and health. The prospective multi-centre EPIC project involves more than half a million people. Initially, 17 centres located in seven European countries were involved in the study: France, Germany, Greece, Italy, The Netherlands, Spain and the UK. Subsequently, these were joined by another six centres in Denmark, Sweden, Norway and Italy, all of which were conducting similar prospective studies. The study was initiated in 1992 and the aim was to investigate relationships between diet, nutritional status, lifestyle and environmental factors and the incidence of cancer and other chronic diseases [79]. The cohorts used in this thesis, namely the EPIC-Norfolk cohort and the Västerbotten Intervention Program (VIP) cohort of the Northern Sweden Health and Disease study (NSHDS), are both parts of the international EPIC project.

4.1.1 EPIC–Norfolk

The UK EPIC-Norfolk study includes over 25 000 men and women aged 45-74 years. Participants were recruited from local collaborating General Practices in the county of Norfolk. Recruitment started in 1993 and was completed by the end of 1997. Study invitations were sent to 77 630 possible participants, of which 30 447 agreed to participate. Of those who agreed to participate, 25 633 attended the health examination [80]. Apart from cancer endpoints, the EPIC-Norfolk study also includes other endpoints like MI. The EPIC-Norfolk cohort was used in paper I. The study was approved by the Norfolk and Norwich Hospital Ethics Committee.

4.1.2 The Northern Sweden Health and Disease Study

The NSHDS is the overall name of three study cohorts: VIP, the Northern Sweden Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) cohort and the mammary screening cohort [81]. The mammary screening cohort did not include assessment of dietary intake, hence material from this cohort is not used in this thesis.

The Västerbotten Intervention Program (VIP)

The VIP started in 1985 in the municipality of Norsjö, as a small-scale community based cardiovascular disease preventive program [82]. The project was initiated because it was recognized that the county of Västerbotten had the highest mortality from MI in Sweden. The project expanded and since 1991 the entire Västerbotten county is covered by the project. The project is still ongoing and inhabitants of Västerbotten county are invited to a health examination at their local health care centre the year they turn 40, 50 and 60 years of age. Until 1995 those turning 30 were also invited. The participation rate has varied during the years and was 48-57% between 1991 and 1995. After excluding invitations to those turning 30, the participation rate raised and is since 2005 66-67% [82]. Investigation of participants and non-participants revealed small differences in social characteristics between them. Non-participation tended to be associated with younger age, lower income and unemployment [83]. At the end of 2006, a total of 86 242 subjects had participated in the program and around 27 000 of those had participated twice [82]. In this thesis, data from 1992-2005 was used in paper III and data from 1991-2006 was used in paper IV. The validation study, used in paper II, took place in 1992 within the VIP project.

The Northern Sweden MONICA Project

The Northern Sweden MONICA Project is part of the international WHO MONICA project, which was initiated in 1982. The aim of the WHO MONICA project was to investigate mortality trends and changes in cardiovascular risk factors during ten years in 38 populations in 26 countries [84]. Many centers have continued to survey beyond these ten years. The first Northern Sweden MONICA survey took place in 1986, and has then been repeated in 1990, 1994, 1999, 2004 and 2009. In each survey, a random sample of between 2000 and 2500 subjecs, from sex- and age-stratified groups, were invited to participate. The survey is population-based and covers Norrbotten and Västerbotten counties in northern Sweden. Between 70% and 80% of the invited subjects have participated [84].

Within the MONICA project, all MI events in northern Sweden are registered in the population-based MONICA registry. The event registration is standardized according to WHO and MONICA criteria and based on reports from hospitals or general practitioners as well as hospital discharge records and death certificates [84].

Data from the MONICA surveys in 1986, 1994 and 1999, representing ages from 25 to 74 years, was used in paper IV.

The Regional Ethical Review Board in Göteborg, Sweden approved the studies on NSHDS presented in this thesis.

4.2 Study design and subjects

Tabel 1 gives an overview of the four papers and the study subjects.

Table 1. *Overview of the four papers of the thesis and their respective study cohort and study subjects.*

	Paper I	Paper II	Paper III	Paper IV
Study cohort	EPIC-Norfolk	VIP	VIP	VIP and MONICA
Number of participants included in the analyses	11 227 men 13 571 women	96 men 99 women	37 150 men 40 502 women	995 male cases and 3417 referents 301 female cases and 1117 referents
Data collected	1993-1997	1992	1992-2005	1991-2006 in VIP 1986-2006 in MONICA
Dietary assessment method	130-question FFQ	84-question FFQ and 10 repeated 24-HDRs	84- and 64-question FFQ	84- and 64-question FFQ

4.2.1 Paper I

Paper I was a cross-sectional study of plant sterol intake in the EPIC-Norfolk cohort. Dietary data from food frequency questionnaires (FFQ) were available for 24 838 participants (11 244 men and 13 594 women). Data had already been cleaned for participants with ten or more lines of missing data in the FFQ (n=247) and subjects with the top and bottom 0.5% of food intake level (FIL) (n=250) [85]. Additionally, 40 participants with total plant sterol intake > 750 mg/day were excluded. In total, 24 798 participants (11 227 men and 13 571 women) were included in the analyses.

4.2.2 Paper II

Paper II was a study evaluating the ability to estimate plant sterol intake of the FFQ used within the NSHDS, taking advantage of a validation study that

was conducted within VIP in 1992. An age- and sex- stratified random subsample of subjects, who were invited to participate in the VIP, were asked to take part in a validation study [86]. Of 246 invited subjects, 102 men and 101 women agreed to participate. The validation study was administered during a year starting with a first FFQ (FFQ1), followed by ten 24-hour dietary recalls (24-HDRs) and finally at the end of the year, a second FFQ was administered (FFQ2). Six participants did not complete all 24-HDRs and two did not return FFQ1 and were excluded. In total, 96 men and 99 women completed FFQ1 and all ten 24-HDRs and thus form the sample size of the paper included in this thesis. All but two women also completed the FFQ2, hence yielding 96 men and 97 women in the analyses regarding reproducibility of the FFQ.

4.2.3 Paper III

Paper III was a cross-sectional study on plant sterol intake and serum levels of cholesterol within VIP. During 1992 to 2005, 83 013 health examinations took place within VIP. Six third-time visits were excluded whereas 71 367 first-time visits and 11 640 second-time visits were included and treated as separate observations. Further exclusions were made because of incorrect age (<29 years or >61 years, n=95), incomplete dietary data (n=3 771), and missing or unrealistic serum level of total cholesterol (<2.5 mmol/L and >20.0 mmol/L, n=708). Additionally, participants with the highest or lowest 0.5% of food intake level (FIL, further described in the methods section) were excluded within sex and version of FFQ (64 or 84 questions) (n=781). In total, 77 652 visits (37 150 by men and 40 502 by women) were included in the analyses presented in the thesis.

4.2.4 Paper IV

Paper IV was a nested case referent study of a first MI within VIP and MONICA. All prospective cases of first MI occurring in VIP participants between 1991 and 2006 and in MONICA participants between 1986 and 2006 were identified via the MONICA registry. Inclusion criterion for cases was no previous MI at baseline examination which took place between 1991 and 2006 in VIP participants and in 1986, 1994 and 1999 in MONICA participants. To each case, up to four referents were selected, matched by sex, age (+/- 2 years), year of health examination and study cohort. Inclusion criteria for referents were being alive and free of MI at the time of diagnosis of the case. In total, 1367 cases of first MI were identified and 4818 matched referents were selected. Seventy-one cases and 284 referents had responded to an FFQ version with 49 questions within MONICA 1990 and were excluded. Therefore, the dataset consisted of 1296 cases (301 female, 995

male) and 4534 referents (1117 female, 3417 male). Eighty-nine percent of the cases were identified from the VIP.

4.3 The plant sterol database

Plant sterol analyses of more than 330 food items were performed at the Clinical Nutrition laboratory at the University of Gothenburg between 1996 and 2006. The results from these analyses were collected in a database, comprising data on campesterol, stigmasterol, β-sitosterol, campestanol, β-sitostanol and the sum of these five sterols, for each analysed food item. The database includes data on vegetables, fruits, cereals, bread, fats, nuts, confectionaries, beverages and specific UK food items. Data on vegetables, fruits, cereals and fatty foods have been published [66-68]. Fruits and vegetables were bought in 1996, cereals in 1997 and fatty foods in 1997 and 2001 in two shops in the Gothenburg area. Specific British food items were bought in Cambridge, UK, in 2004 and 2005. A mix of two different samples was used in the analyses of fruits and vegetables, while cereals and fatty foods were made from a mix of two to seven samples. Specific British food items were analysed from a single sample. All food samples were analysed in duplicates.

Plant sterol analyses

Analyses of the food items were performed using a gas-liquid chromatography procedure. This procedure was validated with gas chromatography coupled to mass spectrometry [87]. In short, the method comprised acid hydrolysis (6 mol/L HCl), alkaline saponification (96% ethanolic potassium hydroxide), lipid extraction with toluene, and a final washing in deionized water to neutral pH. Internal standard, containing 5α-cholestane, was added to all samples before saponification to quantify the sterols. Samples were dehydrated with sodium sulphate, filtered, and evaporated under vacuum at 50 °C. The residue was dissolved in chloroform and stored at -20 °C. Silylation of sterols to trimethylsilyl ether derivatives was performed before analysis by gas-liquid chromatography. The limit of detection was set to 0.01 mg/100 g fresh material of the tested product. The concentrations of the five most frequently occurring plant sterols were measured: the unsaturated plant sterols campesterol, stigmasterol, and β-sitosterol, and the saturated plant stanols campestanol and β-sitostanol. For fatty foods, 5-avenasterol and brassicasterol were also quantified. However these were not accounted for in the estimates of the total plant sterol intake.

4.4 Methods

4.4.1 The EPIC-Norfolk study

The health check-up consisted of a questionnaire about health and lifestyle, an FFQ, a 7-day food diary (not used in this study), a health examination including measurement of anthropometry and blood pressure, and donation of blood. [80]. The FFQ was posted in advance to participants and checked for completeness by nursing staff at the health examination [85].

Food frequency questionnaire

The FFQ used in the EPIC-Norfolk study was a semi-quantitative FFQ consisting of 130 questionnaire lines covering 275 food items. For each questionnaire line, the respondent should estimate how often the foods were eaten on average, during the past year, by choosing one of nine categories ranging from never to more than six times per day. The FFQ also consisted of 16 additional questions, e.g. questions on consumption of other foods, amount of milk consumed, name and type of most often consumed breakfast cereal, and type of fat used for cooking and baking. Standard portions were used without distinction of sex and age.

Calculation of food and nutrient intake was performed using the Compositional Analyses from Frequency Estimate (CAFE) program, especially developed for the EPIC-Norfolk FFQ [85]. As plant sterol content of foods is not included in the ordinary nutritional databases, information from the Swedish plant sterol database was added for this study.

Assignment of plant sterol values

Each of the 275 foods representing the 130 questionnaire lines were assigned a plant sterol value as follows: 63 foods were set to zero due to pure animal origin or due to ingredients not containing plant sterols; 119 foods were based on direct analyses; 56 foods were assigned values from similar analysed products or proportions of analysed products; 23 foods were assigned values based on calculations of UK standard recipes with analysed ingredients; and 14 foods were assigned plant sterol values from the sixth edition of the UK food composition database . The UK standard receipts were obtained from supplements of the UK food composition database [88-94].

Dietary variables

Dietary variables were reported as absolute intake per day (foods, food groups, energy and plant sterols) and as energy-adjusted intake per day (plant

sterols). Energy-adjustment was performed by the energy density model, i.e. by dividing nutrient intake with total energy intake [95].

Background variables

Age

Age was derived from the birth registration number as reported in the questionnaire.

4.4.2 The Northern Sweden Health and Disease Study

The health check-up in VIP as well as MONICA included both a health examination with measurement of weight and height, blood pressure, analyses of blood lipids and plasma glucose, an oral glucose tolerance test (OGGT) and a comprehensive questionnaire. The questionnaire included questions on socioeconomic and psychosocial conditions, self-rated health, personal health history and family history of CVD and diabetes, quality of life, social network and support, working conditions, physical activity, alcohol problems, tobacco use, eating habits and an FFQ [82]. In VIP the FFQ was completed at the health care centre, while in MONICA the FFQ was posted in advance to participants.

Food frequency questionnaire

Four versions of a semi quantitative FFQs were used in the Northern Sweden Health and Disease Study. Three of them were used in VIP: the original 84-item VIP FFQ (used 1992-1996); an older 84-item FFQ (used 1990-1992) and a 64-item FFQ (in use since 1996), and one 84-item FFQ used in MONICA [96]. The three 84-item FFQs were nearly identical. The shorter 64-item FFQ captured essentially the same foods but with fewer questions. In Table 2, the similarities and differences between the questionnaires have been illustrated by Lena Nilsson [96]. In the left column, 47 questions on foods or food groups, present in all FFQs, are shown. In the right column, questions differing in the old 84-item FFQ and the 64-item FFQ are shown, compared to the original 84-item FFQ. In the old 84-item FFQ, one question was not included, six questions were merged to three questions, four questions covered foods from two questions, three questions included more food items and one question included fewer foods, all compared to the original 84-item FFQ. In the 64-item FFQ, nine questions were excluded, 20 questions were merged to nine questions and one question included fewer food items, all compared to the original 84-item FFQ.

Four colour photos for estimation of consumed portions were used for potatoes, rice, pasta, meat, fish and vegetables.

For each questionnaire line in the FFQ, the respondent should answer how often, on average during the past year, the food or foods were eaten. Consumption frequency could be answered with one of nine frequencies varying from never to more than four times a day. Portion sizes were age and sex-specific and determined from actual portion sizes in the 24-HDRs in the validation study [86]. Nutrient intakes were calculated by multiplying frequency of intake by portion size using the food composition database at the Swedish National Food Administration, Uppsala, Sweden, using the software MATs (Rudans Lättdata, Västerås, Sweden). As for the UK nutrient database, plant sterol content is not included in the Swedish food composition database. Thus, plant sterol values were added especially for the studies in this thesis.

Table 2. *Differences and similarities between three FFQ types used within the VIP project 1990-2008. In the left column, questions identical in all three FFQs are listed. In the right column, questions differing (X) or missing (-) within the older 84-item FFQ (old) and the most recent 64-item FFQ (64) are listed [96]. (Reprinted with permission).*

Food items included in all FFQs (n=47)
1. 68% butter/32% canola oil, on bread,
2. butter on bread
3. light margarine (40% fat) on bread
4. margarine (80% fat) on bread
5. butter in cooking
6. margarine (80%fat) in cooking
7. oil in cooking
8. salad dressing with oil, vinaigrette
9. cream, crème fraiche, sour cream
10. whole grain, high-fibre crisp bread, e.g. husmans bread (rye)
11. whole grain high-fibre soft bread,
14. sweet buns, rusk
20. meat as sandwich topping
24. rosemary or juice soup, fruit cream
25. soured milk, yoghurt, 3% fat
26. soured milk, yoghurt, 0,5% fat
29. berries, fresh or deep frozen
32. Banana
34. root vegetables, carrots
35. tomato, cucumber
39. potato, boiled or baked
44. Rice
45. Pasta
46. baked beans, pea soup
50. Pizza
51. ground meat dishes
52. beef stew
53. steak, chop, cuts of meat
54. bacon, pork belly, ham
55. sausage dishes
56. Hamburger
57. Poultry
60. lean fish (perch, cod)
61. fatty fish (herring, whitefish, salmon),
63. salted fish (herring)
64. smoked fish/smoked meat
65. ice-cream
66. sweets, e.g. chocolate, candy
69. cakes, cookies, pastry
70. salted snacks (chips, popcorn, nuts)
72. milk, 1-1,5% fat
79. Tea
80. beer, < 2.25 % alcohol
81. beer, 2.8 – 3.5% alcohol
82. beer, ≥ 4,5% alcohol
83. Wine84. spirits

Food items differing in Old[1] and/or 64[2] (n=37)	Old[1]	64[2]
12. white soft bread	x	x[4]
13. white crisp/flat bread (northern Swedish type)	x	
15. hard cheese, 28% fat (medium fat)	x[4]	x
16. hard cheese, 10-17 % fat (low fat)		x
17. cream cheese, soft cheese spread	x	-
18. whey cheese	x	
19. sausage as sandwich topping	x	x[4]
21. liver paté	x	
22. porridge, oatmeal	x	x[4]
23. porridge, graham-, rye- or barley	x	
27. whole grain cereals, e.g. musli	x[4]	x
28. Corn flakes, low-fibre cereals.		x
30. apples, pears, peaches	x[5]	x[4]
31. orange, mandarin, grapefruit	x	
33. cabbage	x	x[4]
36. lettuce, Chinese cabbage	x[5]	
37. spinach, kale	x	
38. frozen mixed vegetables	x	-
40. fried potatoes	x	x[4]
41. french fries	x	
42. mashed potatoes	x	
43. potato salad	-	-
47. broth + flat bread	x	
48. pancakes, waffles	x[5]	x[4]
49. potato dumplings	x	
58. blood dishes	x[6]	
59. liver, kidney	x	-
62. seafood (shrimp, mussels/clams)	x	
67. sugar cubes, sugar, honey	x	x[4]
68. marmalade, jam	x	
71. milk, ≤ 0,5% fat	x[3]	x[6]
73. milk, ≥ 3% fat	x[3]	x
74. syrups of fruit or berries	x	x[4]
75. carbonated soft drinks, coca cola	x	
76. juice	x	
77. drip-filtered coffee	x[4]	x
78. boiled, unfiltered coffee		x

Food items not included in the original 84-item VIP FFQ (n=3):
Egg dishes (Old + 64)
Pie, e.g. gound meat pie, vegetable pie (Old)
water (64)

[1] the 84-item VIP FFQ, in use before 1992 [2] the 64-item VIP FFQs, in use since 1997 [3] Separate items, [4] Merged with other items, [5] More items included, [6] Fewer items included

Assignment of plant sterol values

Each question in the FFQs could represent either a single food item, an aggregate of items (e.g. tomato and cucumber), or a food group (e.g. berries). Data from a single 24-hour recall from 3000 subjects in a calibration study within VIP [97] or from ten repeated 24-HDR in 195 persons participating in the validation study [86] were used to estimate the distribution in intake in aggregated questions and food group questions. Figure 7 illustrates an example of the procedure of assigning plant sterol values to an aggregated question. In the 84-item FFQ, aggregates of foods were calculated for 28 questions from the calibration data and for three questions from the validation data. In the 64-item FFQ, aggregates of foods were calculated from the calibration data for 22 questions and from the validation data for three questions.

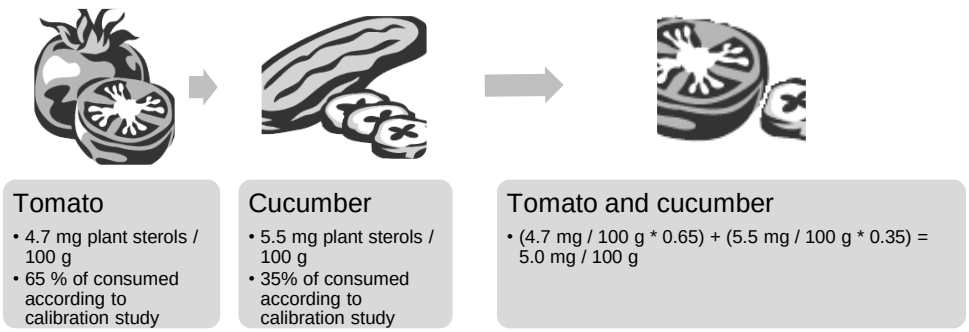

Figure 7. Description of the calculation of the plant sterol value for an aggregated question.

In the 84-item FFQ, 28 questions regarding foods without any plant sterol content were set to zero, 40 questions were based on plant sterol content of direct analyses, 13 questions were based on plant sterol content of calculations of standard recipes with analysed ingredients, one question used the proportion of an analysed food item and for two questions a combination of direct analysis and recipe calculations was used. Standard Swedish recipes were obtained from Vår kokbok [98].

In the 64-item FFQ, 20 questions regarding foods without any plant sterol content were set to zero, 33 questions were based on plant sterol content of direct analyses, seven questions were based on plant sterol content of calculations of standard recipes with analysed ingredients and for four

questions a combination of direct analysis and recipe calculations was used. Table 3 shows a comparison of the EPIC-Norfolk 130-question FFQ and the NSHDS 84- and 64-question FFQ, with regard to number of questions within each food group. In relation to number of questions, the EPIC-Norfolk questionnaire had more questions on vegetables, fruits, snacks and sweets, sauces and other, while the NSHDS questionnaires had more questions on bread and cereals, meat and fish, dairy products and fats.

Table 3. *Comparison of numbers of questions (q) within different food groups in the EPIC-Norfolk 130-question FFQ and the NSHDS 84- and 64-question FFQ.*

	EPIC-Norfolk 130 q.		**NSHDS 84 q.**		**NSHDS 64 q.**	
	No. of q.	**% of total**	**No. of q.**	**% of total**	**No. of q.**	**% of total**
Vegetables	25	19.2	6	7.1	3	4.7
Fruits	11	8.5	4	4.8	3	4.7
Potatoes	4	3.1	5	6.0	2	3.1
Bread and cereals	11	8.5	11	13.1	9	14.1
Meat and fish	16	12.3	16	19.0	12	18.8
Dairy products	7	5.4	10	11.9	8	12.5
Fats	7	5.4	8	9.5	8	12.5
Mixed dishes	8	6.2	6	7.1	4	6.3
Snacks and sweets	19	14.6	6	7.1	5	7.8
Drinks	14	10.8	12	14.3	10	15.6
Sauces and other	8	6.2	-	-	-	-

24-hour dietary recalls

Ten repeated 24-HDRs were conducted by trained interviewers over telephone [86]. The ten 24-HDRs were unannounced, equally dispersed over the year and covered all days of the week. To help in estimation of portion sizes, full-size portion pictures [99] were mailed to participants in advance. Energy and nutrient intakes were calculated using the software MATs (Rudans Lättdata, Västerås, Sweden) and the food composition database at the Swedish National Food Administration, Uppsala, Sweden. Plant sterol values were assigned to each consumed food based on the plant sterol content of direct analyses, calculations of standard recipes with analysed ingredients or proportions of an analysed food item.

Dietary variables

Dietary variables were reported as absolute intakes per day (plant sterols, energy, macronutrients, cholesterol and fiber), as energy-adjusted intakes per day (plant sterols, macronutrients, cholesterol and fiber) or as percentage of total energy intake (E%) (macronutrients). Energy-adjustment was performed by the energy density model, i.e. by division of the nutrient with total energy intake [95].

To handle the strong collinearity between intakes of saturated fat, unsaturated fat and fiber, a new composite categorical fat-fiber variable was constructed. First, each variable was recoded into high or low. According to the Nordic Nutrition Recommendations 2004 [100], the cutoffs were set to 10% of energy intake for saturated fat, 15% of energy intake for unsaturated fat and 3 mg/MJ for energy-adjusted fiber intake. Secondly, for each subject these three variables were combined into a new composite categorical fat-fiber variable, which could take eight values.

Background and lifestyle variables

All background and lifestyle variables were derived from the questionnaire.

Age

Age was derived from the birth registration number as reported in the questionnaire.

Education

Education was categorized based on the highest educational level attained: elementary school; junior high school; high school and college/university.

Smoking

Smoking was categorised into a dichotomous variable in paper III: current smokers and ex/never smokers.

In paper IV, smoking was categorised into: current smokers, ex-smokers and never smokers.

Physical activity

In paper III, a categorical physical activity variable was created based on physical activity at work, leisure time activity and mode of travel to work. This composite physical activity variable could take values from zero to six. Subjects with a physical activity of four, five or six were classified as having a high physical activity.

In paper IV, physical activity level (PAL) was estimated from a combination of two questions, one on physical activity at work and one on leisure time activity, as described and validated by Johansson et al. [101]. For the additional analyses regarding underreporters in Paper III, PAL was also calculated.

Medical variables

Anthropometry

Height was measured to the nearest cm without shoes. Weight was measured without shoes in light clothing to the nearest kg in VIP [82] and to the nearest 0.2 kg in MONICA [84]. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Serum lipids

Serum levels of cholesterol were measured using bench-top analysers (Reflotron®, Boehringer Mannheim GmbH Diagnostica, Germany). In VIP the analyses were performed at each health care center, while the analyses in MONICA were performed at a core laboratory of Umeå University. HDL-cholesterol was measured on a subsample with high total cholesterol, according to the study protocol. HDL-cholesterol was measured after precipitation of the other lipoproteins. LDL-cholesterol was calculated according to Friedewald et al. [102].

Hypertension

In VIP, blood pressure was measured once, in supine position after 5 minutes rest, using a sphygmomanometer [82]. In MONICA, blood pressure was measured twice, in sitting position, and the mean of the two measurements were used [84]. The blood pressure measured in supine position in VIP was corrected to be comparable to the blood pressure measured in sitting position. The correction was performed by equations derived from measurements of blood pressure in both sitting and supine position in more than 600 participants [103].

Participants were classified as hypertensive if systolic blood pressure was ≥ 140 mmHg and/or diastolic blood pressure was ≥ 90 mmHg and/or if they were taking antihypertensive medication. If participants were classified as diabetic the limits were systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 80 mmHg.

Diabetes

Until 2004, plasma glucose was analysed using Reflotron® bench-top analysers (Reflotron®, Boehringer Mannheim GmbH Diagnostica, Germany).

Thereafter, a HemoCue bench-top analyser (Quest Diagnostics) has been used for analyses of plasma glucose values. In VIP, plasma glucose was measured on capillary plasma, while venous plasma was used in MONICA. Fasting plasma glucose was measured after a minimum of 4 hour fast. An OGGT was performed with a 75 g glucose load, on a majority of the non-diabetic participants, according to WHO standards. Participants were regarded as diabetic if fasting plasma glucose was ≥ 7.0 mmol/L and/or two hour capillary plasma glucose ≥ 12.2 or two hour venous plasma glucose ≥ 11.0 and/or self-reported diabetes.

Medication

Participants who answered that they had been using medication the last 14 days were classified as medication users. Medication for hyperlipidaemia, high blood pressure and/or angina/other cardiac conditions were considered in this thesis.

Previously healthy

A binary variable *Previously healthy* (yes/no) was constructed and included current medication for hyperlipidaemia, high blood pressure and/or angina/other cardiac conditions and/or ever having been diagnosed with high blood pressure and/or diabetes prior to the health examination. If all these variables were negative, participants were classified as previously healthy.

4.5 Statistics

All statistical tests were performed using SPSS for WINDOWS, versions 11.5, 14.0 and 18.0 (SPSS Inc, Chicago, IL), except the weighted kappa statistics in paper IV which were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). A p-value less than 0.05 was considered significant.

For continuous variables, independent samples t-test *(Paper I and III)* or Mann-Whitney U-test *(Paper IV)* were used to analyse differences between two groups. One-way analysis of variance (ANOVA) was used to analyse differences in mean values between age groups. When the overall P value from ANOVA was <0.05, the post hoc Bonferroni test was performed *(Paper I)*. For categorical variables, the chi square test was performed to investigate differences in occurrence between groups *(Paper III and IV)*.

Pearson correlation coefficient, Bland-Altman plots and weighted kappa statistics were used to investigate the level of agreement between two methods *(Paper II)*.

Participants were classified into quintiles *(Paper III)* or quartiles *(Paper II and IV)* according to their plant sterol intake, depending on sample size. The construction of quintiles and quartiles were performed separately for men and women and FFQ version (64 or 84 questions).

Multivariate linear regression was used to investigate the relation between plant sterol intake quintile, as the independent variable, and serum levels of cholesterol, as the dependent variable, adjusting for confounders *(Paper III)*.

Odds ratios (OR) with 95% confidence intervals (CI) for a first MI were calculated by multivariate conditional logistic regression, with plant sterol intake quartile as the main predictor, adjusting for confounders *(Paper IV)*.

4.6 Calculations

Attenuation factor

To correct the correlation coefficient for within- and between-individual variation, an attenuation factor was calculated according to Willet [78] as

$$a_{factor}=([1 + (CV_w / CV_b)_x / n_x] [1 + (CV_w / CV_b)_y / n_y])^{0.5}$$

Where CV_w is within-individual variation, CV_b is between-individual variation, n is number of repeated measurements. FFQ is represented by x and the 24-HDRs are represented by y.

Calibration coefficient

A calibration coefficient was calculated by linear regression of the plant sterol intake estimated with the repeated 24-HDRs on the plant sterol intake estimated with FFQ1.

Food intake level (FIL)

FIL was used to identify the most extreme outliers of energy intake. FIL was calculated as reported total energy intake divided by basal metabolic rate (BMR). BMR was estimated from sex, age and body weight according to Schofield [104]. In paper I and III, participants below the 0.5^{th} or above the 99.5^{th} percentile were excluded. In paper IV participants below the 5^{th} or above the 99^{th} percentile were excluded prior to the analyses.

Goldberg cut-off for investigation of underreporting

The Goldberg cut-off for investigation of underreporting is based on the agreement between energy intake and energy expenditure when body weight is stable [105]. Reported energy intake (EI) can be compared to estimated energy expenditure (EE) by comparing FIL with estimated PAL, calculated as EE divided by BMR. Confidence limits for the estimated PAL are calculated as:

Lower limit: $PAL * e^{[s.d.min * (S/100)/\sqrt{n}]}$

Upper limit: $PAL * e^{[s.d.max * (S/100)/\sqrt{n}]}$

In additional analyses related to paper III, confidence limits of 95% were used ($s.d._{min}$= -1.96 and $s.d._{max}$=1.96). In paper IV, confidence limits of 99% were used ($s.d._{min}$= -2.58 and $s.d._{max}$=2.58). The variations in estimated energy intake, estimated BMR and energy requirements are accounted for in S and is given by:

$$S = \sqrt{(CV^2_{wEI}/d + CV^2_{wB} + CV^2_{tP})}$$

CV_{wEI} is the within-subject coefficient of variation in energy intake and in this case it was set to 28.6%, as calculated from 10 repeated 24-hour recalls performed in a subsample of the study population. The number of days (d) is infinite for an FFQ. This simplifies the equation to:

$$S = \sqrt{(CV^2_{wB} + CV^2_{tP})}$$

The precision of estimated BMR is given by CV_{wB} and was set to 9.8% for men and 8.8% for women. CV_{tP} is the between-subject variation in PAL and was set to 15% [106].

Under- and overreporters were classified by comparing FIL with CI limits for PAL:

Underreporter if: $FIL \leq PAL * e^{[s.d.min * (S/100)/\sqrt{n}]}$

Overreporter if: $FIL \geq PAL * e^{[s.d.max * (S/100)/\sqrt{n}]}$

In paper III, the lower 95% CI limit for men corresponded to PAL*0.70 and the higher 95% CI limit corresponded to PAL*1.42. For women corresponding figures were PAL*0.71 and PAL*1.41. PAL level for each subject was derived from questions about physical activity at work and leisure time according to Johansson and Westerterp [101]. Accordingly, 53% of the men were classified as under reporters and 1% as over reporters. The corresponding figures for women were 55% and 0.8 %, respectively.

In paper IV, the lower 99% CI limit for men corresponded to PAL*0.63 and the higher corresponded to PAL*1.59. For women the lower limit was PAL*0.64 and the higher limit was PAL*1.57. Forty-two percent of the men and 51% of the women were classified as under reporters, while none was classified as over reporters.

5 RESULTS

5.1 Dietary intake of naturally occurring plant sterols in UK

The reported dietary intake of naturally occurring plant sterols in the UK was 300 mg/d for men and 293 mg/d for women. Energy-adjusted plant sterol intake was lower in men than in women (33 *v.* 36 mg/MJ). β-sitosterol contributed with 66% of total plant sterol intake, while campesterol and stigmasterol contributed with 22 and 8%, respectively. The plant stanols, campestanol and β-sitostanol together accounted for 4% of the total plant sterol intake.

Bread and other cereals, vegetables and added fats were identified as the three main dietary sources of plant sterols in the EPIC-Norfolk population, representing 19, 18 and 17% of the total plant sterol intake, respectively. Additionally, cakes, scones and chocolate contributed with 14% and fruits with 12%. Together these five sources represented 80% of the plant sterol intake. Energy-adjusted plant sterol intake from these five main sources differed between men and women (Figure 8). Women had higher energy-adjusted reported intakes from vegetables, bread and other cereals, added fats and fruits, while men had a higher reported intake from cakes, scones and chocolate.

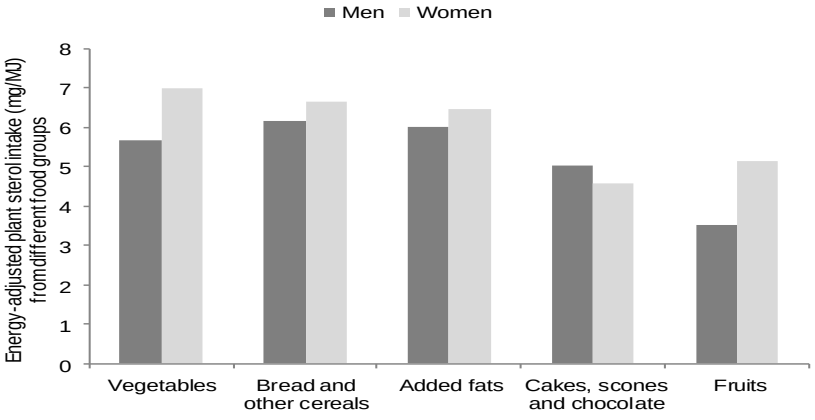

Figure 8. Energy-adjusted plant sterol intake (mg/MJ) from the five main sources of plant sterol intake in 11 227 men and 13 571 women participating in EPIC-Norfolk. All differences between men and women are significant (independent samples t-test, $p<0.001$).

Figure 9 jointly presents box plots of absolute and energy-adjusted plant sterol intake in EPIC-Norfolk and VIP.

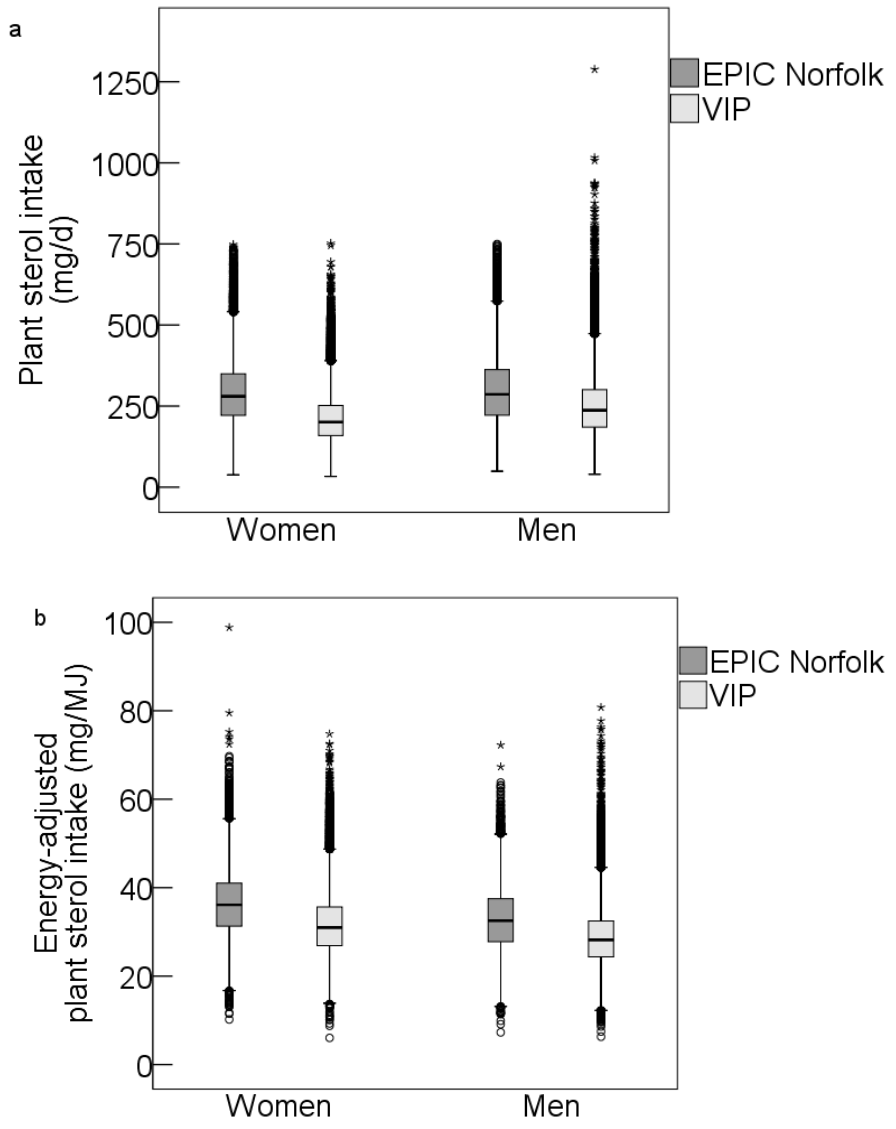

Figure 9. Absolute (a) and energy-adjusted (b) plant sterol intakes by sex and cohort. Boxes represent median, first and third quartile of intake. Minimum and maximum of intakes are also displayed.

5.2 Dietary intake of naturally occurring plant sterols in northern Sweden

Men in northern Sweden had a reported mean plant sterol intake of 252 mg/d, while women had a reported mean intake of 212 mg/d. β -sitosterol was the major contributor to total plant sterol intake and represented 64% of the total plant sterol intake, followed by campesterol which contributed with 24% and stigmasterol which contributed with 5%. The plant stanols, campestanol and β-sitostanol, together represented 6% of the total plant sterol intake. The energy-adjusted plant sterol intake was lower in men than in women (29 *v*. 32 mg/MJ). In both men and women, a higher energy-adjusted plant sterol intake was associated with higher absolute intake of plant sterols, higher intake of unsaturated fat and higher intake of fiber. A higher energy-adjusted plant sterol intake was also associated with lower intakes of energy, saturated fat, alcohol and cholesterol.

Differences in food consumption between those with a low and those with a high intake of energy-adjusted plant sterols were also investigated. Figure 10 shows consumption frequencies (portions/d) of different foods and food groups by the lowest and highest quintile of energy-adjusted plant sterol intake. Men and women with a high energy-adjusted plant sterol intake had higher intakes of low-fat spread, butter, vegetable oil, fruits, vegetables, bread and cereals, potatoes, pasta and rice, whilst men and women with a low energy-adjusted intake of plant sterols had higher intakes of high-fat spread, dairy products, meat and sweets. Fish intake differed marginally (although statistically significant) between quintile one and five, and compared to quintile one men in quintile five had a somewhat higher fish intake while women had a lower intake.

Figure 11 shows the co-variation between foods and absolute and energy-adjusted intakes. Intake of vegetable oil was highly correlated to both absolute and energy-adjusted intake of naturally occurring plant sterols in both men and women. Moderately high significant correlations were found between absolute intake of plant sterols and consumption frequencies of bread and cereals; potatoes, rice and pasta and vegetables in both men and women, and between energy-adjusted plant sterol intake and consumption frequency of vegetables in women.

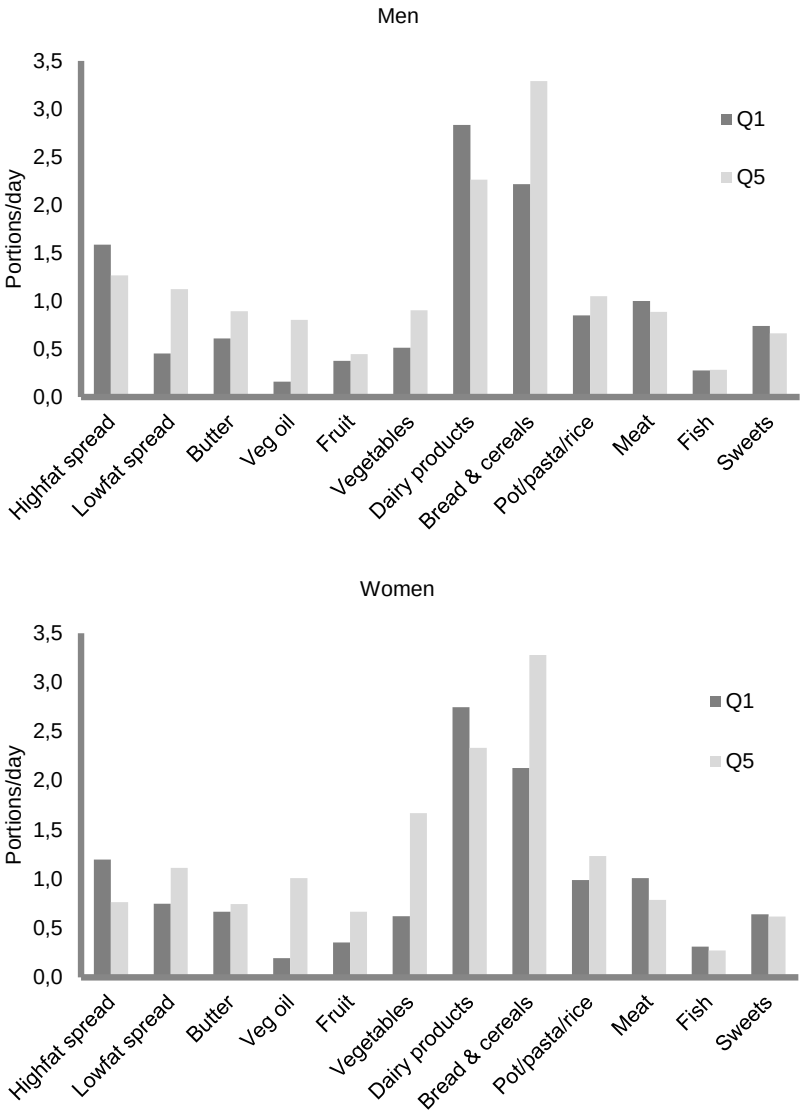

Figure 10. Consumption frequency (portions/d) by the quintile with the lowest (Q1) and highest (Q5) energy-adjusted plant sterol intake (mg/MJ). All differences between Q1 and Q5 are significant (independent samples t-test, $p<0.05$).

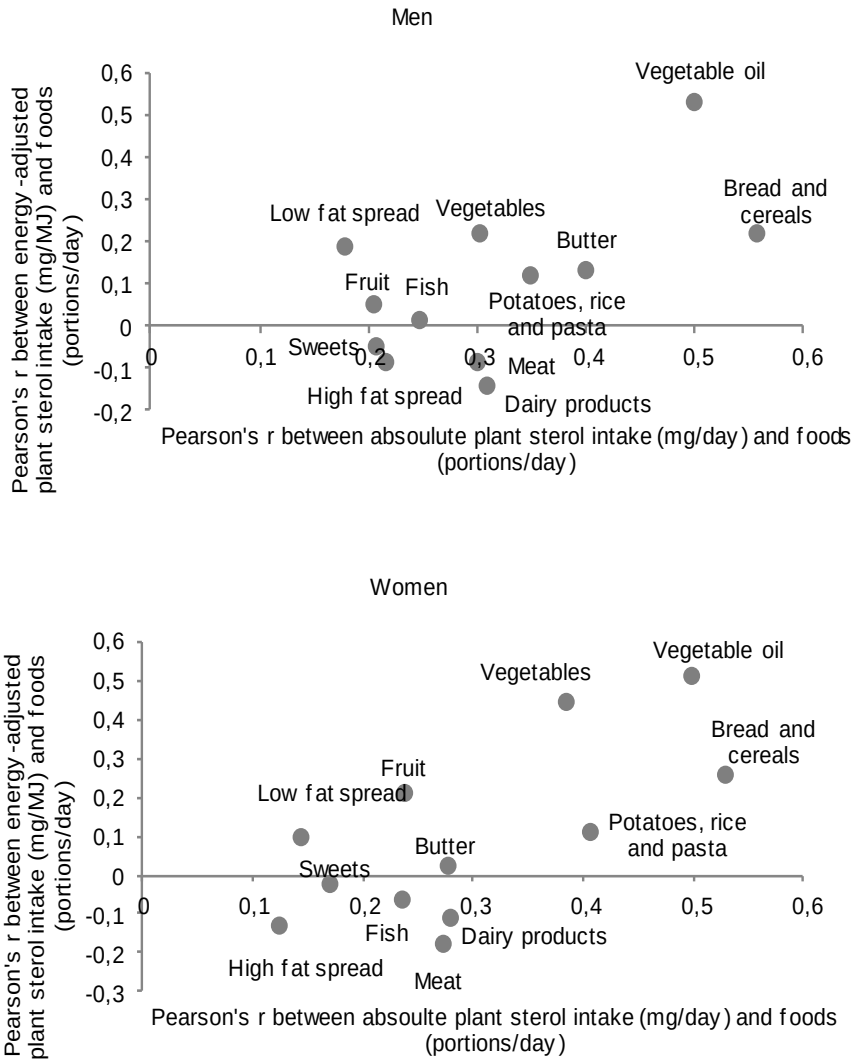

Figure 11. Correlations between intake of food groups and absolute intake (mg/day, x-axis) and energy-adjusted intake (mg/MJ, y-axis) of naturally occurring plant sterols. (Pearson's r=Pearson's correlation coefficient)

5.3 Evaluation of the ability to measure plant sterol intake by the Northern Sweden FFQ

Table 4 shows results from the evaluation of plant sterol intake estimated with the Northern Sweden FFQ with ten repeated 24-HDRs as reference method. The absolute intake of plant sterols was 19% higher estimated for men estimated with FFQ1 than with 24-HDRs, and 17% higher for women. Correlation coefficients between total plant sterol intake estimated with FFQ1 and 24-HDR was 0.58 and 0.55 for men and women, respectively.

Energy-adjusted total plant sterol intake was 17% higher for men estimated with FFQ1 than with 24-HDRs, while it was 7% higher for women. Correlation coefficients between energy-adjusted plant sterol intake estimated with FFQ1 and 24-HDRs were lower than for absolute plant sterol intake. Figure 12 shows the results of cross-classification of participants in quartiles of absolute and energy-adjusted plant sterol intake estimated with FFQ1 and 24-HDR. Forty-seven per cent of the men and 44% of the women were classified with exact agreement and about as many were classified in an adjacent quartile according to absolute plant sterol intake. Severe misclassification was rare. Cross classification of energy-adjusted plant sterol intake showed a somewhat lower agreement. Fewer subjects were classified with exact agreement and severe misclassification was also more prevalent.

The reproducibility of the FFQ was also investigated. For men, absolute plant sterol intake was 9% higher estimated with FFQ1 compared to FFQ2, while it was 6% higher for women. Energy-adjusted plant sterol intake did not differ between the two measurements. Correlation coefficients were over 0.6 for both absolute and energy-adjusted plant sterol intake for both sexes. Figure 13 shows that about 50% of the subjects were classified in the same quartile according to both absolute and energy-adjusted plant sterol intake and that very few were severely misclassified.

Table 4. *Absolute and energy-adjusted plant sterol intake estimated with the first administration of the Northern Sweden FFQ (FFQ1) and ten repeated 24-hour dietary recalls (24-HDR) in 96 men and 99 women participating in the Västerbotten validation study.*

Plant sterol intake		FFQ1		24_HDR		Ratio FFQ1:24HDR*		Pearson's correlation coefficient†			Calibration coefficient‡	
	Gender	Mean	95% CI	Mean	95%CI	Mean	95%CI	Crude	P value	De-attenuated	Λ-value	95%CI
Absolute	Men	257	238, 276	226	211, 242	1.19	1.11, 1.28	0.58	<0.001	0.68	0.47	0.33, 0.61
	Women	219	204, 233	197	185, 209	1.17	1.09, 1.24	0.55	<0.001	0.64	0.46	0.32, 0.60
Energy-adjusted	Men	28	27, 29	25	24, 26	1.17	1.11, 1.23	0.28	<0.01	0.34	0.24	0.07, 0.42
	Women	31	30, 32	29	28, 30	1.07	1.03, 1.11	0.46	<0.001	0.56	0.39	0.24, 0.54

* Mean (95% CI) of individual ratios between absolute and energy-adjusted plant sterol intakes estimated with FFQ1 and 24-HDRs

† The Pearson correlation coefficient between absolute and energy-adjusted plant sterol intakes estimated with FFQ1 and 24-HDRs, crude coefficients and after de-attenuation

‡ The calibration coefficient, with 95% CI corresponding to the slope of the regression of absolute and energy-adjusted plant sterol intake estimated with 24-HDRs on the intake estimated with FFQ1

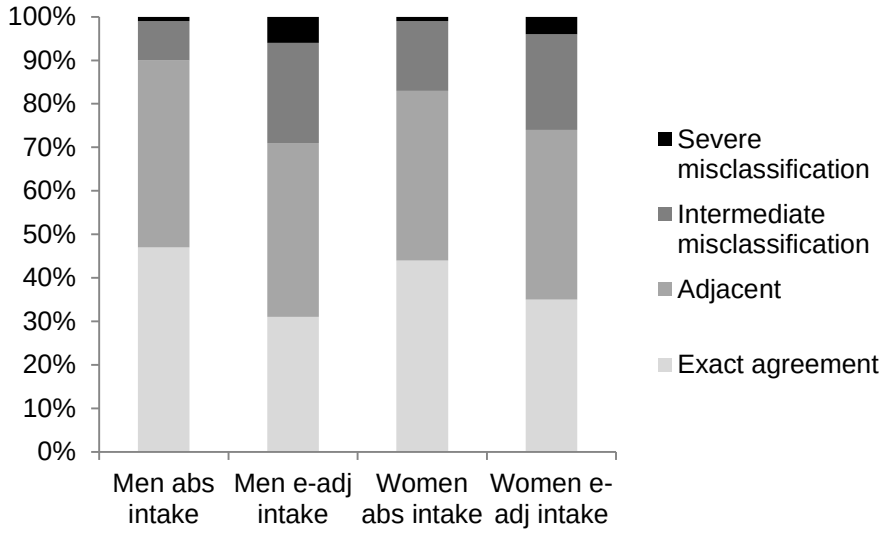

Figure 12. Illustration of the agreement of cross-classification of the absolute (abs) plant sterol intake and the energy-adjusted (e-adj) plant sterol intake estimated with FFQ1 and ten repeated 24-HDRs in 96 men and 99 women.

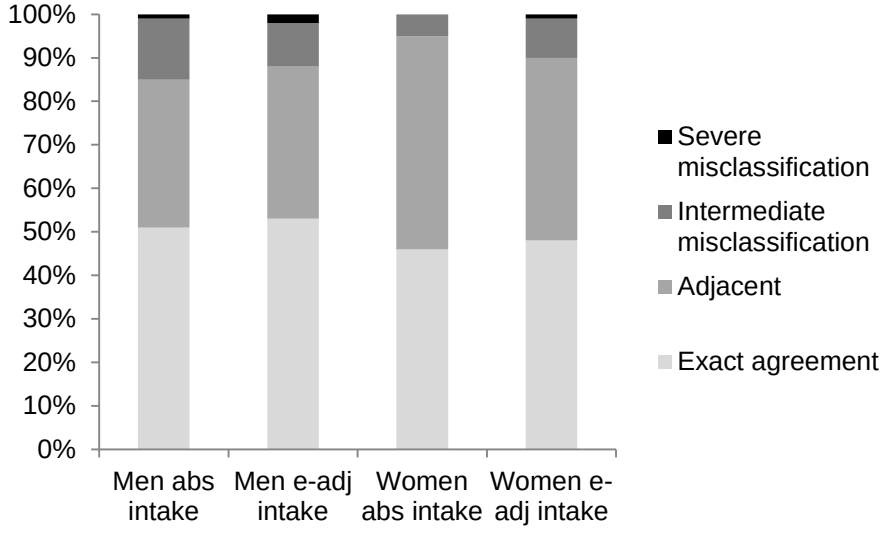

Figure 13. Illustration of the agreement of cross-classification of the absolute (abs) plant sterol intake and the energy-adjusted (e-adj) plant sterol intake estimated with FFQ1 and FFQ2 in 96 men and 97 women.

5.4 Dietary intake of plant sterols in relation to serum cholesterol in northern Sweden

In crude linear regression analyses with energy-adjusted plant sterol quintile as predictor, there were significant inverse linear trends between energy-adjusted plant sterol quintile and total and LDL-cholesterol in both men and women (Table 5). After adjustments for age, BMI, saturated fat intake, unsaturated fat intake, fiber intake, alcohol intake, smoking, high physical activity, lipid-lowering medication and (in women) menopausal status, the significant trends remained for serum levels of total cholesterol in both men and women and for LDL-cholesterol in women. In the crude analyses, serum levels of total cholesterol in quintile 5 was, compared to quintile 1, 0.16 mmol/L (2.8%) lower in men and 0.21 mmol/L (3.7%) lower in women, while serum levels of LDL-cholesterol was 0.16 mmol/L (3.8%) and 0.13 mmol/L (3.2%) lower in men and women, respectively. After adjustment for the above mentioned confounders the differences between quintile 5 and quintile 1 did not essentially change.

A multiple linear regression with energy-adjusted plant sterol intake as a continuous predictor was also performed, adjusted for the confounders mentioned above. These analyses suggested that if energy-adjusted plant sterol intake is increased by 20 mg/MJ total serum levels of total cholesterol decreases with 0.06 mmol/L in men and 0.22 mmol/L in women. Increasing energy-adjusted plant sterol intake with 20 mg/MJ implies a decrease in serum levels of LDL-cholesterol by 0.06 mmol/L in men (not statistically significant) and by 0.12 mmol/L in women.

A higher energy-adjusted plant sterol intake was associated with a higher prevalence of high physical activity and lipid-lowering medication and a lower prevalence of smoking in both men and women.

Table 5. *Serum levels of total and LDL cholesterol by energy-adjusted plant sterol intake quintile (Q).*

		Energy-adjusted plant sterol intake					
	β-coefficient of plant sterol quintile[1]	Q1	Q2	Q3	Q4	Q5	P for linear trend
Men							
Total cholesterol (mmol/L)		n=7430	n=7430	n=7430	n=7430	n=7430	
Unadjusted	-0.036 (0.004)	5.70 (1.16)[2]	5.63 (1.15)	5.59 (1.13)	5.58 (1.14)	5.54 (1.13)	<0.001
Adjusted[3]	-0.019 (0.004)	5.69 (0.22)	5.64 (0.20)	5.60 (0.19)	5.57 (0.18)	5.54 (0.17)	<0.001
Adjusted[4]	-0.011 (0.004)	5.69 (0.27)	5.63 (0.26)	5.60 (0.25)	5.57 (0.23)	5.54 (0.22)	0.008
LDL cholesterol (mmol/L)		n=2012	n=1878	n=1858	n=1846	n=1859	
Unadjusted	-0.033 (0.008)	4.19 (1.15)	4.15 (1.17)	4.13 (1.16)	4.14 (1.16)	4.03 (1.15)	<0.001
Adjusted[3]	-0.016 (0.008)	4.20 (0.17)	4.16 (0.16)	4.12 (0.15)	4.09 (0.14)	4.07 (0.13)	0.062
Adjusted[4]	-0.008 (0.008)	4.20 (0.24)	4.15 (0.24)	4.12 (0.23)	4.09 (0.21)	4.07 (0.22)	0.337
Women							
Total cholesterol (mmol/L)		n=8100	n=8101	n=8101	n=8100	n=8100	
Unadjusted	-0.048 (0.004)	5.65 (1.18)	5.56 (1.18)	5.51 (1.13)	5.51 (1.14)	5.44 (1.09)	<0.001
Adjusted[3]	-0.061 (0.004)	5.65 (0.44)	5.57 (0.46)	5.53 (0.47)	5.48 (0.46)	5.45 (0.45)	<0.001
Adjusted[4]	-0.052 (0.004)	5.64 (0.46)	5.56 (0.48)	5.52 (0.48)	5.48 (0.48)	5.45 (0.46)	<0.001
LDL cholesterol (mmol/L)		n=1962	n=1882	n=1958	n=1858	n=1872	
Unadjusted	-0.030 (0.009)	4.05 (1.18)	4.00 (1.26)	3.97 (1.18)	3.98 (1.20)	3.92 (1.20)	<0.001
Adjusted[3]	-0.038 (0.008)	4.06 (0.39)	4.00 (0.42)	3.98 (0.43)	3.96 (0.42)	3.93 (0.41)	<0.001
Adjusted[4]	-0.026 (0.008)	4.05 (0.43)	4.00 (0.45)	3.98 (0.46)	3.96 (0.45)	3.93 (0.45)	0.002

[1]Mean (SE)

[2]Mean (SD) (all such values)

[3] Adjusted for age and BMI

[4] Adjusted for age, BMI, saturated fat (E%), unsaturated fat (E%), fiber (g/MJ), alcohol (E%), smoking, high physical activity, cholesterol-lowering medication and (in women) menopausal status.

Table 6. *Serum levels of total and LDL cholesterol by absolute plant sterol intake quintile (Q).*

		Absolute plant sterol intake					
	β-coefficient of plant sterol quintile	Q1	Q2	Q3	Q4	Q5	P for linear trend
Men							
Total cholesterol (mmol/L)		n=7429	n=7431	n=7430	n=7431	n=7429	
Unadjusted	-0.055 (0.004)	5.71 (1.16)	5.67 (1.13)	5.61 (1.14)	5.57 (1.14)	5.48 (1.14)	<0.001
Adjusted[3]	-0.031 (0.004)	5.72 (0.19)	5.66 (0.19)	5.61 (0.19)	5.56 (0.19)	5.49 (0.18)	<0.001
Adjusted[4]	-0.022 (0.004)	5.72 (0.25)	5.65 (0.24)	5.61 (0.24)	5.55 (0.24)	5.50 (0.23)	<0.001
LDL cholesterol (mmol/L)		n=1964	n=1932	n=1861	n=1854	n=1842	
Unadjusted	-0.039 (0.008)	4.21 (1.14)	4.14 (1.16)	4.14 (1.18)	4.12 (1.18)	4.02 (1.14)	<0.001
Adjusted[3]	-0.018 (0.009)	4.20 (0.15)	4.16 (0.15)	4.13 (0.15)	4.10 (0.15)	4.05 (0.15)	0.039
Adjusted[4]	-0.011 (0.009)	4.20 (0.23)	4.16 (0.23)	4.12 (0.23)	4.09 (0.23)	4.05 (0.21)	0.227
Women							
Total cholesterol (mmol/L)		n=8100	n=8101	n=8100	n=8101	n=8100	
Unadjusted	-0.094 (0.004)	5.72 (1.18)	5.63 (1.15)	5.55 (1.13)	5.45 (1.13)	5.33 (1.10)	<0.001
Adjusted[3]	-0.030 (0.004)	5.72 (0.43)	5.62 (0.43)	5.54 (0.44)	5.46 (0.43)	5.33 (0.44)	<0.001
Adjusted[4]	-0.029 (0.004)	5.72 (0.46)	5.62 (0.45)	5.54 (0.46)	5.45 (0.45)	5.33 (0.45)	<0.001
LDL cholesterol (mmol/L)		n=2016	n=1951	n=1898	n=1824	n=1843	
Unadjusted	-0.087 (0.009)	4.13 (1.20)	4.10 (1.18)	3.98 (1.20)	3.91 (1.22)	3.79 (1.18)	<0.001
Adjusted[3]	-0.021 (0.008)	4.14 (0.36)	4.07 (0.38)	4.00 (0.39)	3.92 (0.40)	3.78 (0.44)	0.013
Adjusted[4]	-0.021 (0.009)	4.15 (0.41)	4.07 (0.42)	4.00 (0.43)	3.92 (0.44)	3.78 (0.46)	0.013

[1]Mean (SE)

[2]Mean (SD) (all such values)

[3] Adjusted for age and BMI

[4] Adjusted for age, BMI, saturated fat (E%), unsaturated fat (E%), fiber (g/MJ), alcohol (E%), smoking, high physical activity, cholesterol-lowering medication and (in women) menopausal status.

Additional analyses were performed with absolute, instead of energy-adjusted, plant sterol quintile as main predictor, and the results from these analyses are presented in table 6. The effect of absolute plant sterol intake on serum levels of total and LDL-cholesterol was higher, especially for women, compared to the effect of energy-adjusted plant sterol intake. In women, serum levels of total cholesterol was 0.39 mmol/L (6.8%) lower in quintile 5 compared to quintile 1, and serum levels of LDL-cholesterol was 0.34 mmol/L (8.2%) lower in the fully adjusted model.

To investigate if the inclusion also of second-time visits had any impact on the results, analyses were performed with only first-time visits. Analyses with energy-adjusted plant sterol quintile as main predictor showed similar differences between quintile one and quintile five regarding total and LDL-cholesterol (data not shown). However, in the fully adjusted model, the only significant trend that remained was for total cholesterol in women. This may be an effect of the smaller sample size.

After exclusion of those classified as under reporters, analyses revealed essentially the same results as in the original analyses when all were included. However, the statistical significance differed, probably as an effect of the smaller sample size. Unadjusted regressions of energy-adjusted plant sterol quintile on total and LDL-cholesterol were all significant for both men and women. After the first adjustment for age, BMI and (in women) menopausal status, the p-value increased to borderline significance for total cholesterol in men, while it still was significant in women. For LDL, energy-adjusted plant sterol quintile was no longer a significant predictor in men, while it was borderline significant for women. Further adjustments for fat intake, fiber intake, alcohol intake, smoking, physical activity and cholesterol-lowering medication did not affect total cholesterol in women, while the p-value increased further for all other regressions.

5.5 Dietary intake of naturally occurring plant sterols and the risk of a first MI

Median age at first MI diagnosis was 62 and 63 years in men and women, respectively. The median interval from baseline examination to diagnosis was six years and varied between zero and 21 years. At baseline, both male and female cases had, compared to the referents, higher BMI, higher total cholesterol and higher triglycerides and a higher prevalence of smoking, hypertension and diabetes. Male cases were also more likely to be on lipid-lowering medication.

In crude logistic regression analyses, with absolute plant sterol quartile as predictor, plant sterol intake showed a protective effect in men, but not in women (Table 7). For men, OR of the fourth compared to the first quartile was 0.70. Adjustment for BMI and fat and fiber intake did not change the results. Further adjustment for alcohol intake, hypertension, lipid-lowering medication and education somewhat attenuated the OR to 0.76, and although the 95% CI of the OR of the fourth compared to the first quartile was significant, the trend was no longer significant. Adjustment with the regression calibration coefficient (as reported in paper II), decreased the OR to 0.56. Analyses with energy-adjusted plant sterol quartiles did not show any significant trends.

Analyses were also performed solely on participants who at baseline were classified as previously healthy (Table 8). ORs were consistently lower for both men and women, but especially for women. P for trend was statistically significant for absolute plant sterol quartile for men in the crude analyses and after the first set of adjustments, but not in the fully adjusted model.

Table 7. *Risk of a first myocardial infarction by plant sterol intake quartile*[1]

		Quartiles				
		1	2	3	4	P for trend
Absolute plant sterol intake						
Women (n cases/referents)		82/279	78/280	71/279	70/279	
	Crude	1.0	0.94 (0.66-1.35)	0.89 (0.62-1.28)	0.88 (0.61-1.28)	0.907
	Adjusted[2]	1.0	0.90 (0.63-1.29)	0.84 (0.58-1.22)	0.82 (0.56-1.21)	0.757
	Adjusted[3]	1.0	1.02 (0.69-1.50)	1.09 (0.72-1.64)	1.08 (0.71-1.64)	0.974
Men (n cases/referents)		305/854	245/855	235/855	210/853	
	Crude	1.0	0.80 (0.66-0.98)	0.78 (0.64-0.95)	0.70 (0.57-0.86)	0.004
	Adjusted[2]	1.0	0.80 (0.66-0.98)	0.77 (0.63-0.94)	0.70 (0.57-0.86)	0.006
	Adjusted[3]	1.0	0.83 (0.68-1.02)	0.84 (0.68-1.04)	0.76 (0.61-0.95)	0.091
Energy-adjusted plant sterol intake						
Women (n cases/referents)		64/279	75/280	76/279	86/279	
	Crude	1.0	1.18 (0.81-1.71)	1.24 (0.85-1.80)	1.39 (0.96-2.02)	0.372
	Adjusted[2]	1.0	1.13 (0.77-1.65)	1.17 (0.78-1.75)	1.35 (0.86-2.12)	0.622
	Adjusted[3]	1.0	1.29 (0.85-1.95)	1.44 (0.92-2.24)	1.80 (1.11-2.94)	0.129
Men (n cases/referents)		268/854	263/855	220/855	244/853	
	Crude	1.0	0.99 (0.81-1.21)	0.84 (0.68-1.03)	0.95 (0.77-1.18)	0.306
	Adjusted[2]	1.0	0.97 (0.79-1.18)	0.81 (0.65-1.02)	0.92 (0.72-1.18)	0.290
	Adjusted[3]	1.0	1.04 (0.84-1.28)	0.90 (0.71-1.14)	1.02 (0.79-1.32)	0.579

[1] OR and 95% CI calculated by conditional logistic regression.
[2]Adjusted for BMI, fat and fiber intake
[3]Adjusted for BMI, fat and fiber intake, alcohol intake, smoking, hypertension, medication for hyperlipidemia, education

Table 8. *Risk of a first myocardial infarction by plant sterol intake quartile in participants who classified at baseline as previously healthy*[1, 2]

		Quartiles				
		1	2	3	4	P for trend
Absolute plant sterol intake						
Women (n cases/referents)		56/117	35/129	38/109	36/116	
	Crude	1.0	0.56 (0.34-0.93)	0.69 (0.41-1.14)	0.64 (0.37-1.09)	0.135
	Adjusted[3]	1.0	0.56 (0.33-0.93)	0.62 (0.37-1.04)	0.58 (0.33-1.01)	0.096
	Adjusted[4]	1.0	0.63 (0.35-1.12)	0.86 (0.49-1.51)	0.74 (0.40-1.38)	0.452
Men (n cases/referents)		197/448	158/445	154/430	135/453	
	Crude	1.0	0.81 (0.63-1.04)	0.80 (0.61-1.03)	0.66 (0.50-0.86)	0.022
	Adjusted[3]	1.0	0.81 (0.63-1.05)	0.81 (0.62-1.05)	0.66 (0.50-0.87)	0.031
	Adjusted[4]	1.0	0.84 (0.64-1.09)	0.87 (0.66-1.15)	0.70 (0.52-0.94)	0.119
Energy-adjusted plant sterol intake						
Women (n cases/referents)		45/124	35/119	43/120	42/108	
	Crude	1.0	0.81 (0.48-1.36)	1.06 (0.64-1.75)	1.11 (0.67-1.83)	0.690
	Adjusted[3]	1.0	0.71 (0.41-1.23)	0.88 (0.51-1.53)	0.87 (0.47-1.61)	0.674
	Adjusted[4]	1.0	0.65 (0.35-1.20)	0.84 (0.45-1.57)	0.82 (0.41-1.65)	0.586
Men (n cases/referents)		179/434	161/417	146/454	158/471	
	Crude	1.0	0.94 (0.73-1.22)	0.79 (0.60-1.04)	0.86 (0.65-1.14)	0.369
	Adjusted[3]	1.0	0.92 (0.70-1.20)	0.79 (0.59-1.06)	0.86 (0.63-1.18)	0.468
	Adjusted[4]	1.0	0.92 (0.70-1.22)	0.82 (0.60-1.11)	0.92 (0.66-1.29)	0.609

[1] OR and 95% CI calculated by conditional logistic regression.
[2] Participants regarded as previously healthy if no reported history of hypertension or current diabetes and no current medication for hypertension, hyperlipidemia and/or angina/other cardiac conditions at baseline
[3]Adjusted for BMI, fat and fiber intake
[4]Adjusted for BMI, fat and fiber intake, alcohol intake, smoking, hypertension, education

6 DISCUSSION

Dietary intake of naturally occurring plant sterols have previously been sparsely studied, likely because of the lack of easily accessible nutrient values, but also because they have been thought to be biologically inert. However, the studies presented in this thesis suggest important roles for naturally occurring plant sterols in the prevention of CVDs. The starting point of the present thesis has been the unique opportunity to combine large population based cohort studies of dietary intake with an extensive plant sterol database. Within the frame of this thesis, we have examined several steps of the theoretical causal chain between dietary intake of naturally occurring plant sterols, serum levels of cholesterol and the risk of contracting a first MI.

Below strengths and weaknesses of the studies are discussed and the results related to existing knowledge and plausibility.

6.1 Methodology

Are we able to measure plant sterol intake?
A central issue is if we were able to measure plant sterol intake properly, because this is the basis of all research in the thesis. This issue depends firstly on the correctness of chemical analyses of foods, secondly on the assignment of correct plant sterol values to the foods in the FFQ and thirdly on the ability of the FFQ to estimate intake of foods containing plant sterols.

Chemical analyses
The methodology used to analyse plant sterol content in foods in the present database has been carefully evaluated, and was considered to be accurate in the quantification of different common plant sterols and stanols [87].

There are however not many extensive publications on plant sterol content of foods. Comparison of plant sterol values of vegetables and fruits in the present database [68], with values in other publications [71, 107-109], shows little difference for most items. As an example, the plant sterol value of orange is 24 mg/100g in the present database, while it varies from 23 to 27 mg/100 g in the other publications. Cauliflower, on the other hand, varied more. The plant sterol value of cauliflower was 40 mg/100g in this database while it varied from 18 mg/100g to 43 mg/100g in other studies [71, 107-109]. It could be speculated that differences of this magnitude could depend on what was considered the edible part, and in this case how much of the

stem was included in the sample for analysis, as the stem might have a higher or a lower plant sterol content. The variation between 27 and 41 mg/100 g in three samples of cauliflower in one study [108] also shows that there is a variation between different samples that cannot be explained by methodological differences. As for cereals, analysed food items are poorly specified in other publications [107], making comparisons with values from the present database unreliable. Plant sterol values for nuts and seeds in the present database [66] are similar to values presented in two recent publications [71, 110], while values from an older are shifting for some items [107]. Compared to other publications with plant sterol data on vegetable oils [107, 111, 112], values from the present database [66] seem to agree well for most oils. However, the variation is wide for some oils. Sunflower oil contains 436 mg/100 g according to our database, while it is reported to contain between 263 mg/100 g and 725 mg/100g according to others [71, 107, 111, 112]. It could be speculated that these differences depend on the level of refinement of the oils. The process of oil refining has been shown to reduce the level of plant sterols by between 10% and 70% [29]. A more recent publication showed that compared to the crude oil, the plant sterol content of the totally refined oil of corn, soybean and rapeseed was decreased by 36%, 18% and 24%, respectively [113].

As noted in the introduction, there are numerous plant sterols, while the present database only presents five plant sterols for most foods. This means that the dietary intake of naturally occurring plant sterols likely is slightly higher than that reported. However, in the present studies this unknown fraction has not been quantified. In other publications the unknown fraction has been estimated to between 23% and 26% of the total intake [71, 114]. There is however no reason to believe that this unknown fraction differs between subjects with high or low serum levels of cholesterol or between subjects with high or low risk of contracting a first MI. The dietary intake of naturally occurring plant sterols is probably underestimated to the same extent for all subjects, thus not affecting the ranking of the subjects.

In summary, the plant sterol values used in the present thesis seem to agree fairly well with plant sterol values presented by others. Still, the impact of the variations in plant sterol content of foods in different publications, on the estimation of plant sterol intake, is difficult to quantify. Presumably, these variations partly mirror true differences between food samples: levels of plant sterols may alter with for example maturity level and storage [108] although, plant sterols in vegetables and fruits seem to be practically unaffected by cooking [68]. These differences in plant sterol content in different food

samples is however not unique for plant sterols but probably present for most nutrients.

Assignment of plant sterol values

Assignment of the plant sterol values to the foods in the EPIC-Norfolk FFQ was performed by Susan Andersson and co-authors [5]. In questions regarding specific UK foods, analysed values of such were used. In NSHDS, the assignment of plant sterol was performed by the author. Data from the calibration and validation studies performed within VIP was used when assigning plant sterol values to aggregated questions. When needed, calculations of standard recipes with analysed ingredients were performed. These strategies helped in assigning the most correct plant sterol value of each question. Although, individual variation within each question, such as preference for peaches over pears, or special recipes as opposed to standard recipes, is not possible to take into account within FFQs. Thus, the assignment of plant sterol values to the questions was done as precisely as possible, but the true variation between individuals is impossible to capture with the present FFQs.

Ability of the FFQs to estimate plant sterol intake

The ability to measure plant sterol intake of the FFQ used in the Swedish studies was evaluated in paper II with ten repeated 24-HDRs as the reference method. The FFQ has, compared to the repeated 24-HDRs, been shown to give higher estimates of intake frequencies of bread and cereals, vegetables, fruits, potatoes, pasta and rice [86], which probably contribute to the higher estimation of plant sterol intake by the FFQ method. Portion sizes were sex- and age-specific within VIP, while they were not within EPIC-Norfolk. This could probably explain why the difference in mean intake is so low between men and women in EPIC-Norfolk. Still, even though the estimated intake according to the FFQ was somewhat higher than according to the ten repeated 24-HDRs, the FFQ was judged to have the ability to estimate plant sterol intake to the same degree as it does other nutrients and to rank participants according to their intake with an acceptable precision.

What does this mean for the interpretation of plant sterol data?

If dietary intake of naturally occurring plant sterols in different populations is estimated with different plant sterol values, then differences between populations must be interpreted with caution. Part of a difference in plant sterol intake between populations could be explained by differing plant sterol values in the database used for estimation of plant sterol intake. In addition, part of a difference could be explained by differing dietary assessment methods. On the other hand, when investigating relations between dietary

intake of naturally occurring plant sterols and health outcome within a population, this insecurity in the true plant sterol intake is of less concern, since the ranking of individuals would probably not be affected.

Measurement error in nutritional epidemiology

All dietary intake assessments are prone to measurement errors. Measurement errors can appear both within persons and between persons, and be either random or systematic [78]. A random within person error, implies that if diet is measured repeatedly, the reported intake will sometimes be higher than the true intake, and sometimes lower than the true intake. A systematic within person error will, on the other hand, systematically cause a higher or a lower reported intake compared to the true intake. Random between person errors mean that in a group of people, some will report a higher intake and some a lower intake compared to the true intake. Systematic between person errors, imply that reported intake is systematically under or over reported in a group of people. These types of errors will affect the outcome differently within different studies. Within a descriptive study, random errors, within or between persons, will probably not affect the outcome of mean intake. Systematic errors in this type of study will cause an under- or over-estimation of the intake. In studies of intake versus outcome, random errors, within or between persons, are likely to attenuate associations. Systematic errors that affect all persons to the same extent will not affect risk estimates, while systematic errors that affect cases and non-cases differentially seriously can violate associations between intake and outcome.

To investigate random error, the dietary assessment method needs to be repeated, which was done in paper II. Investigation of the reproducibility of the FFQ showed good agreement between the first and the second FFQ, implying that the random error is not substantial. To investigate systematic within-person error, the dietary assessment method needs to be validated by a superior method which can measure true intake. Unfortunately, no dietary assessment method is able to measure true dietary intake in free-living people. In paper II, the FFQ was evaluated against ten repeated 24-HDRs, as suggested by others [115]. The evaluation showed no evident signs of differential systematic errors regarding low versus high plant sterol intake.

In additional analyses related to paper III and in paper IV, subjects classified as under reporters were identified. It is however possible that too many subjects were classified as under reporters, as the present FFQs probably are not able to capture the entire energy intake. A study evaluating design characteristics of FFQs reported that, in relation to doubly labeled water the

energy intake was underestimated by between 11% and 36% [116]. In paper III and IV, the prevalence of underreporting was between 40% and 55%, as identified by the Goldberg cut-off corresponding to an underreporting of 30-35% of energy. This high prevalence of under reporting could be an effect of the number of questions in the present FFQs, since the FFQs in the study mentioned above had between 124 and 180 FFQ items. It is however not likely that the ranking of individuals in our studies was markedly affected.

Absolute intake versus energy-adjusted intake

Another key issue in the present thesis is the use of absolute versus energy-adjusted plant sterol intake, in analyses of their relation to serum levels of cholesterol and risk of a first MI. Plant sterol intake is strongly correlated to energy intake, which makes it problematic to assign observed effects of high intake of plant sterols to these very compounds and not simply to a high overall dietary intake. It could be speculated that by relating plant sterol intake to energy intake, the quality of diet with respect to plant sterols would be better captured.

However, because of the strong co-variation between plant sterol intake and energy intake, energy-adjustment may transpose subjects between quintiles when classifying them by absolute intake or energy-adjusted intake. Subjects with a high intake of both plant sterols and energy and subjects with a low intake of both plant sterols and energy, will after energy-adjustment all have about the same energy-adjusted plant sterol intake.

Additionally, although the intake range is rather wide, the majority have an intake between 150 and 350 mg/day. Adjustment for energy intake makes the distribution even narrower which could make it more difficult to find an association between intake and outcome. Energy-adjustment may also introduce measurement error from the estimated energy intake.

It must also be taken into consideration if it is the absolute plant sterol intake or the energy-adjusted plant sterol intake that is of highest biological importance. As plant sterols act on both endogenous, bilary secreted, cholesterol and exogenous, dietary, cholesterol, it could be speculated that the absolute intake is the most important exposure information.

6.2 Findings

Main findings

We found that the intake of naturally occurring plant sterols was around 250 mg/day for men and 210 mg/day for women in northern Sweden. A previous publication from our group showed that the intake in the UK was around 300 mg/day for both men and women in the UK [5]. Main dietary sources in the UK were vegetables, bread and other cereals and added fats. We also found an inverse relation between dietary intake of naturally occurring plant sterols and serum levels of total cholesterol in both Swedish men and women, and LDL-cholesterol in women. In Swedish men, we also found an inverse relation between dietary intake of naturally occurring plant sterols and the risk of contracting a first MI.

Intake

The estimated intakes of naturally occurring plant sterols presented in this thesis are essentially on the same level as the previously reported intakes of 280-340 mg/day in men and 220-260 mg/day in women reported from other European populations [69-72]. Men and women in northern Sweden have a somewhat lower estimated intake than that seen in other European populations, which in part may be attributed to the number of questions in the FFQs used. Although, also the energy-adjusted plant sterol intake was somewhat lower in Sweden compared to the UK, Spain and Finland [70, 71]. Morton et al. reported in 1995 that the intake of naturally occurring plant sterols in the British diet was 186 mg/day [117]. This is considerably lower than the presently estimated intake of the EPIC-Norfolk population. However, this discrepancy can be explained by methodological differences. In the study by Morton et al. [117] the estimation was done by analyses of food samples from a national food survey, but at that time the survey did not include foods consumed outside home.

Food sources

There is a strong consistency across different populations concerning the main dietary sources of naturally occurring plant sterols. Bread and cereals and vegetable fats seem to be the most important dietary sources of naturally occurring plant sterols in several European countries [69, 71, 72]. In the EPIC-Norfolk population vegetables were equally important as a source of plant sterols as were bread and cereals and added fats. In other populations vegetables have contributed to a much lesser extent to the total intake of naturally occurring plant sterols. Perhaps this could reflect the amount of questions on vegetables in the EPIC-Norfolk FFQ, as well as the

overestimation of vegetable intake by the FFQ compared to a 7-day diary [118].

Relations to serum cholesterol and risk of a first MI

An obvious question is, if the observed relations between dietary intake of naturally occurring plant sterols and serum levels of cholesterol and risk of a first MI really could be attributed to the very effect of the intake of naturally occurring plant sterols. With the present study design, where other factors are not experimentally controlled for, it is not possible to eliminate the risk that the observed effect is due to some other nutrient or some other factor. However, in the regression analyses, our attempt was to include every relevant confounder that we could theoretically identify to be related to both plant sterol intake and serum levels of cholesterol and the risk of a first MI, respectively. Still, there may be risk of residual confounding, by possible confounders not accounted for or by incomplete control of variables included in the fully adjusted models.

Another problem is collinearity between different dietary variables, like fat, fibre and plant sterols, and between intake of plant sterols and other confounders, like smoking and physical activity or education. This could potentially result in over-adjustment, which could cause an imprecision and attenuation in the estimation of the effect of plant sterol intake on serum levels of cholesterol and the risk of a first MI. In the current thesis, the problem with collinearity between fat and fibre intake was handled by the construction of a composite categorical variable, accounting for the intake of unsaturated fat, saturated fat and fibre. This approach reduced the risk of over-adjustment, which could have occurred if intakes of fat and fibre had been included as separate continuous variables.

In the fully adjusted model, serum levels of total cholesterol was 0.15 mmol/L lower for men in the fifth compared to the first quintile of absolute plant sterol intake, while it was 0.19 mmol/L lower for women. Mean plant sterol intake was 140 mg/day and 110 mg/day higher in quintile 5 compared to quintile 1 in men and women, respectively. Since plant sterols affect serum levels of LDL-cholesterol and not HDL-cholesterol, these figures can be compared with the effect on serum levels of LDL-cholesterol when substituting 4-6% of energy intake from saturated fat by carbohydrates [20]. The addition of 5-7 g of soluble fibre gives the same effect on serum levels of total cholesterol [23]. It has to be considered that these effects originate from clinical trials, where other dietary factors are controlled for. In observational studies, as well as in the every-day life, it is not possible to fully distinguish the effect of one nutrient from the effect of another, since nutrients are

consumed within foods. A higher intake of one nutrient, is related to a higher intake of some other nutrients, and may also be related to lower intake of yet other nutrients. This means that an intake of a nutrient positively affecting a health factor could be related to intakes of other nutrients also affecting this health factor, either positively or negatively.

Other observational studies have shown that serum levels of LDL-cholesterol have been between 0.12 mmol/L and 0.26 mmol/L lower in the highest compared to the lowest tertile, quartile or quintile of plant sterol intake [5, 73, 114]. Although controlled feeding studies with plant sterol doses achievable with normal diet have shown effects on cholesterol absorption [3, 4, 119, 120], such studies have failed to show significant effects on serum levels of cholesterol [119, 121]. This can however possibly be explained by too few subjects since a non-significant trend was observed in one of the studies, showing a decline in serum levels of LDL-cholesterol by 5% when increasing the intake of plant sterols from 59 mg/day to 459 mg/day [119].

Even though the difference in serum levels of total and LDL-cholesterol between the highest and the lowest quintile of plant sterol intake in the present thesis is rather small, this difference could imply a reduced risk of ischaemic heart disease of 4-16% depending on age [122]. According to the results in study IV, the reduction in risk related to a high intake of naturally occurring plant sterol could be even higher. Men in quartile four had an OR of 0.76 compared to men in quartile one, implying a reduced risk of 24%. After adjustment for within and between individual variation, the OR decreased to 0.56, implying a risk reduction of 44% when comparing quartile four with quartile one. However, no significant effect was seen in women, despite the fact that the difference in LDL-cholesterol between quintile five and quintile one in paper III was larger for women than for men. Perhaps this contradiction could be explained by the lower sample size in women in paper IV, but also by the fact that serum levels cholesterol as a risk factor for MI generally is not as strong for women as for men [123, 124].

Previously healthy

In paper IV, the exclusion of participants not classified as previously healthy at baseline examination clarified the relationship between intake of naturally occurring plant sterols and risk of a first MI. In the analyses, ORs decreased considerably, especially for women. This could imply that participants who at baseline are aware of some risk factor associated with MI, in some way report their intake differently than participants without awareness of such a risk factor. This difference in reported intake could either reflect a truly different intake or simply a desire to eat differently, and these possibilities are

impossible to separate. Another study of the VIP population found that food patterns are associated with health problems that existed prior to the health examination and dietary data collection occasion [125]. A study of a southern Swedish cohort reported a positive relationship between individuals' reporting a past food habit change and several risk factors for CVDs [126]. This implies, that in prospective studies of diet and disease, the responses of subjects with prior awareness of a risk factor related to the study outcome, are likely to be biased and attenuate possible associations between diet and disease. Ways to handle this bias by including variables that may capture such awareness are thus needed.

7 CONCLUSIONS

In the EPIC-Norfolk population, the three main dietary sources of naturally occurring plant sterols were bread and other cereals, vegetables and added fats, together contributing with more than 50% of the intake. The most important food sources found in the EPIC-Norfolk population applies to findings from other European populations.

The Northern Sweden FFQ was able to estimate absolute intake of naturally occurring plant sterols and to rank subjects according to both their absolute and their energy-adjusted plant sterol intake. The reproducibility of the FFQ over one year was high, in terms of estimating both absolute and energy-adjusted plant sterol intake.

In a population in northern Sweden, the dietary intake of naturally occurring plant sterols was inversely related to serum levels of total cholesterol in both men and women, and to serum levels of LDL-cholesterol in women. These results are in accordance with findings in other populations.

A high intake of naturally occurring plant sterols was related to a 24-44% lower risk of contracting a first MI in men in northern Sweden. This is, to our knowledge the first study showing a relation between dietary intake of naturally occurring plant sterols and the risk of contracting a first MI. In women, however, no effect was found.

8 FUTURE PERSPECTIVES

CVDs are serious threats to human well-being and health, yet a large part of all CVDs could be prevented. Diet is one truly modifiable variable to which all humans are exposed, making diet fundamental in the preventive work against CVDs. The contemporary Nordic nutrition recommendations recommend an increased intake of fruits, vegetables, wholegrain cereals, nuts and seeds and replacement of saturated fats by unsaturated fats [127]. These recommendations are not essentially different from the dietary recommendations regarding nutritional treatment of hyperlipidaemia. Adherence to these recommendations will to some extent increase the intake of naturally occurring plant sterols. It is however possible to further enhance the intake of plant sterols by choosing foods with a high plant sterol content, and thereby optimizing the favorable effects of a healthy diet on serum levels of cholesterol.

A high dietary intake of naturally occurring plant sterols has been shown to be related to lower serum levels of total and LDL-cholesterol in several epidemiological studies. Still, it would be beneficial if the causal relationship between naturally occurring plant sterols and serum levels of cholesterol was confirmed by dietary intervention trials.

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