Diet and the development of atherosclerosis: 
a whole-diet approach from childhood to adulthood
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To my family
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Abstract

Cardiovascular diseases (CVDs) are the leading cause of mortality in the world. Studies of the impact of single nutrients on the risk for CVD have often provided inconclusive results, and recent research in nutritional epidemiology with a more holistic whole-diet approach has proven fruitful. Moreover, dietary habits in childhood and adolescence may play a role in later health and disease, either independently or by tracking into adulthood. The main aims of this study were to find childhood and adulthood determinants of adulthood diet, to identify dietary patterns present among the study population and to study the associations between long-term food choices and cardiovascular health in young Finnish adults.

The study is a part of the multidisciplinary Cardiovascular Risk in Young Finns study, which is an ongoing, prospective cohort study with a 21-year follow-up. At baseline in 1980, the subjects were children and adolescents aged 3 to 18 years (n included in this study = 1768), and young adults aged 24 to 39 years at the latest follow-up study in 2001 (n = 1037). Food consumption and nutrient intakes were assessed with repeated 48-hour dietary recalls. Other determinations have included comprehensive risk factor assessments using blood tests, physical measurements and questionnaires. In the latest follow-up, ultrasound examinations were performed to study early atherosclerotic vascular changes.

The average intakes showed substantial changes since 1980. Intakes of fat and saturated fat had decreased, whereas the consumption of fruits and vegetables had increased. Intake of fat and consumption of vegetables in childhood and physical activity in adulthood were important health behavioural determinants of adult diet. Additionally, a principal component analysis was conducted to identify major dietary patterns at each study point. A similar set of two major patterns was recognised throughout the study. The traditional dietary pattern positively correlated with the consumption of traditional Finnish foods, such as rye, potatoes, milk, butter, sausages and coffee, and negatively correlated with fruit, berries and dairy products other than milk. This type of diet was independently associated with several risk factors of CVD, such as total and low-density lipoprotein cholesterol, apolipoprotein B and C-reactive protein concentrations among both genders, as well as with systolic blood pressure and insulin levels among women. The traditional pattern was also independently associated with intima media thickness (IMT), a subclinical predictor of CVD, in men but not in women. The health-conscious pattern, predominant among female subjects, non-smokers and urbanites, was characterised by more health-conscious food choices such as vegetables, legumes and nuts, tea, rye, cheese and other dairy products, as well as by the consumption of
alcoholic beverages. This pattern was inversely, but less strongly, associated with cardiovascular risk factors. Tracking of the dietary pattern scores was observed, particularly among subjects who were adolescents at baseline. Moreover, a long-term high intake of protein concurrent with a low intake of fat was positively associated with IMT.

These findings suggest that food behaviour and food choices are to some extent established as early as in childhood or adolescence and may significantly track into adulthood. Long-term adherence to traditional food choices seems to increase the risk for developing CVD, especially among men. Those with intentional or unintentional low fat diets, but with high intake of protein may also be at increased risk for CVD. The findings offer practical, food-based information on the relationship between diet and CVD and encourage further use of the whole-diet approach in epidemiological research. The results support earlier findings that long-term food choices play a role in the development of CVD. The apparent influence of childhood habits is important to bear in mind when planning educational strategies for the primary prevention of CVD. Further studies on food choices over the entire lifespan are needed.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>CA</td>
<td>cluster analysis</td>
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<td>CHD</td>
<td>coronary heart disease</td>
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<td>CI</td>
<td>confidence interval</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
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<td>FA</td>
<td>factor analysis</td>
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<td>FFQ</td>
<td>food frequency questionnaire</td>
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<td>HDL</td>
<td>high-density lipoprotein</td>
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<td>HR</td>
<td>hazard ratio</td>
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<tr>
<td>IMT</td>
<td>intima media thickness</td>
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<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
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<tr>
<td>MI</td>
<td>myocardial infarction</td>
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<tr>
<td>NS</td>
<td>non significant</td>
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<tr>
<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>PCA</td>
<td>principal component analysis</td>
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<tr>
<td>REML</td>
<td>restricted maximum likelihood</td>
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<tr>
<td>RR</td>
<td>relative risk</td>
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<tr>
<td>SBP</td>
<td>systolic blood pressure</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<tr>
<td>sICAM-1</td>
<td>soluble intercellular adhesion molecule 1</td>
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<tr>
<td>sVCAM-1</td>
<td>soluble vascular adhesion molecule 1</td>
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<tr>
<td>TC</td>
<td>total cholesterol</td>
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**List of original publications**

This thesis is based on the following original publications and articles, which are referred to in the text by Roman numerals I-IV.


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1 Introduction

The Cardiovascular Risk in Young Finns study is one of the largest follow-up studies in the world on cardiovascular risk and its determinants from childhood to adulthood (Juonala et al. 2004). It is a multidisciplinary, prospective multi-centre project with extensive measurements and a follow-up period of more than two decades. For nutrition science, it provides an exceptional opportunity to study dietary factors, their determinants and shaping over the course of a lifetime. Nutrition can be considered a wide spectrum of behavioural and physiological elements of different levels. Therefore, if studied comprehensively, the nutrition of an individual from childhood into adulthood must be measured with various tools and analysed with various approaches. In the Cardiovascular Risk in Young Finns cohort, nutritional factors have been and are assessed from the level of detailed information on nutritional biomarkers up to the subjective conception of health-related issues. In addition to the Cardiovascular Risk in Young Finns study, the results of nutritional studies from such a long-term setting ranging from childhood into adulthood have been published on only a few cardiovascular study cohorts, the most well known being the Bogalusa Heart Study (Nicklas 1995, Demory-Luce et al. 2004). Given the culture-dependency of diet, the Cardiovascular Risk in Young Finns study provides unique information on the long-term dietary issues in Finland which may be applicable in similar societies in Europe.

Cardiovascular diseases (CVD), including atherosclerosis, are a group of widely prevalent non-communicable diseases of the heart and blood vessels with a multifactorial etiology and long progression time. Although partly genetically determined, the risk for CVD is highly influenced by lifestyle factors during the life-course (Yusuf et al. 2001). The possible effects of diet and nutrients have been widely studied in past decades, and compelling evidence on the mechanisms of nutrients and bioactive dietary compounds has accumulated, such as the harmful effects of saturated fatty acids or sodium and the beneficial role of fibre, unsaturated fatty acids or folate (World Health Organization 2003). However, outside statistical tables and experimental laboratories, these assumptions on the effects do not seem to work all that well in real life. Clinical trials on the benefits of nutrients, shown in ecological settings, have yielded inconclusive results for the supplementation of β-carotene, vitamin E and other antioxidants (Ommen et al. 1996, Stephens et al. 1996, Rapola et al. 1997, Yusuf et al. 2000), while population studies on the nutritional effects, shown experimentally, have not always shown consistent effects of e.g. ω-3 fatty acids (Ascherio et al. 1995, Knekt et al. 2004) or flavonols and flavones (Hirvonen et al. 2001). The development of CVD is a long process taking decades, and subclinical atherosclerosis occurs even in children and adolescents. Hence, numerous dietary factors influence the pathogenesis in the
context of the entire risk factor profile all throughout one's life, and a single measurement of one exposure is unlikely to be a good predictor of outcome (Yusuf et al. 2001).

People do not eat nutrients; they eat foods in different combinations which, along with countless other factors, may affect the risk for CVD (Hegsted 1994, Messina et al. 2001, Voutilainen et al. 2006, Jacobs and Tapsell 2007). Nutrients act in their natural matrices, either plant or animal, in interactions with each other and with other bioactive components, and are likely to do so in the human biological system as well. A single nutrient if isolated from its natural matrix may lose its bioactivity or affect human physiology unexpectedly. Additionally, food items in the diet may also interact with each other and should be considered as parts of the entire diet rather than as single foods. While obtaining information on the role and mechanisms of single components of the diet in the disease process is essential, it may be insufficient to understand such phenomenon in the whole context. Much has been resolved of the CVD-diet relationship, but much remains to be learned. In a complicated picture such as this, with numerous predictors with different pathways and decades of pathological processes, a large, prospective, observational follow-up study with a comprehensive approach provides a well-reasoned setting to achieve a broader view of the complex relations between diet and cardiovascular health.

In this series of studies, the diets of young Finnish adults are investigated longitudinally and holistically, with the primary focus on the relationship between long-term diet and cardiovascular health.
2 Review of the literature

2.1 Cardiovascular diseases

CVDs are the leading cause of mortality in the world, especially in the high-income world, but increasingly in low-income countries as well (World Health Organization 2003). Approximately 17 million deaths (30% of all deaths) result from CVD every year, and at least an additional 20 million people survive non-fatal CVD events. CVDs are a prime example of lifestyle diseases and estimates indicate that more than half of the deaths and disabilities from CVD could be preventable by changes in lifestyle and modifiable environment.

CVDs are a group of disorders of the heart and blood vessels. The most prevalent are coronary heart disease (CHD), cerebrovascular disease, peripheral arterial diseases and hypertension. In CHD, the coronary arteries narrow and blood flow to the heart is reduced. The heart muscle suffers from ischemia due to inadequate blood flow, leading to angina pectoris or even myocardial infarction (Tortora and Grabowski 2000). Other common CVDs include inflammatory heart diseases and congenital heart disorders.

2.2 Atherosclerosis

Atherosclerosis (in Greek athēre = gruel and sklerōs = hard), the underlying cause of many of the most prevalent CVDs, is a pathological process of thickening and loss of elasticity of the arterial wall. This process is present to some extent at almost all ages and in both men and women (Awtry and Loscalzo 2000). Injury to the vascular endothelium is the initiating event of the atherogenetic process. The normal endothelium is an important modulator of vascular tone, producing vasoactive substances, and is also involved in the local control of intravascular thrombosis (Ross 1999). Hypertension, dyslipidemia, smoking and local hemodynamic abnormalities produce endothelial injury leading to endothelial dysfunction, which is the earliest measurable abnormality in atherosclerotic vessels. Macrophages and lipids, predominantly low-density lipoprotein (LDL), accumulate at the site of injury. LDL is oxidised and ingested by macrophages, which produce foam cells. These foam cells aggregate and compose the first lesions of atherosclerosis: the fatty streaks.

This process can affect any artery in the body and is to some extent reversible. Such a lesion does not occlude the arterial lumen and therefore does not involve clinical symptoms. As the lesion expands, more smooth muscle cells migrate, leading to the
transition of the fatty streak into an atherosclerotic plaque, or atheroma, which may then undergo a marked increase in fibrous tissue. At this stage, the fibrous plaque leads to the narrowing of the lumen and may manifest as a clinically symptomatic disease. The significance of a plaque varies depending on the length and morphology of the lesion. However, a 70% decrease in the luminal diameter of a coronary artery is generally adequate to restrict blood flow during increased demand (e.g. exercise) (Awtry and Loscalzo 2000). As lipids accumulate in the macrophages, cell necrosis occurs, resulting in a vulnerable plaque with a free lipid pool in the core and a weakened fibrous cap. If the fibrous plaque ruptures, its highly thrombogenic contents activate the coagulation system in the arterial lumen leading to the formation of an intraluminal thrombus and partially or totally occluding the lumen. Typically, the atherosclerotic process occurs simultaneously in several locations in the artery or arteries, and the occlusion results from several ruptures (Ross 1999, Falk 2006).

Although the clinical symptoms usually occur only in middle-age or later, the field of cardiovascular research now widely acknowledges that the atherogenetic process commonly begins as early as in childhood. Atherosclerotic lesions of an early stage are present in all humans from the second decade of life, sometimes even earlier (Stary et al 1994). By the age of 30 years, it is common in humans to have up to 30% of the aortic intimal surface covered by fatty streak lesions, possibly reversible (Gerrity and Antonov 1997). However, the progression of fatty streaks to more advanced atheromas may also begin early in life. This progression does not occur solely with aging, but requires other intrinsic or extrinsic stimuli.

The arterial wall comprises three layers: the intima (inner layer), media and adventitia. The arterial intima media thickness (IMT), measured with ultrasound imaging, first served as a surrogate marker of atherosclerosis more than two decades ago (Pignoli et al. 1986). Large observational studies have later shown carotid IMT to be an independent marker of the risk for future cardiovascular events in large observational studies (Bots et al. 1997, Chambless et al. 1997 and 2000, O'Leary et al. 1999, Fathi et al. 2004). Abnormal, premature thickening of the arterial walls is an early indicator of vascular disease, and the thickness of the intima media layer of the carotid artery, in particular, is a good predictor of later events (Akosah et al. 2007). Ultrasonic measurements can also detect early stage non-occlusive plaques that may be vulnerable to rupture and increasingly serve as a useful tool in research as well as in clinical evaluation to recognise patients at high risk for CVD (De Backer et al. 2004).

The pathology of the transition of fatty streaks into occlusive fibrous plaques is not fully understood. Much of our present understanding of the pathobiology of plaque
development is based on animal models and cell cultures (Gerrity and Antonov 1997). Again, most of the risk factors considered relevant in the progression of the disease have been established on the basis of epidemiological studies with no certainty of the causal effect of the risk factor on the disease or its progression. Risk factors that have most conclusively been strong, independent predictors of atherosclerosis in different populations are often referred to as the traditional or conventional risk factors. These risk factors include age, male gender, family history of CVD, smoking, hypertension, dyslipidemia and glucose intolerance, and have proved to be competent predictors of CVD in, for instance, the Framingham Heart Study, an extensive longitudinal population-based prospective study on the etiology of CHD with a large cohort and more than 50 years of follow-up (Lloyd-Jones et al. 2004). This study has produced a prediction algorithm (Framingham prognostic score) that provides estimates of total CHD risk over a ten-year period based on present traditional risk factors, such as age and blood cholesterol. Relative risk for CHD is estimated by comparison to low-risk Framingham Heart Study participants. In addition, similar models have since been introduced, such as the SCORE risk evaluation for total risk estimation based on European data (Conroy et al. 2003). Although criticised for their impreciseness, particularly in predicting future outcomes among the young (Nasir et al. 2005, Beswick and Brindle 2006), these predictive models have served in clinical use and risk management. A direct relationship between the number of traditional risk factors present and the severity of atherosclerotic lesions in the arteries has also been shown in autopsy studies of children and adolescents who have died accidentally (Berenson et al. 1998).

However, a large proportion of cardiovascular events occur in individuals with no or few traditional risk factors (Hackam and Anand 2003). During the past decade, new emerging biological risk factors for atherosclerosis and other CVDs have been suggested. In particular, clinical interest has focused on lipid parameters such as lipoprotein(a) and apolipoproteins A-I and B, on inflammatory biomarkers such as C-reactive protein (CRP) and fibrinogen, and on nutritional markers associated with atherothrombosis, such as plasma homocysteine (Ridker 2001, Mallika et al. 2007). Of these, the markers for inflammation, particularly for elevated levels of CRP, have gained the widest acceptance due to the body of evidence supporting their use in clinical risk prediction. Numerous studies have shown low socio-economic status and other psychosocial factors to associate with CVD-related outcomes (Rozanski et al. 1999), but results are difficult to interpret and to apply in clinical practice, since most of these factors are in a complex association with each other as well as with traditional cardiovascular risk factors (Bairey Merz et al. 2002). Moreover, CVDs have both a multifactorial and a multigenetic basis, involving a number of genes and environmental
factors. The inherited genes may predispose an individual to an increased or decreased risk for the disease, but it is the interaction between environmental factors (e.g. diet) and the genes that generally determines whether the disease develops (World Health Organization 2003).

### 2.3 Diet in the development of atherosclerosis

#### 2.3.1 Dietary fatty acids

Our understanding of the role of nutrition in the development of atherosclerosis has a long history. Nutritional factors act mostly via traditional or emerging risk factors, but may also have other, direct mechanisms on the pathology of atherosclerosis and of other CVDs. While the effect of elevated blood cholesterol levels on CVD risk was first demonstrated in experimental studies only in the 1950s (Awtry and Loscalzo 2000), the impact of nutrition on cholesterol levels was understood much earlier. A Dutch physician, Dr DeLangen, showed in 1916 that the blood cholesterol levels of the natives in Indonesia were considerably lower than those of the Dutch colonists (for a historical review see Steinberg 2005). Speculating that this could be due to differences in diet, he performed the first reported controlled study, showing the now broadly approved effects of dietary cholesterol and fatty acids on blood cholesterol. A few decades later, the detrimental effects of saturated fats and the beneficial effects of polyunsaturated fats in particular were acknowledged after several controlled feeding studies (Steinberg 2005).

Dr Keys, the initiator and principal investigator of the subsequent Seven Countries Study, wrote in 1957: 'It is clear that it is unnecessary to prescribe a diet extremely low in total fats to lower the serum cholesterol; exclusion of the saturated fats (in butterfat and meat fats) has the greatest effect, and this effect may be enhanced by substitution of such oils as corn oil and cottonseed oil.' (Keys 1957).

Even today, dietary fatty acids are widely believed to play a significant role in the relationship between diet and atherosclerosis via several different mechanisms of which the anti-inflammatory, antiarrythmic, antithrombotic and, above all, lipid-related effects are the most important. Saturated fatty acids raise total and LDL cholesterol, but individual fatty acids yield different effects (Katan et al. 1994). Myristic and palmitic acids found in abundance in meat and dairy products as well as lauric acid found in coconuts yield the greatest effect, which is attributed mainly to the impaired removal of LDL from the circulation (Denke 2006). Saturated fatty acids with shorter chains are absorbed directly into the portal circulation, and therefore have no effect on serum cholesterol levels. Similarly, longer-chain saturated fatty acids (e.g. stearic acid found in
abundance in meat and cocoa) have only a minimal effect due to their high rate of conversion to monounsaturated fatty acids. Although not all saturated fats negatively affect cardiovascular health, equations formulated to predict the effects of changes in the fatty acid profile of the diet on serum lipids focus on total saturated fats. Fatty acids with double bonds in the *trans* configuration (found naturally in small amounts in meat and in dairy products and also created industrially in the partial hydrogenation of oils) appear to increase LDL cholesterol levels similarly to saturated fatty acids. In contrast to saturated fats, *trans* fatty acids decrease high-density lipoprotein (HDL) cholesterol concentrations, thus perhaps posing a greater risk to cardiovascular health (Mozaffarian et al. 2006).

Unsaturated fatty acids, when displacing carbohydrates or saturated fatty acids in the diet as well as independently, are associated with decreased risk for CVD. Mono- and, to a lesser extent, polyunsaturated fatty acids decrease serum LDL and increase HDL cholesterol levels and lower the total-to-HDL cholesterol ratio (Moreno and Mitjavila 2003, Denke 2006). An increasing body of evidence suggests that dietary monounsaturated fatty acids yield healthy benefits beyond cholesterol, such as effects on lipoprotein oxidation, coagulation, fibrinolysis and the endothelium (Pérez-Jiménez et al. 2002). Moreover, while polyunsaturated fatty acids affect LDL or HDL cholesterol levels, they also play several roles in the inflammation processes and, especially fatty acids of the ω-3 series also appear to suppress cardiac arrhythmias and to reduce triglycerides (Sacks and Katan 2002, de Lorgeril and Salen 2007). The most important sources of unsaturated fatty acids are vegetable oils; polyunsaturated fatty acids, especially ω-3 fatty acids, are also found in fish and shellfish.

The associations between dietary fatty acids and CVD, especially CHD, have been examined in observational and experimental epidemiological settings. An inverse association between the intake of unsaturated fat, especially of long-chain polyunsaturated fatty acids, and CVD and its risk factors has been observed in many (e.g. Erkkilä et al. 2003, Whelton et al. 2004, Oh et al. 2005), though not in all studies (Geleijnse et al. 2002, Osler et al. 2003, Brouwer et al. 2006, Wennberg et al. 2007). Moreover, a Finnish prospective intervention project for children (STRIP), with repeated counselling of a saturated fat- restricted diet initiated in infancy, has observed lowered serum cholesterol levels, improved insulin sensitivity and enhanced endothelial function even a decade later (Raitakari et al. 2005, Kaitosaari et al. 2003, Niinikoski et al. 2007). Despite the widely accepted role of saturated fat in the development of CVD and findings linking the high intake of saturated fat to increased risk for CVD (McGee et al. 1984, Kushi et al. 1985, Hu et al. 1997, Xu et al. 2006), it is often difficult to distinguish whether the observed effect is due to saturated fat *per se* or to the small
amounts of unsaturated fat in diets rich in saturated fat. In the 20-year follow-up of the Nurses’ Health Study, with more than 70,000 female participants and repeated measures of diet, higher intakes of trans fatty acids and, to a lesser extent, of saturated fats were associated with increased risk, whereas higher intakes of nonhydrogenated polyunsaturated and monounsaturated fats were associated with decreased risk (Oh et al. 2005). Many other studies, in both prospective and case-control designs, support a major role of trans fatty acids in the risk for CVD, with a stronger effect than that of saturated fats (Aro et al. 1995, Hu et al. 1997, Pietinen et al. 1997, Oomen et al. 2001, Oh et al. 2005). These studies have been conducted in various populations with various food cultures and diet compositions. Therefore, it is somewhat debatable whether it is possible to extract findings on a single component or on a few components from the entity of the diet in order to make universally generalisable conclusions. However, the nutrition research community is in general consensus on the importance of dietary fatty acids in the etiology of atherosclerosis.

2.3.2 Other dietary components

Sodium and potassium are the most influential dietary factors affecting blood pressure (Pickering 2006). Dietary sodium is associated with elevated blood pressure, whereas dietary potassium lowers the risk for hypertension (Reddy and Katan 2004). This association has been shown in cross-population comparisons (Beevers 2002), and the causality of these findings is supported by the results of several clinical trials with sodium restrictions or potassium supplements (Whelton et al. 1997, Geleijnse et al. 2003). Since hypertension is one of the established risk factors of CVD, the relevance of dietary sodium and potassium in the etiology of atherosclerosis is of interest. In a large cohort of Finnish men and women, 24-hour urinary sodium excretion was directly associated with the risk for CHD and CVD (Tuomilehto et al. 2001). The possible underlying physiological mechanism is clear: high intakes of sodium leads to its reabsorption in the kidneys, and thus to a loss of potassium. This results in increased contraction and reduced vasodilatation in vascular muscle cells (Adrogué and Madias 2007). Results from observational studies on sodium intake and CVD outcomes are controversial (Alderman et al. 1995, Tunstall-Pedoe et al. 1997, Alderman et al. 1998, Nagata et al. 2004, Geleijnse et al. 2007). However, some of the studies showing beneficial health effects of sodium intake have been questioned for their methods and interpretation (see e.g. debate in International Journal of Epidemiology 2002;31).

Numerous epidemiological studies have shown a significant protective effect of dietary fibre on CVD as well as negative associations between dietary fibre and CVD-related risk factors (King 2005). Several clinical trials have reported most soluble and some
insoluble fibres to reduce total and LDL cholesterol concentrations (Anderson and Hanna 1999) and to prevent hypertension especially in populations with a relatively low background intake of dietary fibre (Streppel et al. 2005). Moreover, many large cohort studies have shown significant inverse associations between the intake of fibre or foods rich in fibre and CVD or its risk factors, including plasma lipids, blood pressure, fibrinogen and type 2 diabetes (Pietinen et al. 1996a, Rimm et al. 1996, Liu et al. 1999, Ludwig et al. 1999, Truswell 1999, Montonen et al. 2003), and these results have been confirmed in a large pooling analysis (Pereira et al. 2004). The major sources of dietary fibre vary in different food cultures. In Finland, more than half of the intake of fibre is attributed to the consumption of whole grains, especially rye (Männistö et al. 2003), while in many other cultures fruits and vegetables play a more significant role. One must keep in mind, however, that foods rich in fibre contain several other bioactive components with possible known or unknown beneficial effects on cardiovascular health. Therefore, these results may be partly confounded by other, unmeasured or uncontrolled factors, and would best be derived to food-based recommendations rather than to an indication of the health benefits of fibre exclusively.

Other significant findings that have been repeated in different populations include the inverse associations of antioxidative compounds, folate, flavonoids and other phytochemicals in diet and CVD outcomes (see e.g. reviews by Cherubini et al. 2005, Graf et al. 2005, Voutilainen et al. 2006, McCully 2007). The results of clinical trials and of other experimental studies on single nutrients are quite inconclusive or even conflicting, possibly due to confounding, interactions, synergistic activity or the different activity of different isomers (Reddy and Katan 2004). Many of the nutrients or other bioactive elements of the diet that have been suggested to be protective against CVD are present in fruits and vegetables in particular. Therefore, recommendations for high consumption of plant foods are universally accepted.

### 2.4 The whole-diet approach

#### 2.4.1 Rationale and methodology

In past decades, modern nutrition science has identified numerous relationships between health and nutrients or other bioactive components in the diet. These findings have made a true difference from a public health point of view, providing important knowledge on the prevention of deficiencies, such as scurvy or pellagra in the 19th century. Decades later, when multifactorial and non-communicable diseases, such as CVD, cancer and osteoporosis, had overpowered the simpler deficiency diseases as
cause of morbidity and mortality in high-income countries, a majority of the research on nutrition and health outcomes continued to focus on the effects of single nutrients (Jacobs and Tapsell 2007). Even today, most nutritional recommendations are based on the best current evidence of the optimal intakes of the known essential nutrients in order to maintain health and to avoid disease. On the basis of these intakes, national or international bodies and authorities have often constructed additional food-based guidelines for public use. That is, these seemingly food-based approaches are not truly based on the research on the effects of foods, but on those of nutrients.

In recent years, however, the nutrient-based approach has faced constantly growing criticism. The often-repeated mantra "People do not eat nutrients; they eat foods" has gained attention, especially in nutritional epidemiology. Some say that concentrating only on single nutrients and omitting the context of food and of the whole diet will inevitably lead to oversimplifying the relationship under study, and the information obtained will likely be incomplete or, in some cases, even false (Hu 2002, Newby and Tucker 2004, Jacobs and Tapsell 2007). In its natural context, a nutrient acts as a part of the physiology of its source (i.e. a plant or animal). Studying its effects in isolation may provide unique information on the mechanisms behind possible effects, but may be insufficient to observe the entire phenomenon. All nutrients and other bioactive components of the diet have or may have synergistic, antagonistic or other interaction effects in the food matrix or in the whole diet. Many of these effects of interaction remain unknown and are therefore uncontrollable during data collection or analyses in epidemiological research. Therefore, assessing and employing the diet as a whole in the analytical nutritional epidemiology provides a holistic means to overcome many of the drawbacks of the single nutrient approach. In addition, a whole-diet approach is a powerful tool for use in descriptive nutritional epidemiology. The exploratorily derived dietary patterns of a given population represent a holistic means to describe existing dietary habits and to compare these habits in subgroups of subjects or over time.

Although based on a more down-to-earth standpoint, the whole-diet approach entails many methodological challenges. As in the single-nutrient approach, where observationally showing the unconfounded effects of a nutrient on a given health outcome is extremely difficult, the whole-diet approach in nutritional epidemiology also offers several analytical issues for consideration (Kant 2004, Newby and Tucker 2004). Most epidemiological, observational, whole-diet based studies from recent years have used food consumption data obtained with common methods (i.e. food frequency questionnaires (FFQ), food records or dietary recalls). Evidence shows that, when using the whole-diet approach, the food consumption method does not play as a significant
role as do other, mostly analytical or statistical methodological decisions (Hu et al. 1999, Togo et al. 2003, Khani et al. 2004, McNaughton et al. 2005, Crozier et al. 2008). The dietary pattern, as a holistic view of what and how much an individual eats and drinks, can be assessed in different ways. Two categorically distinct approaches are in wide use (Figure 1). A priori approaches are based on previous knowledge of the effects of nutrients or foods or both in the diet. Such approaches include the use of particular, scripted indices that normally provide a quantitative measure expressing the composition of the diet, such as the Healthy Eating Index (HEI), the Diet Quality Index or the Mediterranean Diet Score (Patterson et al. 1994, Kennedy et al. 1995, Knoops et al. 2004, Lagiou et al. 2006). In contrast, a posteriori methods are carried out with no a priori conception of the data. Based on the food consumption data obtained, a posteriori methods provide a description of distinct diets that truly exist among the subjects (Hu 2002).

![Figure 1](adapted from Schulze and Hoffmann 2006).

Most of the dietary pattern studies in recent years have used exploratory, a posteriori approaches of which the main techniques include statistical multivariate methods, such as cluster, factor (principal component) or reduced rank regression analysis. In a cluster analysis (CA), researchers seek, within a given study population or group of subjects, a set of homogenous subgroups of subjects with minimal within-group and maximal between-group variation. Thus, the analysis produces groups with significantly distinct food consumption (see e.g. Huijbregts et al. 1995, Schroll et al. 1996, Wirfält and Jeffery 1997).
The factor analysis (FA) is a frequently used form of exploratory dietary pattern analysis. It entails a data reduction technique that aims to establish new variables that are linear combinations of existing variables, and to explain as much as possible of the variation in the original variables. Thus, the derived factors (i.e. dietary patterns in the case of nutritional epidemiology) are based on the correlation between food groups used in the analysis (see e.g. Hu et al. 2000, Williams et al. 2000, Osler et al. 2001, Fung et al. 2001, Schulze et al. 2001). FA includes no independent and dependent variables (Benigni and Giuliani 1994). Its main purpose is to transform a dataset with innumerable variables and correlations between variables into a small set of underlying factors, which represent orthogonal, linear combinations of the original variables, without losing significant information. The first step in dietary pattern analysis using FA is to group the food items found in the dataset into distinctive food groups (either according to their nutrient composition or role in food behaviour). The mean daily consumption figures of these food groups serve usually as the input values in the FA. There should be enough significant correlations between these values for the dataset to be appropriate for FA; such is usually the case in dietary pattern analyses. Whether the consumption figures should be standardised or energy-adjusted before the FA is debatable, but these adjustments seem to affect the patterns to be derived only slightly (Balder et al. 2003).

The principal component analysis (PCA) is often used for the initial factor extraction. PCA studies the spatial distribution of the input variables and produces a given number (up to the number of the input variables) uncorrelated factors. The next step is to decide how many factors will be extracted in the final analysis. The choice of the number of factors can be based on the Kaiser criterion, namely eigenvalues over one. The eigenvalue for a given factor expresses the overall variance in food consumption accounted for by that factor. The number of factors to be extracted can also be based on the eigenvalue plot (scree plot), which graphically plots the factors on the X axis and the corresponding eigenvalues on the Y axis and sketches a curve from the highest eigenvalues on the left, declining to lower eigenvalues on the right. In the scree plot test, all factors after a clear elbow in the curve will be dropped from further analyses. The final decision on the factors chosen for further analyses must be based on the subject discretion of the researcher. The number of factors, in this case dietary patterns, is usually limited to those whose meaning and content is readily comprehensible and identifiable.

The FA is then usually rerun with the decided number of factors, which may then be rotated to facilitate the interpretation. The factor loadings are the correlation coefficients between the extracted factors and the food consumption variables. A positive loading
indicates that the food group is positively associated with the factor, while a negative loading indicates an inverse association. The higher the factor loading of a food group is, the greater the contribution of that group to the factor, since the square of the factor loading corresponds to the variance of the food group explained by the factor. A factor score is then calculated for each subject, showing the position of the subject's combination of food consumption in factor space. This score is mostly standardised (with a mean value of zero and a standard deviation of one) and may also be energy-adjusted; it represents the congruence of the subject's food consumption with that dietary pattern. The higher the pattern score for an individual, the more his or her diet resembles that particular dietary pattern. These factor scores will also serve as explanatory variables in further analyses on diet and health outcomes.

A more recent tool for dietary patterns analysis, reduced rank regression analysis, introduced by Hoffmann et al. (2004a) diverges slightly from CA and FA in its basic theory. This type of analysis aims not to explain as much as possible of the variance in food consumption, but to identify factors of foods or of food groups that explain as much variation as possible in the outcome variables, for example, nutrient intakes, biomarkers or biological risk factors (Hoffmann et al. 2004a and 2004b). Thus, although mathematically similar to exclusively data-driven methods, this approach may not be considered purely exploratory or a posteriori, because it also requires a priori information on other factors associated with food consumption.

While the data-driven methods require no a priori information on diets or on their relations to other factors, some aspects in the analyses require the subjective decision making of the researcher. These aspects include the possible subgrouping of the subjects by, for instance, gender or age, the grouping of foods with which the analysis will be carried out, standardisation or other mathematical adjustment of the food consumption figures, the number of clusters or factors to be derived, and the criteria with which the clusters or factors will be chosen for further analyses, among others. In addition, dietary patterns identified in one population may be non-existent or irrelevant in another one. Therefore, the results of studies reporting existing dietary patterns or associations between dietary patterns and health outcomes may not be generalisable to other populations or with different analytical decisions (Newby and Tucker 2004, Schulze and Hoffmann 2006).

However, although the derived dietary patterns greatly depend on the underlying population and its food choices, many studies have reported relatively similar findings. In most studies carried out on populations in high-income countries, among the identified patterns are the 'Western' dietary pattern (typically characterised by the high
consumption of red meat, processed red meat, high-fat dairy products, refined grains, processed potato products and desserts) and the 'prudent', 'healthy' or 'vegetable/salad' pattern (with higher consumption of fruits, vegetables, legumes, fish, poultry, and whole grains). Outside the United States, a 'traditional' dietary pattern often differs from the 'Western' and is derived separately. Although similar in label, the detailed food composition of the traditional dietary pattern naturally varies according to the traditional food culture in that particular population. More strongly manifesting local aspects in food choices emerge mainly in additional patterns (i.e. those identified along with the two or three aforementioned patterns), which include, for example, 'alcohol' in different variations prevalent in Sweden (Newby et al. 2006), France (Kesse-Guyot et al. 2008), and Ireland (Villegas et al. 2004); 'sweet' or 'confectionery' in Italy (Pala et al. 2006), China (Shi et al. 2006), and Sweden (Newby et al. 2006); 'salty' in Japan (Hirose et al. 2004); and 'ethnic' in the United Kingdom (McNaughton et al. 2007).

2.4.2 Stability of dietary patterns

The stability (i.e. tracking) of diet over a given period of time has traditionally been studied with the relative intakes of a set of selected nutrients as the variables representing an individual's diet. Results have often shown only low to moderate tracking from childhood to adulthood or in long-term analyses (Boulton et al. 1995, Welten et al. 1997, Bertheke Post et al. 2001, Gallagher et al. 2006), but somewhat higher tracking for a shorter period of time (Stein et al. 1991, Singer et al. 1995, Dunn et al. 2000). Some of the studies with a longitudinal setting have examined the stability of the (relative) consumption of single food items of food groups, a few of which indicate a significant tracking (Lien et al. 2001, Wang et al. 2002), whereas others have found only weak stability from childhood or adolescence to adulthood (Kvaavik et al. 2005, Lake et al. 2006, te Velde et al. 2007). A few recent papers in nutritional epidemiology have used dietary patterns to report results on the long-term stability of food choices. In another, longer-term study of adult women and men in Britain, an exploratory dietary pattern analysis was carried out using data collected from the same individuals on three occasions over a 17-year follow-up period in adult life (McNaughton et al. 2007). Four dietary patterns were identified, namely 'ethnic foods and alcohol' (women and men), 'meat, potatoes, and sweet foods' (women only), 'fruit, vegetables, and dairy' (women only), and 'mixed' (men only). Further examination of pattern score stability on an individual level suggests that healthier eating behaviours (the 'fruit, vegetables and dairy' pattern) track more than do other eating behaviours (Mishra et al. 2006). In a Swedish cohort of 33 840 women, 'healthy', 'Western', 'alcohol' and 'sweets' patterns were identified and found to be only moderately stable over a ten-year follow-up period (Newby et al. 2006). With a shorter examination
period and fewer subjects, the Southampton Women's Survey derived dietary patterns of a group of 94 women aged 20-34 (Borland et al 2008). Adherence to the two patterns ('prudent' and 'high energy') found showed significant tracking after two years. In addition to the pure consistency or tracking analyses, several researchers have published reports on the reproducibility of exploratorily driven dietary patterns with different time intervals between the measurements, thus showing good reproducibility (Hu et al. 1999, Khani et al. 2004, Lau et al. 2008).

2.4.3 Dietary patterns and the development of atherosclerosis

Knowledge of the importance of the whole diet in the development of CVD is not new; the concept of the Mediterranean diet was noted as early as in the 1950s (Keys 1957). Paradoxically, however, although the idea of the beneficial effects of Mediterranean diets was deduced from the simple observation that the prevalence of CVD was exceptionally low in populations that followed the traditional, plant-rich Mediterranean diet, this finding was relatively quickly transformed into a serum cholesterol prediction equation based only on the intakes of saturated and polyunsaturated fats and dietary cholesterol (i.e. nutrients, not foods) (Fetcher et al. 1967). This equation became widely implemented in both cardiovascular research and public health policy in the United States and other countries and, in part, served in favour of the nutrient-based view of diet-health issues. Later, the prediction formula received criticism for oversimplifying the issue.

In the Seven Countries Study, launched in 1958 and hailed as a landmark in CVD epidemiology, lifestyle cardiovascular risk factors, especially diet, and CVD rates appeared to differ considerably between seven countries (USA, Finland, the Netherlands, Italy, the former Yugoslavia, Greece and Japan) (Kromhout et al. 1994). This study provided further evidence that the differences in the consumption of foods and combinations of foods were significant and independent predictors of differences in cardiovascular morbidity and mortality (Kromhout 1989, Menotti et al. 1999). Above all, while such studies showed the important role of the fatty acid profile of the diet, they also emphasised the protective effects of vegetable foods against the risk for disease (Keys et al. 1986, Menotti et al. 1999). A whole-diet approach was implemented in the Seven Countries Study cross-population examination in which the country-specific average dietary score, representing high consumption of meat and low consumption of vegetables, was in a positive linear relationship with the 25-year CHD death rate (Menotti et al. 1999).
Of the individual food groups, the beneficial effects of vegetables and fruit on cardiovascular risk have been examined relatively widely. In their systematic review of numerous reports from ecological, cohort, case-control and controlled intervention studies, Ness and Powles (1997) conclude that the results are consistent with a strong protective effect of fruits and vegetables against stroke, and a weaker, but significant, protective effect against CHD. The discussion on the possible underlying mechanisms suggests it is inevitable to consider the diet as a whole. In an isoenergetic situation, a diet with large amounts of fruits, vegetables and vegetable foods is low in other foods. Therefore, even in a randomised controlled trial, it is difficult to show causality between high consumption of plant foods and health outcomes.

The renaissance of the exploratory diet-based approach in nutritional epidemiology has since shown promising results in the field of cardiovascular research (Table 1). One of the first studies involved a prospective cohort of 44,875 men participating in the American Health Professionals Follow-up Study, where the 'Western' dietary pattern (characterised by the high consumption of red and processed meat, refined grains, sweets and desserts) positively, and the 'prudent' dietary pattern (with higher intakes of vegetables, fruits, legumes, whole grains, fish, and poultry) negatively, associated with the risk for fatal or nonfatal CHD incidence (Hu et al. 2000). Both associations had a highly significant dose-response ($p < 0.001$). To argue for this approach, the authors concluded that dietary patterns may be easier for the public to interpret and could therefore be useful in nutrition intervention and for education purposes. Several other publications have followed this study report with cohort studies reporting the 'Western' dietary pattern to be positively, and the 'prudent' or 'healthy' dietary pattern to be negatively associated with CVD-related outcomes. The Nurses' Health Study, a large American prospective cohort with women, identified 'Western' and 'prudent' patterns and their associations with CHD incidence nearly identical to those of the American Health Professionals Follow-up Study (Fung et al. 2001). The 'Western' pattern was later linked to elevated rates of stroke among the Nurses' Health Study subjects (Fung et al. 2004), and in Denmark, women with high 'prudent' pattern scores had significantly lower mortality rates for CVD than did those with low scores (Osler et al. 2001). The 'Western' pattern has also been observed to associate with CVD risk factors such as markers of insulin resistance (Kerver et al. 2003) and of inflammation (Lopez-Garcia et al. 2004).

Of the hypothesis-based, \textit{a priori} whole-diet approaches, the theory of the heart-healthiness of the traditional Mediterranean diet has been the most popular in nutrition science as well as in public debate since the early findings of Dr Keys. The Mediterranean diet is usually defined as a diet rich in plant-based foods, fish and olive
oil; moderate in wine, dairy products, potatoes, poultry and eggs; and low in sweets, meat and meat products (Bach et al. 2006). Variations of the Mediterranean diet or different indices representing adherence to it have been implemented in both observational and intervention cardiovascular studies. The cardioprotective properties of the Mediterranean diet were well acknowledged in three recent reviews (Bautista and Engler 2005, de Lorgeril and Salen 2006, Willett 2006). The conclusion is based on increasing epidemiological evidence from numerous cohort and case-control studies, thus indicating that high adherence to the Mediterranean diet is significantly associated with lower risk for CVD mortality (Knoops et al. 2004), non-fatal CVD (Martinez-Gonzalez et al. 2002, Ciccarone et al. 2003, Bilenko et al. 2005) or with lower levels of CVD risk factors, such as hypertension (Psaltopoulou et al. 2004), obesity (Schröder et al. 2004) and serum lipids (Goulet et al. 2003, Panagiotakos et al. 2004). However, in their critical review, Waijers et al. (2007), while accepting the likely beneficial components of the Mediterranean diet, question the general use of Mediterranean diet scores and consider it particularly debatable whether using these scores and their population-specific cut-off points in non-Mediterranean populations is appropriate.

While the vast majority of epidemiological diet-based research has been conducted in observational settings, this approach has also been implemented in intervention studies of which the successful Dietary Approaches to Stop Hypertension (DASH) study is the most well known and cited (Appel et al. 1997). DASH was an intervention study with 459 normotensive participants and was based on the hypothesis that patterns of food consumption rather than individual nutrients efficiently affect blood pressure. The DASH diet, high in fruits and vegetables and low-fat dairy products, reduced systolic and diastolic blood pressure significantly more than did two other diets (a typical American diet and a diet rich in fruits and vegetables; all three diets had equal levels of sodium). The DASH diet also proved to decrease the levels of total, LDL and HDL cholesterol levels, thus indicating a cardioprotective effect, but with some reservations due to its effect on HDL cholesterol (Obarzanek et al. 2001). A few studies have implemented a Mediterranean diet approach in intervention studies, mostly among subjects at high risk for CVD, and reported a beneficial effect of intervention diets: decreased cholesterol levels and improved insulin sensitivity (Pérez-Jiménez et al. 2001) as well as reduced body mass index (BMI), plasma lipids (Vincent-Baudry et al. 2005), markers of inflammation (Esposito et al. 2004) and blood pressure (Estruch et al. 2006). The Lyon Heart Study diet, rich in vegetable oils, legumes, bread, fruits and vegetables, and low in meat, butter and cream, was so effective at reducing secondary CVD mortality, that the trial was terminated in advance (de Lorgeril et al. 1999).
Table 1. A summary of observational studies on the associations of exploratory dietary patterns and cardiovascular diseases and/or their risk factors among adults.

<table>
<thead>
<tr>
<th>Study</th>
<th>Country and study population</th>
<th>Study design</th>
<th>Follow-up period</th>
<th>Dietary pattern method</th>
<th>Identified dietary patterns</th>
<th>Outcomes</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health Professionals Follow-up study (Hu et al. 2000)</td>
<td>USA; 44 875 men aged 40-75 years</td>
<td>Prospective cohort</td>
<td>8 years</td>
<td>FA</td>
<td>'Prudent' vegetables, fruit, legumes, whole grains, fish, and poultry 'Western' red meat, processed meat, refined grains, sweets and desserts</td>
<td>CHD incidence</td>
<td>Adjusted RR for highest vs. lowest quintile 'Prudent' 0.70 (95% CI 0.56, 0.86) 'Western' 1.64 (95% CI 1.24, 2.17)</td>
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<td>Nurses' Health Study (Fung et al. 2001)</td>
<td>USA; 69 017 women aged 38-63 years</td>
<td>Prospective cohort</td>
<td>12 years</td>
<td>FA</td>
<td>'Prudent' fruit, vegetables, whole grains, legumes, poultry, and fish 'Western' refined grains, red and processed meat, desserts</td>
<td>CHD incidence</td>
<td>Adjusted RR for highest vs. lowest quintile 'Prudent' 0.76 (95% CI 0.60, 0.98) 'Western' 1.46 (95% CI 1.07, 1.99)</td>
</tr>
<tr>
<td>Nurses' Health Study (Fung et al. 2004)</td>
<td>USA; 71 768 women aged 38-63 years</td>
<td>Prospective cohort</td>
<td>14 years</td>
<td>FA</td>
<td>See above</td>
<td>Stroke incidence</td>
<td>Adjusted RR for highest vs. lowest quintile 'Prudent' NS 'Western' 1.58 (95% CI 1.15, 2.15)</td>
</tr>
<tr>
<td>Nurses' Health Study (Lopez-Garcia et al. 2004)</td>
<td>USA; 732 women aged 43-69 years</td>
<td>Prospective cohort</td>
<td>4 years</td>
<td>FA</td>
<td>See above</td>
<td>Markers of inflammation and endothelial dysfunction: plasmaCRP, interleukin 6, E-selectin, sICAM-1, sVCAM-1</td>
<td>Adjusted regression coefficients with p &lt; 0.05 'Prudent' inverse association with CRP and E-selectin 'Western' positive association with CRP, E-selectin, sICAM-1 and sVCAM-1</td>
</tr>
<tr>
<td>Study</td>
<td>Country and study population</td>
<td>Study design</td>
<td>Follow-up period</td>
<td>Dietary pattern method</td>
<td>Identified dietary patterns</td>
<td>Outcomes¹</td>
<td>Main results¹</td>
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<tr>
<td>MONICA (Osler et al. 2001)</td>
<td>Denmark; 5872 men and women aged 30-60 years</td>
<td>Prospective cohort</td>
<td>15 years (median)</td>
<td>FA</td>
<td>'Prudent' wholemeal bread, pasta, rice, oatmeal products, fruit, vegetables, and fish 'Western' meat, sausages, potatoes, butter, and white bread</td>
<td>CVD mortality</td>
<td>Adjusted RR for 1 SD increase 'Prudent' Men 0.87 NS; Women 0.63 (95% CI 0.44, 0.90) 'Western' NS</td>
</tr>
<tr>
<td>MONICA (Osler et al. 2002)</td>
<td>Denmark; 7316 men and women aged 30-60 years</td>
<td>Prospective cohort</td>
<td>14 years</td>
<td>FA</td>
<td>See above</td>
<td>CHD incidence (fatal and non-fatal)</td>
<td>NS</td>
</tr>
<tr>
<td>Framingham offspring/spouse study (Millen et al. 2002)</td>
<td>USA; 1423 women aged 18-76 years</td>
<td>Prospective cohort</td>
<td>12 years</td>
<td>CA</td>
<td>'Heart healthy' fruit, vegetables, lean protein sources, whole grains, low-fat dairy products; four other clusters 'Empty calorie' sweetened beverages, high-fat sweets and snacks</td>
<td>Carotid artery stenosis</td>
<td>Adjusted OR for 'Empty calorie' vs. 'Heart healthy' 2.28 (95% CI 1.12, 4.62)</td>
</tr>
<tr>
<td>MORGEN Study (van Dam et al. 2003)</td>
<td>Netherlands; 19750 men and women aged 20-65 years</td>
<td>Cross-sectional</td>
<td>-</td>
<td>FA</td>
<td>'Cosmopolitan' fried vegetables, salad, rice, chicken, fish, and wine 'Traditional' red meat and potatoes 'Refined-foods' chips, high-sugar beverages, and white bread</td>
<td>SBP, serum TC, HDL-C and glucose</td>
<td>Adjusted regression coefficients with p &lt; 0.05 'Cosmopolitan' positive association with HDL-C, inverse association with SBP 'Traditional' positive association with SBP, TC and HDL-C, glucose 'Refined-foods' positive association with TC</td>
</tr>
<tr>
<td>Study</td>
<td>Country and study population</td>
<td>Study design</td>
<td>Follow-up period</td>
<td>Dietary pattern method&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Identified dietary patterns</td>
<td>Outcomes&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Main results&lt;sup&gt;3&lt;/sup&gt;</td>
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<tr>
<td>NHANES III (Kerver et al. 2003)</td>
<td>USA; 13 130 men and women aged ≥ 20 years</td>
<td>Cross-sectional survey</td>
<td>-</td>
<td>FA</td>
<td>'Western' red and processed meats, eggs, and high-fat dairy products; 'American healthy' green and leafy vegetables, salad dressings, tomatoes, other vegetables, and tea</td>
<td>BMI, serum lipids, red blood cell folate, glycated hemoglobin, homocysteine, C-peptide, insulin</td>
<td>Adjusted linear models with p &lt; 0.05 'Western' positive association for serum C-peptide, serum insulin, and glycated hemoglobin; inverse association with red blood cell folate 'American healthy' NS</td>
</tr>
<tr>
<td>Ohsaki National Health Insurance (NHI) Cohort study (Shimazu et al. 2007)</td>
<td>Japan; 40 547 Japanese men and women aged 40-79</td>
<td>Prospective cohort</td>
<td>7 years</td>
<td>FA</td>
<td>'Japanese' soybean products, fish, seaweeds, vegetables, fruits and green tea; 'Animal food' beef, pork, ham, sausage, chicken, liver, butter, coffee and alcoholic beverages; 'DFA' dairy products, fruit and vegetables, low in alcohol</td>
<td>CVD mortality</td>
<td>Adjusted HR for highest vs. lowest quartile 'Japanese' 0.74 (95% CI 0.59, 0.91) 'Animal food' 1.24 (1.00, 1.54) 'DFA' NS</td>
</tr>
<tr>
<td>Swedish Mammography Cohort (Åkesson et al. 2007)</td>
<td>Sweden; 24 444 postmenopausal women</td>
<td>Prospective cohort</td>
<td>6 years</td>
<td>FA</td>
<td>'Healthy' vegetables, fruit, whole grains, fish, and legumes; 'Alcohol' alcoholic beverages, salty snacks, chocolate; 'Western' meat, processed meat, liver, refined grains, potatoes, eggs</td>
<td>MI incidence</td>
<td>Adjusted RR for lowest vs. highest quintile 'Healthy' 1.71 (1.14, 2.55) 'Alcohol' 1.64 (1.09, 2.47) 'Western' NS</td>
</tr>
</tbody>
</table>

<sup>1</sup> FA = factor analysis; CA = cluster analysis.<br><sup>2</sup> CHD = coronary heart disease; CRP = C-reactive protein; sICAM-1 = soluble intercellular adhesion molecule 1; sVCAM-1 = soluble vascular adhesion molecule 1; CVD = cardiovascular disease; SBP = systolic blood pressure; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; BMI = body mass index; MI = myocardial infarction.<br><sup>3</sup> RR = relative risk; CI = confidence interval; NS = non significant; SD = standard deviation; OR = odds ratio; HR = hazard ratio.
2.4.4 Summary of the whole-diet approach

A whole-diet approach in nutritional epidemiology has proven useful, and as the results from such studies exceedingly accumulate, such results can be interpreted and applied to public health purposes more efficiently than findings from single-nutrient-based research. Hypothesis-derived indices representing a certain characteristic of an underlying diet may be helpful in describing food choices or in linking them to health outcomes. However, such indices have limitations in that they are based on \textit{a priori} knowledge and may therefore omit unknown yet relevant aspects of a diet. On the contrary, data-derived methods face set research questions with an open mind and are thus more efficient in explaining true differences in food consumption and disease. The exploratory analyses entail methodological challenges. Since the dietary patterns identified may be nonexistent or irrelevant in other populations, it is necessary to identify population-specific patterns. Moreover, further studies are needed on the implementation and validity of pattern analyses in cardiovascular epidemiology.
3 Aims of the study

The main objective of this study was to investigate the role of diet in the development of atherosclerosis, with a special focus on the holistic and longitudinal view of the diet of young Finnish adults.

The specific aims of the study were

- to assess the nutrient intakes relevant to the risk for CVD among young Finnish adults, and to find childhood and adulthood determinants of dietary intakes (Study I);

- to identify data-driven dietary patterns present among the study population from childhood to adulthood (Study II);

- to examine the stability of food choices from childhood to adulthood (Study II);

- to study the associations between long-term dietary patterns and risk factors for atherosclerosis (Study III); and

- to study the associations between long-term diet and early atherosclerotic vascular changes (Study IV).
4 Subjects and methods

4.1 The Cardiovascular Risk in Young Finns study

The Cardiovascular Risk in Young Finns (the Young Finns) study is a large multicentre longitudinal study of CVD risk factors and their determinants among children, adolescents and young adults in Finland. Determinations include serum cholesterol and other lipids, blood pressure and anthropometric measurements, as well as dietary interviews. Data have been collected with questionnaires on diet, smoking, alcohol use, physical activity and other lifestyle factors, and on subjects’ and their family’s socio-demographic situation and history. The ethics committees of the participating centres have approved the study protocol and all subjects or their guardians have given their written consent. Details of the project have been described previously (Åkerblom et al. 1999, Raitakari et al. 2003, Juonala et al. 2004).

The baseline study was conducted in 1980. The total sample size was 4320 boys and girls in six equal age cohorts (aged 3, 6, 9, 12, 15 and 18 years). These subjects were randomly chosen from the national register in five university towns (Helsinki, Turku, Tampere, Kuopio and Oulu) and rural localities in their surroundings. A total of 3596 subjects (83% of those invited) participated in 1980, and all but those who had died (by 2001 $n = 51$), become severely disabled ($n = 7$), emigrated ($n = 64$) or withdrawn their earlier consent ($n = 18$), were invited to the follow-up studies. Between 1980 and 1992, the cohorts were followed-up in three-year intervals, and again in 2001. Participation rates in the follow-ups varied between 66 and 76%. The progression of the study appears in Figure 2.

4.2 Subjects

The present study is based on the information on the dietary factors of the Young Finns project. In the first study year in 1980, a 50% random sample of the subjects was chosen to participate in a 48-hour dietary recall interview. In 1980, the dietary information was obtained from 1768 children and adolescents, all of whom were invited to the follow-up dietary study in 1986; 1200 (68%) participated. After 21 years, those who had participated in 1980, who were not excluded from the study (see reasons above) and who still had a permanent address in Finland ($n = 1748$) were invited to the 21-year follow-up field study, which again included the dietary interview; 1049 (60%) participated, of whom 1037 subjects provided reliable data. In other follow-ups (i.e. in
1983, 1989 and 1992), a smaller-scale dietary study was carried out in the Young Finns study with a smaller subgroup of subjects and with a limited protocol. Consequently, data from these follow-ups were excluded from this study. The numbers of male and female subjects in each study appear in Table 2.

![Figure 2.](image_url)

**Figure 2.** Progression of the Cardiovascular Risk in Young Finns study. Study years, numbers of participants and participation rates appear on the X-axis; ages of the participants appear on the Y-axis. In 1980, a total of 4320 children and adolescents were invited; those who participated in 1980 were then invited to later follow-ups.
### Table 2. Numbers of subjects in analyses of Studies I-IV.

<table>
<thead>
<tr>
<th></th>
<th>Girls/women</th>
<th>Boys/men</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrient intakes and</td>
<td>421</td>
<td>352</td>
<td>773</td>
</tr>
<tr>
<td>longitudinal analyses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet quality 2001</td>
<td>571</td>
<td>466</td>
<td>1037</td>
</tr>
<tr>
<td><strong>Study II</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary patterns 1980</td>
<td>893</td>
<td>875</td>
<td>1768</td>
</tr>
<tr>
<td>Dietary patterns 1986</td>
<td>620</td>
<td>580</td>
<td>1200</td>
</tr>
<tr>
<td>Dietary patterns 2001</td>
<td>571</td>
<td>466</td>
<td>1037</td>
</tr>
<tr>
<td>Tracking analysis</td>
<td>421</td>
<td>352</td>
<td>773</td>
</tr>
<tr>
<td><strong>Study III</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk factors in dietary</td>
<td>571</td>
<td>466</td>
<td>1037</td>
</tr>
<tr>
<td>pattern quintiles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivariate analyses</td>
<td>620</td>
<td>580</td>
<td>1200</td>
</tr>
<tr>
<td><strong>Study IV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All analyses</td>
<td>420</td>
<td>365</td>
<td>785</td>
</tr>
</tbody>
</table>

### 4.3 Dietary assessment

Dietary studies have been an essential part of the project from the beginning. The methods of the dietary studies include the 48-hour dietary recall interviews and questionnaires on dietary habits. In the 48-hour recall, dietary interviewers (all trained dietitians), collected information on foods and beverages consumed by subjects during the two days prior to the interview. In 1980 and 1986, 3- to 12-year-old children were interviewed together with their mothers, fathers or other accompanying persons. For the pre-school aged children (3- and 6-year-olds), the child's daytime caregiver was asked to maintain a record of all foods and drinks consumed outside home; these records were then verified during the interview. As detailed information as possible on the type and amount of foods and drinks reported was documented. The food composition data used in the 1980s were based on the Finnish food composition tables (maintained by the University of Helsinki) and on analytical data obtained from the local food industry. Foreign food composition tables were used when no appropriate domestic data were available. Closer details have been described previously (Räsänen et al. 1985 and 1991). In 2001, the National Food Composition Database Fineli (National Public Health Institute, 2003) was used. The latest version (FND2) of the composition database served
to calculate the consumption of foods and intakes of energy and nutrients for each participant.

For all the study years, the participants also received a questionnaire on longer-term dietary habits and food consumption to complete. The questionnaire has been slightly modified over the years to fit the relevant issues according to time and age, but has included the same set of focal questions on food habits, for example, choices of cooking fat and bread spread, consumption of coffee and tea, the amount and type of milk consumed, and others. Long-term food choices have been assessed during all follow-ups with a short, non-quantitative 15- to 20-item FFQ with six response categories ranging from 'daily' to 'never'.

### 4.4 Identification of dietary patterns

Food groupings of the different food consumption databases were unified to obtain 23 standardised food groups (Table 3). Food items were grouped according to their habitual culinary use or to their nutrient composition. Some items (e.g. coffee, tea) were preserved as separate groups because they were considered to represent distinctive food choices. Mean intake (g/d) served as input values in the analysis.

A PCA was then performed separately for each study year to extract the major dietary patterns in the study group. Both the amount of variance (eigenvalue) of each factor and a scree plot analysis (Figure 3) were considered the criteria for deciding the number of factors extracted. The two criteria lead to similar conclusions, so all interpretable factors with eigenvalues greater than one were therefore chosen for further consideration. Two factors were eventually chosen for each study year. A third extracted factor was also carefully considered, but was dropped from further studies in the end due to its inconsistent characterisation. The two factors were then rotated with an orthogonal transformation to force non-correlation of the factors and to enhance the interpretation. Separate analyses for females and males were also attempted, but due to their similar results, the analyses were conducted together for both genders. The extraction and identification of dietary patterns were performed with version 8.2 of the SAS statistical package for Windows (SAS Institute Inc., Cary, NC, USA, 2000).

In addition, in 2001 the subjects were asked to complete a questionnaire on habitual dietary choices including a short FFQ with six response categories. Because of the limited number of foods and food groups included in the FFQ, these data were used not to assess the dietary patterns, but only to evaluate the validity of the dietary patterns by
calculating the mean consumption (obtained by the 48-hour recalls) of relevant food groups in each FFQ category.

**Table 3.** Food groups used in the factor analysis.

<table>
<thead>
<tr>
<th>Food group</th>
<th>Foods included in the group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rye</td>
<td>Rye, rye bread, rye porridge</td>
</tr>
<tr>
<td>Wheat</td>
<td>Wheat, wheat bread, pasta</td>
</tr>
<tr>
<td>Other cereals</td>
<td>Cereals other than rye and wheat, breakfast cereals, biscuits, starch, rice</td>
</tr>
<tr>
<td>Potatoes</td>
<td>Potatoes, potato products</td>
</tr>
<tr>
<td>Root vegetables</td>
<td>Root vegetables</td>
</tr>
<tr>
<td>Legumes and nuts</td>
<td>Peas, beans, other legumes, nuts, seeds, soy products</td>
</tr>
<tr>
<td>Other vegetables</td>
<td>Leaf vegetables, onions, cabbages, tomatoes, cucumber, canned vegetables, mushrooms</td>
</tr>
<tr>
<td>Fruit and berries</td>
<td>Fresh fruits, canned fruits, berries, fruit and berry juices</td>
</tr>
<tr>
<td>Milk</td>
<td>Milk</td>
</tr>
<tr>
<td>Cheese</td>
<td>Cheese</td>
</tr>
<tr>
<td>Other dairy products</td>
<td>Cream, sour milk products, ice cream</td>
</tr>
<tr>
<td>Butter</td>
<td>Butter, butter-oil spreads, lard</td>
</tr>
<tr>
<td>Margarines and oil</td>
<td>Soft margarine, low-fat spreads, oil</td>
</tr>
<tr>
<td>Pork</td>
<td>Pork</td>
</tr>
<tr>
<td>Other meat</td>
<td>Beef, game, poultry, lamb, meat products</td>
</tr>
<tr>
<td>Sausages</td>
<td>Sausages, frankfurters</td>
</tr>
<tr>
<td>Offal</td>
<td>Liver, kidney, other offal</td>
</tr>
<tr>
<td>Fish and shellfish</td>
<td>Fish, shellfish, fish products</td>
</tr>
<tr>
<td>Eggs</td>
<td>Eggs</td>
</tr>
<tr>
<td>Sugars and confectionery</td>
<td>Sugar, syrup, sweets, chocolate</td>
</tr>
<tr>
<td>Coffee</td>
<td>Coffee</td>
</tr>
<tr>
<td>Tea</td>
<td>Tea</td>
</tr>
<tr>
<td>Alcoholic beverages</td>
<td>Alcoholic beverages</td>
</tr>
</tbody>
</table>
Figure 3. A scree plot analysis of the factor derivation with data from the studies in 1980, 1986 and 2001 separately. Factors are plotted on the X-axis and the corresponding eigenvalues on the Y-axis. Factors after a clear elbow in the curve (between the third and the fourth factors) were dropped from further analyses. Eventually, the two first factors for all study years were chosen for further analyses.

4.5 Biochemical measurements

In 2001, venous samples were drawn from the right antecubital vein of recumbent subjects after a 12-h fast (Juonala et al. 2004). Serum cholesterol and triglyceride concentrations were determined enzymatically (Olympus System Reagent; Olympus Diagnostica GmbH, Hamburg, Germany) in a clinical chemistry analyser (AU400; Olympus Optical Ltd, Mishima, Japan). HDL cholesterol was analysed after precipitation of very low density lipoprotein and LDL with dextrane sulphate 500 000 (Kostner 1976). The concentration of LDL cholesterol was calculated using the Friedewald formula (Friedewald et al. 1972). Subjects with triglycerides above 4 mmol L$^{-1}$ ($n=32$ in the total study population) were excluded from this analysis. Serum apolipoproteins A-I and B were analysed immunoturbidometrically (Orion Diagnostica, Espoo, Finland). All the analyses in 2001 were performed as simultaneously as possible in the laboratory of the Research and Development Unit of the Social Insurance Institution, Turku, Finland. The inter-assay coefficient of variation was 2.19% for serum cholesterol, 2.28% for HDL cholesterol, 3.76% for serum
triglycerides, 3.16% for serum apolipoprotein A-I, and 2.78% for serum apolipoprotein B.

Due to changes in determination methods and kits during the study years, lipid levels from 1980 and 1986 were corrected with the following correction factor equations.

Total cholesterol = 1.091*total cholesterol (1980/1986) - 0.271 mmol/l
HDL cholesterol = 1.068*HDL cholesterol (1980/1986) - 0.0277 mmol/l
Triglycerides = 1.00756*triglycerides (1980/1986) + 0.0582 mmol/l

These equations were determined with linear regression analyses utilising standardised principal component adjustments. Details on the methods and on the correction factor equations of lipid levels have been previously published (Porkka et al. 1997).

Serum insulin was determined with fluorescence polarisation immunoassay (Abbott Laboratories, Abbott Park, IL, USA), serum homocysteine with a microparticle enzyme immunoassay kit (Imx assay, Abbott Laboratories, Tokyo, Japan) and fasting plasma high sensitive CRP by a sensitive latex turbidometric immunoassay (Wako Chemicals GmbH, Neuss, Germany).

4.6 Ultrasound measurement of intima media thickness

Ultrasound studies were carried out using ultrasound mainframes (Sequoia 512, Acuson, Mountain View, CA, USA) with 13.0-MHz linear array transducers (Raitakari et al. 2003). The studies were carried out in all five study centres with similar equipment simultaneously between September 2001 and January 2002. Ultrasound technicians scanned the left common carotid artery following a standardised protocol. The image was focused on the posterior (far) wall, and gain settings served to optimise the image quality. A resolution box function (zoom) was used to record an image 25 mm wide and 15 mm high. A magnified image was recorded from an angle that showed the greatest distance between the lumen-intima interface and the media-adventitia interface. A moving scan with a duration of five seconds, which included the beginning of the carotid bifurcation and the common carotid artery, was recorded and stored in digital format on optical disks for subsequent off-line analysis. A single reader blinded to participants' details manually analysed digitally stored scans with ultrasonic callipers. From the five-second clip image, the best-quality end-diastolic frame was selected (incident with the R wave on a continuously recorded electrocardiogram). From this image, at least four measurements of the common carotid far wall were taken.
approximately 10 mm proximal to the bifurcation to derive maximal carotid IMT. To assess the reproducibility of IMT measurements, 60 participants (a 2.5% random sample of the total study population) were re-examined three months after the initial visit. The between-visit coefficient of variation of the IMT measurements was 6.4%. To assess the reproducibility of the IMT image analysis, a second observer reanalysed 113 scans; the between-observer coefficient of variation was 5.2%. As a part of the ultrasound imaging, the carotid artery elasticity and brachial endothelial-dependent flow-mediated dilatation were also measured, but these variables were not included in this study.

4.7 Assessment of other variables

BMI served in the analyses as a surrogate for the relative amount of body fat (i.e. to assess the possible overweight of the subject). A clinical research nurse measured weight and height at each study point and BMI was calculated as the participant’s weight in kilograms divided by the square of the participant's height in metres. The nurse measured blood pressure from three-year-olds with an ultrasound device (Arteriosonde 1020, Roche); otherwise a standard mercury sphygmomanometer was used in 1980. In 1986 and 2001, a random-zero sphygmomanometer was used. Korotkoff's fifth sound served as the sign of the diastolic blood pressure and the first sound as the sign of the systolic blood pressure (SBP). In the three-year old children SBP was recorded as the pressure in the cuff at the point when the pulse sound was first heard (Korotkoff's first sound), the change in the sound was used as the sign of the diastolic blood pressure (Korotkoff's fourth sound). Each study year, at least three measurements were performed and the average of the systolic blood pressure (SBP) measurements served in the statistical analyses.

Information on the socio-demographic characteristics of the subjects or their families and on lifestyle factors other than diet were assessed with extensive questionnaires, completed by the subjects aged 15 years or more and by their parents or other guardian for younger participants. Years of education was chosen as the surrogate of the socio-economic situation of the subject in adulthood. In childhood, the information on the parent with more years of education served as an indicator of family socio-economic status. Based on the reported level of education, a variable with three categories representing the Finnish educational system was established: 9 years or less, from 10 to 12 years, and 13 years or more of education. Residential area was defined on the basis of a question on the subject's current place of residence.
As regards marital status, the participants were divided into two categories: those with a spouse/partner, and those without. Smoking status was assessed with a question about current and former smoking habits. Three categories were then constructed as follows: those who had never been smokers, those who had quit smoking or reported infrequent and irregular smoking, and those who were regular and frequent smokers. A three-class variable served to assess physical activity: those who never exercise, those who exercise once a week or less, and those who exercise more than once a week. Physical exercise was defined as continuous physical exertion for at least 30 minutes.

4.8 Study designs

4.8.1 Study I

In assessing the nutrient intakes among the Young Finns participants, nutrients selected for examination were those considered to be relevant to the risk for CVD. In addition, the consumption of vegetables and fruits relative to the total energy intake was calculated for each participant. Data on the intakes of ω-3 fatty acids, fibre and salt were not available for all study years. Of the official Finnish nutrition recommendations for the time for adults (National Nutrition Council 1999), six items relevant to the prevention of CVD were selected. These items included recommended intake levels of dietary saturated, monounsaturated, polyunsaturated and ω-3 fatty acids, dietary fibre, and salt. The numbers and proportions of subjects (as adults) meeting these recommendations were then calculated. To describe the quality of the diet in terms of risk for CVD, an index was constructed by assigning one point for each recommendation the subject met. In this calculation, only three of the recommendations were used: saturated fatty acids (equal to or less than 10% of total energy), fibre (equal to or more than 3 g/1000 kJ), and salt (equal to or less than 500 mg/1000 kJ). Polyunsaturated, monounsaturated and ω-3 fatty acids were excluded from the index to avoid over-emphasising the role of dietary fats. Of the four recommendations relating to fatty acid intake, the intake of saturated fatty acids was considered the best indicator of a subject’s diet with regard to risk for CVD. Thus, each subject received a total score of zero to three points: where zero meant none of the three recommendations was met (i.e. poor cardiovascular diet quality) and three meant all three recommendations were met (i.e. good cardiovascular diet quality).

Group means and standard deviations of the intakes in 1980, 1986 and 2001 were computed, and the difference between boys and girls/men and women was tested with the unpaired t-test. A longitudinal analysis examining secular changes in the nutrient
intakes was carried out with linear regression analysis. The SAS procedure PROC MIXED was used in the trend analysis. The unstructured covariance was chosen on the basis of a restricted maximum likelihood (REML) test. Gender differences in the cardiovascular quality of the diet were tested with the \( \chi^2 \)-test. The associations between cardiovascular quality of diet in adulthood and socio-demographic and lifestyle factors in both childhood and adulthood were examined with a logistic regression model. All independent variables were entered individually into a univariate model, and the multivariable models were controlled for age and childhood total energy intake.

All the statistical analyses of Study I were carried out with version 6.12 of the SAS statistical package for Windows (SAS Institute Inc., Cary, NC, USA, 1989).

### 4.8.2 Study II

The analyses of Study II are based exclusively on the dietary pattern analyses (see Identification of dietary patterns) and individual scores for these patterns. The factor scores computed for each subject at each study point and for both dietary patterns were standardised to ensure normality. To evaluate the validity of the assessed dietary patterns, the mean consumptions of foods that were the most relevant in the two dietary patterns in 2001 were computed (i.e. foods or food groups with low or high loadings), according to the response categories (frequency of consumption or cups/glasses per day) of the respective food items in the dietary questionnaire completed by the same subjects. Thus, the information obtained with the 48-hour recall was compared to the information obtained with the questionnaire including the short non-quantitative FFQ. These analyses were carried out on food items for which the required data from both methods were available.

For further analyses, the subjects were divided into quintiles according to the standardised dietary pattern scores, for both genders, for both patterns and for all three study points. Before the division, the pattern scores were energy adjusted to eliminate confounding due to variations in the amounts of food consumed (i.e. in body size and energy expenditure) as well as to control possible under- or over-reporting of food consumption. The adjustment for total energy intake was carried out with the residual method (Willett 1998). The mean values of the selected study characteristics or proportions of the subjects were then calculated separately in the lowest quintile, the three middle quintiles and the highest quintile. Values were age-adjusted; means using least squares estimates and proportions using the direct method with six age-cohort groups using the age distribution of the total study sample as the standard.
To evaluate dietary tracking (i.e. the stability of food choices over time), the extent to which the subjects, originally in the lowest or highest quintile, remained in the same category was examined, i.e. the subjects maintained their position relative to the other subjects, over 6 (from 1980 to 1986) and 21 years (from 1980 to 2001). Spearman correlation coefficients between factor scores in different study years within the total study population were computed.

The statistical analyses of Study II were carried out with version 8.2 of the SAS statistical package for Windows (SAS Institute Inc., Cary, NC, USA, 2000).

4.8.3 Study III

Associations between dietary patterns and cardiovascular risk factors were studied using factor scores for two dietary patterns for all three study years. To describe the basic characteristics related to dietary patterns and to obtain univariate associations between dietary patterns and CVD risk factors in a cross-sectional setting (for all study years separately), the subjects were divided into quintiles according to the standardised and energy-adjusted dietary pattern scores, for both genders, for both patterns and for all three study points. The distribution of selected characteristics of the subjects as well as the mean values of the outcome variables were then calculated separately for the lowest quintile, three middle quintiles and the highest quintile. Values were age-adjusted; proportions were standardised using the direct method with six age-cohort groups using the age distribution of the total study sample as the standard.

A multivariable analysis was conducted to examine the independent longitudinal associations between dietary pattern scores and CVD risk factors as repeated measurements by using a mixed linear regression model and by taking time-variant covariates into account. The unstructured covariance was chosen to serve on the basis of a REML log likelihood test. Repeated measurements of the pattern scores were inserted into the model to predict repeated measurements of the outcome variables (risk factors). All outcome variables were standardised into age- and gender-specific z scores with a mean of zero and a standard deviation of one. This was done due to the age-dependence of the CVD risk factors at issue. Multivariable models were adjusted for age, total energy, smoking, physical activity and years from baseline.

The statistical analyses of Study III were carried out with version 9.1 of the SAS statistical package for Windows (SAS Institute Inc., Cary, NC, USA, 2005).
4.3.4 Study IV

Dietary pattern scores for both patterns and all three study points were standardised for a mean of zero and standard deviation of one and energy-adjusted using the residual method (Willett 1998). The adjusted scores were then averaged for the three study years and used as a continuous explanatory variable in linear regression analyses with adulthood carotid artery IMT as the outcome variable. The multivariable dietary pattern analyses were carried out separately for both genders, adjusting for age, total energy, the consumption of vegetables, smoking, education, physical activity, SBP, LDL cholesterol and BMI.

To estimate the energy-adjusted macronutrient intakes for each subject, the three-year average residuals from the regression of protein, fat and carbohydrate intakes on total energy intake was calculated using the dietary intake data assessed with the 48-hour recalls in 1980, 1986 and 2001. Subjects were divided into groups according to the average macronutrient distribution using gender-specific median values as the cut-off points for each macronutrient. Moreover, a multivariable linear regression analysis was carried out using the carotid IMT as the outcome variable and the average energy adjusted intake of energy-yielding nutrients as explanatory variables, controlling for age, gender, total energy, the consumption of vegetables, smoking, education, physical activity, SBP, LDL cholesterol, and BMI. To further examine the associations between IMT and macronutrients, the mean maximum carotid IMT was calculated among subjects in groups representing different combinations of low and high intakes of macronutrients. The IMT mean values were age-adjusted using least squares estimates. The group means were tested for difference with the unpaired t-test. The analyses of the associations between the intake of energy-yielding nutrients and IMT were initially computed separately, but due to the similar effects, the final analyses were carried out together for men and women.

The statistical analyses of Study IV were performed with version 9.1 of the SAS statistical package for Windows (SAS Institute Inc., Cary, NC, USA, 2005).
5 Results

5.1 Loss to follow-up

The effect of the loss to follow-up was analysed by comparing baseline data on the follow-up participants and non-participants in 1986 and in 2001 (Table 4). In 1986, the prevalence of non-participation was highest in the oldest age group (then 24 years of age), whereas in 2001, this prevalence was highest among the youngest (again at 24 years of age). In 2001, men were more likely to be lost to follow-up than were women. The only differences in mean food consumption were found for the consumption of sausages in 1980 between the 1986 participants and non-participants.

Table 4. Comparison of the baseline characteristics of the participants and non-participants in 1986 and 2001.

<table>
<thead>
<tr>
<th></th>
<th>Participants</th>
<th>Non-participants</th>
<th>P(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls/women n (% of the group)</td>
<td>620 (52%)</td>
<td>270 (48%)</td>
<td>0.10</td>
</tr>
<tr>
<td>Boys/men n (% of the group)</td>
<td>580 (48%)</td>
<td>298 (52%)</td>
<td></td>
</tr>
<tr>
<td>Mean age at follow-up, years</td>
<td>15.7</td>
<td>18.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Largest age group, age in years at follow-up</td>
<td>12</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Mean food consumption in 1980, g/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee</td>
<td>61</td>
<td>111</td>
<td>0.07</td>
</tr>
<tr>
<td>Tea</td>
<td>66</td>
<td>74</td>
<td>0.19</td>
</tr>
<tr>
<td>Rye</td>
<td>76</td>
<td>86</td>
<td>0.45</td>
</tr>
<tr>
<td>Wheat</td>
<td>77</td>
<td>85</td>
<td>0.46</td>
</tr>
<tr>
<td>Milk</td>
<td>622</td>
<td>645</td>
<td>0.56</td>
</tr>
<tr>
<td>Cheese</td>
<td>13</td>
<td>14</td>
<td>0.16</td>
</tr>
<tr>
<td>Other dairy products</td>
<td>72</td>
<td>68</td>
<td>0.71</td>
</tr>
<tr>
<td>Potatoes</td>
<td>130</td>
<td>143</td>
<td>0.95</td>
</tr>
<tr>
<td>Legumes and nuts</td>
<td>6</td>
<td>7</td>
<td>0.21</td>
</tr>
<tr>
<td>Other vegetables</td>
<td>46</td>
<td>45</td>
<td>0.65</td>
</tr>
<tr>
<td>Fruit and berries</td>
<td>229</td>
<td>223</td>
<td>0.85</td>
</tr>
<tr>
<td>Pork</td>
<td>17</td>
<td>18</td>
<td>0.41</td>
</tr>
<tr>
<td>Sausages</td>
<td>32</td>
<td>45</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Other meat</td>
<td>43</td>
<td>45</td>
<td>0.18</td>
</tr>
<tr>
<td>Fish and shellfish</td>
<td>12</td>
<td>13</td>
<td>0.41</td>
</tr>
<tr>
<td>Butter</td>
<td>20</td>
<td>23</td>
<td>0.52</td>
</tr>
<tr>
<td>Margarines and oil</td>
<td>16</td>
<td>20</td>
<td>0.45</td>
</tr>
<tr>
<td>Alcoholic beverages(^2)</td>
<td>6</td>
<td>23</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Participants</td>
<td>Non-participants</td>
<td>P^1</td>
</tr>
<tr>
<td>----------------</td>
<td>--------------</td>
<td>------------------</td>
<td>-----</td>
</tr>
<tr>
<td>2001 Girls/women n (% of the group)</td>
<td>571 (55%)</td>
<td>319 (45%)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Boys/men n (% of the group)</td>
<td>466 (45%)</td>
<td>392 (55%)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Mean age at follow-up, years</td>
<td>31.9</td>
<td>31.0</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Largest age group, age in years at follow-up</td>
<td>39</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Mean food consumption in 1980, g/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee</td>
<td>81</td>
<td>71</td>
<td>0.73</td>
</tr>
<tr>
<td>Tea</td>
<td>74</td>
<td>60</td>
<td>0.20</td>
</tr>
<tr>
<td>Rye</td>
<td>82</td>
<td>75</td>
<td>0.27</td>
</tr>
<tr>
<td>Wheat</td>
<td>82</td>
<td>77</td>
<td>0.51</td>
</tr>
<tr>
<td>Milk</td>
<td>615</td>
<td>640</td>
<td>0.37</td>
</tr>
<tr>
<td>Cheese</td>
<td>14</td>
<td>12</td>
<td>0.21</td>
</tr>
<tr>
<td>Other dairy products</td>
<td>69</td>
<td>73</td>
<td>0.56</td>
</tr>
<tr>
<td>Potatoes</td>
<td>132</td>
<td>137</td>
<td>0.12</td>
</tr>
<tr>
<td>Legumes and nuts</td>
<td>7</td>
<td>6</td>
<td>0.82</td>
</tr>
<tr>
<td>Other vegetables</td>
<td>45</td>
<td>45</td>
<td>0.83</td>
</tr>
<tr>
<td>Fruit and berries</td>
<td>227</td>
<td>227</td>
<td>0.76</td>
</tr>
<tr>
<td>Pork</td>
<td>18</td>
<td>17</td>
<td>0.86</td>
</tr>
<tr>
<td>Sausages</td>
<td>36</td>
<td>37</td>
<td>0.33</td>
</tr>
<tr>
<td>Other meat</td>
<td>44</td>
<td>43</td>
<td>0.77</td>
</tr>
<tr>
<td>Fish and shellfish</td>
<td>23</td>
<td>13</td>
<td>0.41</td>
</tr>
<tr>
<td>Butter</td>
<td>22</td>
<td>20</td>
<td>0.43</td>
</tr>
<tr>
<td>Margarines and oil</td>
<td>17</td>
<td>18</td>
<td>0.11</td>
</tr>
<tr>
<td>Alcoholic beverages^2</td>
<td>9</td>
<td>23</td>
<td>0.17</td>
</tr>
</tbody>
</table>

^1Test for difference where appropriate. Age-adjusted (except for age) analysis of variance for continuous variables; χ^2 for categorical variables; H0 = no difference between participants and non-participants.

^2 Only among subjects 15 years or older.

### 5.2 Changes in nutrient intakes from childhood to adulthood

With regard to risk for CVD, the diet of the subjects had changed favourably from 1980 to 2001. In a longitudinal analysis separating the effect of time from the effect of age, it was observed that the decrease in the intake of total, saturated and monounsaturated fatty acids, and the increase in the consumption of vegetables and in the intake of sucrose, were mainly consequences of time (p for the time trend analysis, < 0.001), whereas the increase in the proportional intake of polyunsaturated fatty acids seemed to be attributable more to the effect of age than of time. A considerable disparity existed between nutritional recommendations and the actual diets of subjects assessed with the 48-hour method in 2001, especially for the intake of salt, where less than 1% of the
subjects had diets with salt under the recommended level (equal to or less than 500 mg/1000 kJ).

5.3 Dietary patterns, their characteristics and associations with cardiovascular risk factors

Major dietary patterns were extracted separately using dietary data from each complete study year (i.e. 1980, 1986 and 2001). Thus the results of the factor analyses were completely independent of each other. However, a consistent range emerged in the major dietary patterns across the study years. The loadings of the food groups for the factors in each study year yielded a similar set of two interpretable patterns. These patterns were also the ones with the highest eigenvalues, and together they explained 18, 21 and 17% of the total variance in food consumption in 1980, 1986 and 2001, respectively. Food group loadings for these patterns for each study year appear in Table 5. A positive loading refers to a positive correlation between the food group and the factor, indicating high consumption of foods belonging to that particular food group as an important element of that dietary pattern. A negative loading refers to an inverse association, and values close to zero signify a weak or no correlation between the food group and the pattern. In the validity analysis, the consumption of the food groups with high or low loading values for the two patterns were found to be in concordance with the information on the longer-term food choices obtained with the short FFQ (Table 6).

The dietary patterns were considered to reflect similar entities of two distinct diets over the study years, since the slight differences observed in the loading matrices in 1980, 1986 and 2001 reflected the general trend in the food consumption in Finland. The patterns were labelled on the basis of the behavioural concept assumed to influence the food choices. Because pattern one was evidently loaded with foods traditionally consumed in Finland, such as rye, milk, coffee, potatoes, sausages and butter, it was labelled the 'traditional' dietary pattern. High traditional pattern scores were more common among boys and men, subjects living in rural areas, smokers and those with a lower level of education. High scores also indicated lower intakes of carbohydrates, sucrose and vitamin C, and higher intakes of fat and saturated fatty acids. Female subjects with high traditional pattern scores had on average higher total and LDL cholesterol concentrations throughout the study period than women with lower traditional pattern scores. Among males, this trend was apparent only in childhood. In 2001, a significant trend emerged in the levels of numerous risk factors according to the traditional pattern scores among women, but less pronounced among men. In both genders, those with high traditional pattern scores had significantly higher BMI than
others in adulthood. In longitudinal, adjusted models (see Study III), significant associations of the traditional pattern scores were observed with LDL cholesterol concentrations, apolipoprotein B and SBP among both genders, as well as with CRP among men and insulin among women.

Pattern two had high loadings for tea, vegetables, fish, cheese and other dairy products. In Finland, most of these have not been traditionally eaten, and many represent an alternative to the conventional food items (e.g. tea substituting for coffee, yoghurt substituting for milk). These food choices were interpreted as an indication of conscious intention toward healthy food choices, particularly in the 1980s, when food selection in Finland was much more limited (e.g. not all fruits and vegetables were available throughout the year). This pattern was thus named the 'health-conscious' pattern, and was more predominant among girls and women, non-smokers, the well educated, and urban dwellers. In addition, high health-conscious pattern scores indicated lower intakes of saturated fatty acids and sucrose, and higher intakes of polyunsaturated and ω-3 fatty acids, fibre, alcohol and vitamin C. The health-conscious pattern was less consistently associated with the risk factor levels studied. In a multivariate model (see Study III), significant relations were found with LDL cholesterol concentrations, which were inverse among women but positive among men. Additionally, health-conscious pattern scores were inversely associated with apolipoprotein B and homocysteine concentrations.
Table 5. Factor loading matrix for the two major dietary patterns. A positive loading indicates that the food group is positively associated with the factor; a negative loading denotes an inverse association. To facilitate interpretation of the table, loadings with absolute values of 0.20 or more (considered important items of that factor) appear in bold.

<table>
<thead>
<tr>
<th></th>
<th>1980 Pattern 1</th>
<th>1986 Pattern 2</th>
<th>2001 Pattern 1</th>
<th>2001 Pattern 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rye</td>
<td>0.70</td>
<td>0.26</td>
<td>0.76</td>
<td>0.30</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.19</td>
<td>-0.03</td>
<td>0.60</td>
<td>0.38</td>
</tr>
<tr>
<td>Other cereals</td>
<td>-0.18</td>
<td>-0.09</td>
<td>0.04</td>
<td>-0.17</td>
</tr>
<tr>
<td>Potatoes</td>
<td>0.59</td>
<td>0.10</td>
<td>0.22</td>
<td>-0.44</td>
</tr>
<tr>
<td>Root vegetables</td>
<td>0.10</td>
<td>0.48</td>
<td>-0.16</td>
<td>0.02</td>
</tr>
<tr>
<td>Legumes and nuts</td>
<td>0.03</td>
<td>0.14</td>
<td>0.00</td>
<td>0.27</td>
</tr>
<tr>
<td>Other vegetables</td>
<td>0.10</td>
<td>0.52</td>
<td>0.12</td>
<td>0.42</td>
</tr>
<tr>
<td>Fruit and berries</td>
<td>-0.34</td>
<td>0.15</td>
<td>0.02</td>
<td>0.37</td>
</tr>
<tr>
<td>Milk</td>
<td>0.51</td>
<td>-0.38</td>
<td>0.45</td>
<td>-0.61</td>
</tr>
<tr>
<td>Cheese</td>
<td>0.13</td>
<td>0.55</td>
<td>0.15</td>
<td>0.52</td>
</tr>
<tr>
<td>Other dairy products</td>
<td>-0.23</td>
<td>0.65</td>
<td>-0.01</td>
<td>0.17</td>
</tr>
<tr>
<td>Butter</td>
<td>0.79</td>
<td>0.03</td>
<td>0.84</td>
<td>-0.04</td>
</tr>
<tr>
<td>Margarines and oil</td>
<td>0.02</td>
<td>0.12</td>
<td>-0.15</td>
<td>0.07</td>
</tr>
<tr>
<td>Pork</td>
<td>0.06</td>
<td>0.07</td>
<td>0.09</td>
<td>0.21</td>
</tr>
<tr>
<td>Other meat</td>
<td>0.14</td>
<td>-0.23</td>
<td>0.02</td>
<td>-0.22</td>
</tr>
<tr>
<td>Sausages</td>
<td>0.42</td>
<td>0.01</td>
<td>0.39</td>
<td>-0.09</td>
</tr>
<tr>
<td>Offal</td>
<td>0.05</td>
<td>0.01</td>
<td>0.35</td>
<td>-0.11</td>
</tr>
<tr>
<td>Fish and shellfish</td>
<td>0.04</td>
<td>0.03</td>
<td>-0.02</td>
<td>0.30</td>
</tr>
<tr>
<td>Eggs</td>
<td>0.00</td>
<td>0.05</td>
<td>0.42</td>
<td>0.28</td>
</tr>
<tr>
<td>Sugars and confectionery</td>
<td>0.03</td>
<td>-0.19</td>
<td>0.12</td>
<td>0.04</td>
</tr>
<tr>
<td>Coffee</td>
<td>0.50</td>
<td>0.06</td>
<td>0.25</td>
<td>-0.26</td>
</tr>
<tr>
<td>Tea</td>
<td>0.09</td>
<td>0.28</td>
<td>0.11</td>
<td>0.48</td>
</tr>
<tr>
<td>Alcoholic beverages</td>
<td>0.01</td>
<td>0.02</td>
<td>-0.05</td>
<td>0.02</td>
</tr>
</tbody>
</table>

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Table 6. Consumption of foods relevant to the pattern analyses in 2001. Values are the mean daily consumption of the food (in grams) obtained by the 48-hour recall, according to the response categories of the respective food items in the questionnaire.

| Frequency of consumption | daily | nearly | a few | once | once or | less or | p for trend |
|--------------------------|-------|--------|-------|------|twice   |never   |1         |
| Sausages                 | -     | 86     | 71    | 57   | 47     | 25     | < 0.01    |
| Potatoes                 | 104   | 102    | 95    | 92   | 86     | 29     | 0.02      |
| Vegetables               | 171   | 124    | 101   | 83   | 70     | 65     | < 0.01    |
| Cheese                   | 70    | 58     | 46    | 40   | 41     | 29     | < 0.01    |
| Other dairy products     | 237   | 184    | 122   | 136  | 102    | 81     | < 0.01    |
| Fish and shellfish       | -     | 65     | 67    | 69   | 50     | 51     | 0.25      |
| Meat                     | 80    | 82     | 69    | 69   | 53     | 33     | < 0.01    |
| Fruit and berries        | 322   | 207    | 143   | 142  | 104    | 25     | 0.04      |

<table>
<thead>
<tr>
<th>Glasses or cups /day</th>
<th>5 or more</th>
<th>4</th>
<th>3</th>
<th>2</th>
<th>1</th>
<th>0</th>
<th>p for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffee</td>
<td>1002</td>
<td>563</td>
<td>464</td>
<td>333</td>
<td>219</td>
<td>94</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Milk</td>
<td>877</td>
<td>621</td>
<td>534</td>
<td>401</td>
<td>263</td>
<td>121</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Tea</td>
<td>1080</td>
<td>760</td>
<td>578</td>
<td>371</td>
<td>234</td>
<td>178</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

1Trend analysis performed with regression analysis; response category in the model as a continuous predictor variable; intakes log-transformed for the test where distributions non-normal.

5.4 The role of childhood diet in adulthood food choices

Diet in childhood or adolescence proved to be an independent predictor of adulthood diet (Studies I and II). The composition of diet in childhood and physical activity in adulthood were the most important lifestyle determinants of cardiovascular diet quality: the probability of meeting none of the CVD-related dietary recommendations in adulthood was significantly lower (odds ratio 0.5, 95% confidence interval 0.2-0.8) among subjects with the highest vs. lowest consumption of vegetables in childhood. Moreover, food choices expressed as dietary pattern scores showed tracking over the 21-year period. Spearman correlation coefficients between the pattern scores in 1980 and 2001 were 0.32 for the traditional and 0.38 for the health-conscious pattern. The
stability of food choices was evident among subjects with the highest factor scores (the uppermost quintile), and among subjects who were 15 or older in 1980: 41% remained in the uppermost quintile of the traditional pattern and 38% in that of the health-conscious pattern after 21 years (p for the $\chi^2$ test <0.001 for traditional and 0.041 for the health-conscious pattern).

5.5 Associations between diet and intima media thickness

The energy- and age-adjusted traditional dietary pattern score averaged over all study years was positively associated with IMT in both genders. After adjusting for possible confounders, the association disappeared among women but remained highly significant among men (p < 0.01). Health-conscious dietary pattern scores showed no significant associations with IMT. On the basis of 2001 data, the correlations between the consumption of separate food groups and IMT were also calculated (data not shown). Of the 23 food groups, only the consumptions of rye, meat and coffee were significantly (p < 0.05) associated with IMT, all positively.

The subjects were divided into low and high groups of protein, fat and carbohydrates according to the energy-adjusted intake of the macronutrients averaged through the study years. In multivariable analyses controlled for gender, age, total energy intake, consumption of vegetables, smoking, physical activity, education, SBP, LDL cholesterol and BMI, the intake of protein was found to be in a positive, linear association with IMT among subjects belonging to the low fat intake group (p < 0.03). Among subjects with a higher intake of fat, no significant association emerged between protein intake and IMT. The intake of carbohydrates had neither independent nor interaction associations with IMT. In further analyses, the mean IMT, according to the intake groups was more thoroughly studied. In a pairwise comparison of age-adjusted means of IMT among subjects divided into eight groups, according to the intakes of energy-yielding nutrients, IMT values proved to be lowest among those with a low protein intake and highest among those with a high protein intake (Table 7). However, the association of protein was attenuated by high intakes of fat. In a new analysis of combined groups (see Table 7) the mean IMT among those with a high long-term intake of protein and a low long-term intake of fat was 0.633 mm, and was statistically different (p < 0.05) from the mean IMT values of all other groups. In a further inspection, those belonging to high protein and low fat intake group, were more likely to be women, well educated, residing in Helsinki area, non-smokers and regular exercisers than the subjects in other groups (data not shown). In addition, in the food behaviour questionnaire, they were more likely to report not to use any kind of bread spread.
Table 7. Intima media thickness (mm) in adulthood according to macronutrient intakes averaged over study years. Values represent age-adjusted group means (with standard errors of means).

<table>
<thead>
<tr>
<th>Intake group</th>
<th>n</th>
<th>Intima media thickness</th>
<th>n</th>
<th>Intima media thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low carbohydrate</td>
<td>9</td>
<td>0.606 (0.006)</td>
<td>209</td>
<td>0.607 (0.007)</td>
</tr>
<tr>
<td>high carbohydrate</td>
<td>200</td>
<td>0.607 (0.007)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>low protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low carbohydrate</td>
<td>119</td>
<td>0.610 (0.008)</td>
<td>165</td>
<td>0.610 (0.010)</td>
</tr>
<tr>
<td>high carbohydrate</td>
<td>46</td>
<td>0.609 (0.013)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>high fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low carbohydrate</td>
<td>66</td>
<td>0.633 (0.011)</td>
<td>210</td>
<td>0.633 (0.007)</td>
</tr>
<tr>
<td>high carbohydrate</td>
<td>144</td>
<td>0.632 (0.008)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>high protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low carbohydrate</td>
<td>199</td>
<td>0.613 (0.007)</td>
<td>201</td>
<td>0.613 (0.007)</td>
</tr>
<tr>
<td>high carbohydrate</td>
<td>2</td>
<td>0.613 (0.007)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Low = energy-adjusted average intake below gender-specific median; high = energy-adjusted average intake above gender-specific median.

2 Unable to compute the mean due to the small number of subjects.

3 Significantly different (p < 0.05) from groups with high protein, low fat and low/high carbohydrate intake.

4 Significantly different (p < 0.05) from all other groups.
6 Discussion

6.1 Setting

This study was carried out as a part of the Cardiovascular Risk in Young Finns project. The Young Finns study is one of the largest follow-up studies of cardiovascular risk from childhood to adulthood. The main objective of the project is to determine the role of childhood, adolescence and young adulthood in the development of a disease, that usually presents symptoms only in middle-age or later. The setting from childhood into adulthood is ideal for the study of both environmental effects on CVD and its risk factors and the longitudinal profile (stability and/or change) of lifestyle factors and their determinants on a group level (secular trends) or on an individual level (tracking). Long, prospective study designs are expensive, and thus rare, and are found mainly in Europe and in the United States. Some of the prospective cohort studies on the etiology of CVD have similar childhood-to-adulthood settings to that of the Young Finns, like the Bogalusa Heart Study (Berenson 2001) and the Muscatine Study (Davis et al. 2001). In others, the subjects were adults already at baseline, as in the CARDIA study (Cutter et al. 1991), in the Framingham Heart Study (Lloyd-Jones et al. 2004) and in the Finnish KIHD Study (Salonen 1988). The Northern Finland 1966 Birth Cohort, though not designed as a study on cardiovascular health in particular, has provided a valuable setting for many longitudinal research questions in Finland (Isohanni et al. 2000). More recent cohorts have been initiated over the past decade (see e.g. Magnussen et al. 2008).

The participation rate in the Young Finns baseline study in 1980 was 83%, and in later dietary follow-up examinations (which provided data used in the present study), 68% in 1986 and 60% in 2001. The rates can be considered sufficient, taking into account the exceptionally long follow-up period. However, the possibility of selection bias must be considered. Non-participation appears to be related to health status, risk factor status and social circumstances and to affect estimates of disease prevalence or average risk factor levels but not to distort the estimates of relative risk in Finnish cohort and cross-sectional studies (Haapea et al. 2007, Harald et al. 2007). An analysis of participants and those lost to follow-up among the Young Finns cohort showed no differences in baseline levels of traditional risk factors (Juonala et al. 2004), and only minor differences in food consumption. Although the differences were almost entirely non-significant, the future non-participants had consumed more traditional foods at the baseline than the participants, thus the possible bias caused by lost to follow-up resulted more likely in a dilution than in an overestimation of the identified traditional dietary pattern.
Participants in the population-based Young Finns cohort constitute a geographically and socio-economically representative sample of Finnish young male and female adults (children and adolescents at the baseline). Originally, half of all subjects invited from all six birth cohorts came from Eastern Finland and half from Western parts of the country due to the hypothesised impact of genetically different areas. By the end of the 21-year follow-up period, a large proportion of participants from Eastern research towns and villages had moved to the West and particularly from smaller towns to larger cities. This phenomenon is perfectly congruent with the current type and scale of domestic migration in Finland over the past few decades. However, compared with the Finnish population registers, the Western and the urban areas are slightly overrepresented among the Young Finns study participants. The independent impact of geographical origin on the risk for atherosclerosis has been studied and reported elsewhere (Juonala et al. 2005).

6.2 Methodological considerations

The dietary data used in this thesis are based on food consumption information assessed with 48-hour dietary recall interviews. Short-term methods are suitable for estimating dietary intakes on a group level but fail to take into account intra-individual variability, and are thus inappropriate for evaluating the diet of an individual (Willett 1998). Because the diet quality index constructed for Study I of this thesis is based on data from only two days, the proportion of subjects meeting the recommendations could have been larger and the estimates more accurate if studied for a longer period of time. However, when compared to FFQ, for example, recalls do not rely on the long-term memory of subjects, are unstructured and open-ended, provide more detailed information on the foods and quantities consumed, and exhibit less systematic error on a group level (Hoffmann et al. 2002). Three recent papers have evaluated the use of data from short-term dietary recalls, food diaries and FFQs in deriving exploratory dietary patterns (Togo et al. 2003, McNaughton et al. 2005, Crozier et al. 2008). The overall differences found between FFQs, food diaries and 48-hour recalls were small, with some differences in foods that were consumed relatively rarely and loaded low on their factors. The authors of all three papers concluded that the method of food consumption assessment does not play a critical role in pattern identification.

In the present study, foods or food groups that played an important role as markers in the identified dietary patterns were mostly those consumed daily or several times a week, such as coffee, tea, potatoes, fats, bread, vegetables and fruit. A significant correlation also emerged between information obtained by the 48-hour recall and by the
short FFQ measuring longer-term food choices for all food groups except for fish and shellfish. Previous studies in Finnish populations have showed the fish consumption patterns to be somewhat polarised: the consumption of fish has been associated with an excess risk of CVD mortality, elevated serum cholesterol levels, obesity, smoking and type 2 diabetes incidence in men, but with lowered risk factor levels in women (Salonen et al. 1995, Järvinen et al. 2006). In our study, fish was positively loaded on the health-conscious pattern in 2001. It is possible, that with information on the longer-term food consumption, fish could have played a role in the traditional dietary pattern as well. However, since the trend analyses showed significant correlation between recall and FFQ data for all other investigated food groups, the identified dietary patterns (Studies II, III and IV) can be considered to reflect the true food behaviour styles existing among children and young adults in Finland.

The degree of atherosclerotic processes in the subject was assessed with ultrasound scanning of the arterial structure, namely the thickness of intima media layer of the far wall of the common carotid artery. Ultrasound scanning is a widely accepted, non-invasive method which proved to be a significant predictor of future coronary events among CVD-free adults (O’Leary et al. 1999, Ter Avest et al. 2007). An increase of 0.2 mm in the common carotid artery IMT has been shown to increase one's risk for CHD by more than 30% (O’Leary et al. 1999). Carotid IMT is strongly associated with childhood traditional risk factors in the Young Finns cohort, with a dose-dependent relationship between the number of risk factors present in childhood and the IMT measured in adulthood (Raitakari et al. 2003). It is therefore reasonable to assume that subjects with risk factors have an elevated risk for developing pathological changes in vascular structure in the future. Diet may affect vascular changes via risk factors, directly or in both ways.

6.3 Nutrient intakes among young Finnish adults

With regard to risk for CVD, the nutrient intakes of the participants in the Young Finns cohort changed favourably from 1980 to 2001. These changes appear to happen mostly according to time trends and are in concordance with the changes observed in regularly undertaken cross-sectional studies among adults in Finland (Hasunen et al. 1976, Pietinen et al. 1996b, Puska 2002). This phenomenon has also been observed in dietary studies in other Nordic countries (Bergström et al. 1993, Johansson et al. 1996, Osler et al. 1997). These changes are believed to have occurred as a consequence of a major investment in nutrition education along with community-based dietary interventions, a national monitoring system and nutritional guidelines for institutional kitchens (Puska
Moreover, the deregulation of the importation of foods into the EU has resulted in the improved year-round availability of fruits and vegetables and in the increased variety of food items, including low-fat products, both of which make it easier for people to follow dietary recommendations (Pietinen et al. 2001). People in Finland are, on average, more interested in the healthiness of their food choices than are people in other European countries (De Almeida et al. 1997).

6.4 Identified dietary patterns

In many cases, describing people's diets by assessing existing diets rather than intakes of single nutrients is more informative. The dietary patterns identified in this study represent easily recognisable and interpretable descriptions of distinct food choices in Finland. The dietary pattern derivation analysis was repeated separately in women and men, and similar patterns emerged. The consistency of the patterns throughout the 21-year study period was noteworthy. The small differences reflect the general change in food variety and culture in Finland over the past decades (e.g. the increased use of margarines and wheat instead of butter and rye) and should not be considered solely changes in food choices. During the 21-year follow-up, nutrient intakes have changed towards those recommended in Finland (see e.g. Pietinen et al. 2001), a change that can be seen in this cohort as well. The results suggest that, in order to improve the nutrient composition of the diet, one does not necessarily have to change the actual choices on a food group level, but to make more subtle alterations within a food group towards healthier foods. A great part of these alterations are carried out by the food industry and institutional kitchens as well, thus emphasising the role of the authorities and of the general awareness of a healthy diet.

Food grouping, as well as other steps in identifying dietary patterns, being subject to researchers’ discretion and to the analyses being carried out, may affect the results. In this study, 'milk' and 'other dairy products' were considered two all-inclusive food groups and were not divided into low-fat and high-fat products. At the beginning of the study, the fat content of commonly consumed dairy products was much more homogenous than it is today, and low-fat or fat-free milks, being seldom consumed, would therefore not have been distinguished as a separate food group in the factor analysis (i.e. would have had insufficiently high communality). This could have obscured the identification and interpretation of the dietary patterns. Furthermore, because the main objective was to identify patterns reflecting food choices on a behavioural level rather than on the basis of nutrient intakes, 'milk' and 'other dairy products' were kept as two groups without classifying them according to the fat content.
It is possible, that this may have obscured some of the variation relevant to the truly existing food choices among the study participants.

For each study year, two factors were eventually chosen. A third extracted factor was also carefully considered, but was dropped from further study. In 1980, this third pattern was characterised by sugars and confectionery, wheat, eggs and margarines, and was tentatively called a 'bakery' pattern. In 1986 and in 2001, a similar pattern emerged, but with much lower eigenvalues, thus not representing a strongly distinctive dietary behaviour. In 1986, the third factor had high loadings in root vegetables, pork and potatoes, and in 2001, in other milk products, fruits and berries, sugar and confectionery, and other cereals. Due to their inconsistent characterisation, the third factor was dropped from the analyses at all study points, and further studies were continued with only the two dietary patterns. The two maintained factors together explained 18, 21 and 17% of the total variance in food consumption in 1980, 1986 and 2001, respectively. This can be considered noteworthy, given that the factor analysis was carried out with dietary data. In some disciplines, such as the social sciences, many variables used in factor analyses are categorical, and questionnaires may be constructed so that the variables correlate highly with each other and efficiently distinguish the underlying traits of the subjects. Dietary data are distinctive in that most foods are consumed by the majority of subjects, and food items are therefore unlikely to correlate very strongly with each other.

The dietary patterns identified in this study are clearly interpretable in Finnish society and food culture. In a cohort of Finnish men and women older than the subjects of the present study, two major dietary patterns emerged and were labelled 'conservative' and 'prudent'; both were loaded with very similar food items to the traditional and health-conscious patterns, respectively (Montonen et al. 2005). Moreover, although dietary pattern analyses are subject to several methodological decisions by the researcher and cannot be generalised to other populations, surprising similarities exist in the identified dietary patterns around high-income countries. Although the data-derived patterns are usually mathematically uncorrelated, they often represent very distinct food behaviour. In most cases, when two major patterns are extracted, the stronger one reflects locally typical, conventional and common food choices, and is usually the most predominant among men, older subjects and subjects living in rural areas. Labelling this dietary pattern then depends on the country or population in which the study is being carried out: in the United States and in many West European countries, this pattern is called 'Western', whereas in other parts of the world it is usually labelled 'traditional', as in the present study. The other pattern often reflects more modern dietary habits and is characterised by the high consumption of foods that are newer in the culture: exotic,
fashionable or novelty, and in many cases more expensive, foods. High consumption of these foods is likely to represent a conscious intention to choose foods different from conventional ones (e.g. in Finland, substitution of tea for coffee). This pattern describes the diets among mostly younger, urban subjects. In some cultures, as in Finland, these modern choices are motivated by an awareness of health issues, whereas in other countries they may even reflect disregard for healthiness (e.g. in Mediterranean countries, where traditional food choices may represent a healthier lifestyle than do newer, recently imported food habits) (Sanchez-Villegas et al. 2003). Consequently, one must bear in mind that the label of a particular dietary pattern is always the brainchild of the researcher, and two patterns with the same verbal label may differ entirely in their characteristic foods.

6.5 Associations between childhood and adulthood diets

The childhood diet proved to be a significant determinant of adulthood diet, whereas neither the level of parental education nor the subjects’ own education were associated with the cardiovascular quality of the adulthood diet. In a British longitudinal study on the change of dietary habits between the ages of 11-12 to 32-33 years, food consumption in adolescence was a moderate predictor of adulthood diet (Lake et al. 2006). At the group level, similar to the findings of the present study, dietary intakes improved from the baseline (in 1980) to the follow-up (in 2000), for which the reason the subjects most often gave was their awareness of nutrition-related health messages. A positive parental influence was also detected. In the Boyd Orr cohort with an exceptionally long retrospective follow-up period from the childhood of the subjects (in the 1930s) to the age of 61-80 years, the consumption of vegetables in childhood (measured on a family level) was significantly associated with the healthiness of the adulthood diet even 60 years later (Maynard et al. 2006). Factors affecting diet through decades of life are highly culture-dependent and cannot be generalised over distinct societies. In Finland, the availability of vegetables throughout the year was not nearly as abundant in 1980 as it is today; therefore, high consumption of vegetables at the baseline is likely to be a good indicator of a family’s conscious interest in healthy food choices, which will presumably influence the child’s habits later in life.

Childhood and adulthood diets were also shown to associate in the tracking analysis using the relative dietary pattern scores as a tool to describe the diet. Tracking was especially evident between adolescence and adulthood, among those with high pattern scores and was equally significant in both traditional and health-conscious food choices. A longitudinal Norwegian cohort, followed from adolescence through early adulthood,
showed tracking of the consumption of four food groups chosen to indicate the quality of the whole diet (vegetables, fruits, sweets and soft drinks) (Lien et al. 2001). The relative ranking of the frequency of the consumption was maintained for all food groups. Most studies of the long-term tracking of diet from childhood or adolescence through adulthood have been based on nutrient intakes and have found only little or no tracking at all (Kelder et al. 1994, Boulton et al. 1995, Bertheke Post et al. 2001, Gallagher et al. 2006, te Velde et al. 2007). The present study is probably the first long-term tracking analyses from childhood through adulthood using exploratory dietary patterns. One of the studies on adults with a seven-year follow-up period showed only weak stability (Weismayer et al. 2006), whereas significant tracking was observed in a study with a two-year follow-up among adult women (Borland et al. 2008), in a one-year study on adolescents (Li and Wang 2008) and in an exceptionally long-term study with several dietary pattern assessments from the age of 36 to the age of 53 years (Mishra et al. 2006). The distinct results can at least partly be attributed to methodological differences. A single-nutrient approach in longitudinal dietary studies, although simple and effective to conduct, may be unable to highlight the phenomenon very well. If the ultimate aim is to study the stability or instability of food behaviour at the individual level, it is logical to measure food choices, not nutrient intakes. In addition, an individual’s diet cannot be accurately described with the consumption of only one food or food group. Again, dietary patterns, whether hypothesis- or data-derived, can serve as a useful tool to study the tracking of dietary behaviour. They may also prove useful in describing secular trends in diet at the population level.

When studying diet-health relationships, tracking plays an especially relevant role. If dietary habits, food choices or nutrient intakes show significant tracking, they may have an important effect on health outcomes via two routes. First, if disadvantageous dietary factors are present for a long period of time, their effects accumulate, building up a risk load which may eventually lead to illness or deterioration of health. This is of particular relevance in diseases that develop over a long period of time, such as CVD, cancer or osteoporosis. Accumulation may also occur with protective food habits accumulating beneficial health capital, which may help in maintaining health despite other risk factors. Second, the long-term stability of dietary factors from childhood or adolescence into adulthood means that they are likely to be present at a critical phase in the development of the disease. This is obvious especially in bone health, where adolescence is the critical phase, but may also be relevant in CVD, e.g. obesity or lifestyle factors at puberty may be independently associated with adulthood CVD risk (Perry 1999). The causality of the findings is extremely difficult to study due to the complex life-course pathways between lifestyle and outcomes. In addition, childhood, and especially adolescence, may be critical in the adoption of a long-term lifestyle, as
suggested by the results of the present study, which show stronger tracking of dietary pattern scores from adolescence than from childhood into adulthood. In the present study, participants who were children at baseline were young adults in the latest follow-up and were less often married or had children than subjects who were adolescents already at baseline. It may well be that food choices tend to come full circle back to resemble childhood diet only when one starts his or her own family.

6.6 Dietary patterns and the development of atherosclerosis

The traditional dietary pattern was found to be positively associated with several traditional and emerging CVD risk factors in both men and women, and with the thickening of carotid intima media in men. The health-conscious pattern was significantly, albeit less strongly, in an inverse relationship with cardiovascular risk factors. The associations between exploratory food-based dietary patterns and risk for CVD have been increasingly studied in many countries and with distinct settings, mostly in large observational prospective cohorts. While most of the reported results show significant associations, the possibility of severe publication bias must be kept in mind. Nevertheless, the significant associations seem to follow the same pattern and although some null findings have been reported, no real controversies exist. In the large American Nurses' Health Study among women and in the similar Health Professionals Follow-up Study among men, the associations of two dietary patterns, derived through factor analysis, with the risk for CHD and stroke have been studied (Hu et al. 2000, Fung et al. 2001 and 2004). In both studies, a 'prudent' pattern, characterised by high consumption of fruits and vegetables, whole grains, fish and poultry, formed an inverse association with the risk for CHD. In contrast, a 'Western' dietary pattern (rich in red and processed meat, refined grains, sweets and desserts, French fries, and high-fat dairy products) identified in both studies was positively associated with CHD incidence in both cohorts, and with stroke in the Nurses' Health Study. Additionally, in the cluster analysis in the Framingham Offspring/Spouse Study, women in an 'empty calorie' cluster with high consumptions of sweetened beverages, high-fat sweets and snacks, were at two-fold higher risk for carotid stenosis than were the women in the 'heart healthy' diet cluster, characterised by high intakes of fruits, vegetables, lean protein sources, whole grains and low-fat dairy products (Millen et al. 2002). Women with high scores for the 'prudent' pattern, identified in the MONICA Denmark Study through factor analysis and rich in wholemeal bread, pasta, rice, oatmeal products, fruits, vegetables and fish, had a lower CVD mortality than did those with lower 'prudent' scores; no such associations were found in men nor with the risk for non-fatal CHD (Osler et al. 2001 and 2002). As an example from a very different food culture, in a
cohort of Japanese men and women, subjects with high scores for the 'animal food' dietary pattern were at increased risk for CVD mortality, whereas the traditional 'Japanese' pattern, rich in soybean products, fish, seaweeds, vegetables, fruits and green tea, was associated with a lower risk (Shimazu et al. 2007).

The Lyon Diet Heart Study is thus far the only randomised trial to study the effect of the whole-diet approach in secondary prevention (de Lorgeril et al. 1999). The intervention diet was customised for each patient according to the same principals of the traditional Mediterranean diet: more bread, cereals, legumes and beans, fruits, vegetables, poultry and fish, and less red meat, sweets and animal fats, which were replaced with experimental canola oil-based margarine (chemically like olive oil, but enriched in linoleic acid and alpha-linolenic acid). A remarkable protective effect of the intervention diet was reported with a more than 50% reduction in the risk for CVD recurrence over four years of follow-up.

Studies report a growing number of dietary patterns to be linked to the levels of various CVD risk factors. These studies offer suggestions of possible pathways between diets and CVD. Most effects are thought to be mediated via traditional or emerging risk factors, namely hypertension, dyslipidemia, inflammation and impaired insulin metabolism (Hu and Willett 2002). Perhaps the most solid evidence has been observed in the DASH trial, which has convincingly showed a plant-based diet to reduce blood pressure (Appel et al. 1997). Hence, the beneficial characteristics of dietary patterns rich in fruits and vegetables are likely to show their influence at least partly via effects on blood pressure. A diet rich in plant foods has also been reported to be associated with decreased levels of inflammation markers and increased HDL cholesterol concentrations (Van Dam et al 2003, Esposito et al. 2004, Lopez-Garcia et al. 2004) as well as with a lower risk for type 2 diabetes (Montonen et al. 2005). In their widely cited review of metabolic studies, epidemiological investigations and clinical trials from recent decades, Hu and Willett (2002) concluded that diets with nonhydrogenated unsaturated fats as the predominant form of dietary fat, whole grains as the main form of carbohydrates, an abundance of fruits and vegetables, and adequate ω-3 fatty acids can offer significant protection against CHD. They criticise narrowly focusing only on macronutrient intake limits set by nutritional recommendations, and claim that a variety of options exist for designing heart-healthy diets.

The traditional pattern of the present study showed consistent positive associations with elevated levels of risk factors, namely total and LDL cholesterol, apolipoprotein B and CRP levels among both genders, as well as with insulin levels and SBP among women and homocysteine levels among men. The traditional pattern proved to be low in some
of the foods or nutrients considered heart-healthy, such as fruits and vegetables, but high in some, such as unsaturated fatty acids. Simultaneously, foods with high loadings in the traditional pattern contain high amounts of nutrients considered detrimental to cardiovascular health, namely saturated fatty acids and sodium. Antioxidant vitamins and different fatty acids have been suggested to be among the dietary factors effecting plasma CRP levels, but the true mechanisms remain unclear (Nanri et al. 2007). Higher insulin concentrations independently associated with traditional pattern scores may result from the effects of several macronutrient factors, especially from the quantity and type of fatty acids and carbohydrates in the diet (Riccardi and Rivellese 2000). Moreover, the high sodium content of traditional foods is likely to play an important role in the higher SBP observed among female subjects with high traditional pattern scores. The traditional diet proved to have a positive association with BMI in both women and men, but was significant only in adulthood. This association is likely due to the accumulating effects of food behaviour during life. The role of BMI in the associations between dietary patterns and risk factors was only moderate in general, but expectedly relevant in the association between traditional dietary pattern scores and insulin, which was shown to weaken when BMI was inserted in the model.

The independent association between traditional dietary pattern scores and the IMT was apparent only in men. Risk factors have been shown not to be equally manifested among women and men previously in this cohort (Raitakari et al. 2003, Juonala et al. 2006). The explanation to this remains unclear, but it may partly be due to the protective effect of endogenous estrogen against atherosclerosis in premenopausal women (Clarkson 2007).

Consistent with the results of other studies, the plant food-rich health-conscious dietary pattern among the Young Finns cohort was associated with lower levels of total and LDL cholesterol, apolipoprotein B, CRP and homocysteine, but the associations were weaker than those of the traditional pattern and were evident mostly among women. This seems obvious bearing in mind that the traditional pattern had higher eigenvalues and was therefore bound to show stronger associations with the outcomes at each study point than the health-conscious dietary pattern. Moreover, although the dietary patterns assessed differ in their average nutrient composition, the patterns are based on food groupings, not on nutrient intakes. Therefore, the nutrient intakes of subjects with similar pattern scores may, nevertheless, vary considerably. In the univariate analyses, many associations between risk factor levels and dietary pattern scores among males are apparent only during childhood. This could be attributed to the drastically broadened selection of foods, including those typical of the traditional diet, with more variation in their nutrient contents. It seems that having a high traditional pattern score in 1980
inevitably meant that certain foods and certain nutrient intakes went together. In 2001, it was possible to have a high traditional score with distinct food choices leading to a distinct nutrient profile of the diet. Similarly, the choices within a food group could be the likely explanation for the gender differences in the associations between health-conscious patterns scores and risk factors. A study on the nutrient intakes of the same study population revealed significant gender differences. These results suggest that gender differences exist in food choices within the same food group. While the pattern represents conscious efforts to make healthy food choices, it cannot be considered exclusively health-promoting. In addition to the numerous beneficial micronutrients from vegetables, the possibly effective components of the health-conscious diet include high amounts of polyunsaturated fatty acids and dietary fibre, while the association with high intakes of sucrose can be considered potentially harmful. Moreover, its effect on the intakes of distinct fatty acids is weaker than the effect of the traditional pattern. Thus, these results suggest that the health-conscious dietary pattern is an indicator of an overall health-promoting lifestyle rather than an independent preventive factor for CVD. For similar reasons, Kerver et al. (2003) termed a dietary pattern rich in vegetables, tomatoes, fruits, and tea as 'American-healthy' and not 'healthy'; and similarly, this 'American-healthy' dietary pattern had no association with risk markers of CVD, while among the same subjects, the 'Western' pattern was found to be associated with an elevated level of several risk factors.

Although many of the observed associations can at least partly be attributed to the known effects of nutrients in the pattern diets, it is extremely difficult, if not impossible, to discuss the contribution of single nutrients or of single food items to the associations found. In a separate inspection, only three of the 23 food groups were significantly associated with early vascular changes as individual variables. These three (rye, meat and coffee) were also strongly loaded on the traditional dietary pattern. Therefore, it is unlikely that these foods would account for the association, but are to be considered indicators of wide-ranging traditional food choices and a traditional diet as a whole. Rye, in particular, has been linked to a lowered risk for CVD in studies carried out in Finland (Pietinen et al. 1996a, Leinonen et al. 2000). In Finland and many other countries in the Northern hemisphere, rye contributes a great part of the intakes of fibre, folate and phytoestrogens and is likely to have beneficial rather than adverse health effects. Indeed, rather than drawing conclusions of the effects of individual foods and food groups, the whole-diet approach is more efficient in showing the overall view of the relationship between food choices and the development of atherosclerosis. Moreover, the association found between the traditional dietary pattern and the IMT is stronger than that of the individual food groups, suggesting not only additive but also synergetic effects. One must bear in mind, however, that despite their advantages,
dietary patterns are usually able to explain only a moderate proportion of total variance in the consumption of foods (Schulze et al. 2001). Dietary patterns are unlikely to predict a multifactorial group of diseases, such as CVD, solely by themselves. Even if a pattern has been shown to be associated with CVD, one cannot conclude that the observed pattern is the most beneficial or most detrimental of all possible diets (Schulze and Hu 2002). On the other hand, recognising existing dietary patterns and their relationships with health outcomes in a given population may be useful in understanding the diet-disease phenomenon within the relevant, population-specific context and in identifying susceptible dietary behaviour.

6.7 Energy distribution and the development of atherosclerosis

In this study, subjects with diets low in fat but high in protein had higher values of carotid IMT, than did those with diets low in protein or high in both protein and fat. The positive association remained after controlling for potential confounders, such as the consumption of vegetables, and was unaffected by the intake of carbohydrates. Results should always be interpreted while taking into account cultural differences and different food habits. For instance, in the United States and in many West European cultures, a substantial part of dietary carbohydrates comes from refined grains, sugared cereals and beverages, and processed potatoes, whereas in Finland, the dominant sources of carbohydrates are cooked potatoes and whole-grain products, especially rye bread and porridge (Männistö et al. 2003). Observational epidemiological studies have led to the conclusion that substitution of vegetal protein or unsaturated fat for carbohydrate is beneficial (Appel et al. 2005, Halton et al. 2006), but these conclusions cannot be generalised to populations with different underlying food habits. Although low-carbohydrate diets may be efficient in short-term weight control and have other advantageous effects over the short term, results suggest adverse health outcomes, such as increased cardiovascular and total mortality, of such diets over the long term (Lagiou et al. 2007, Trichopoulou et al. 2007). In a trial by Rankin et al. (2007), a low-carbohydrate, high-fat and high-protein diet increased the levels of CRP, a key marker of inflammation. In many observational studies, low-carbohydrate diets are also high in protein, and the effect may indeed, at least partly, be attributed to protein, as Kelemen et al. (2005) and Trichopoulou et al. (2007) proposed.

Because of the sufficient number of participants, it was possible to carry out a further analysis dividing the subjects into groups according to their macronutrient intake distribution. The positive association between carotid IMT and protein intake was manifested only among those whose mean daily intake of fat was below median, when
averaged over the study years from childhood to adulthood. These subjects differed from other participants in health behaviour choices than can be considered an effort to a healthy lifestyle. Decreasing one's consumption of fat, especially of visible fats included in foods known to be heart-healthy, such as soft margarines and vegetable oils, may thus lead to unintentional deterioration of the fatty acid profile of the diet and to insufficient intakes of essential and other unsaturated fatty acids. Diets higher in fat may actually have advantageous health effects and thus dilute the adverse effects of high intake of protein. While carbohydrates had no significant associations, substituting fat, especially unsaturated fat, with protein may put one at risk for CVD.

In addition, those with a higher intake of protein may also have different intakes of other nutrients. The main sources of protein in the cohort of the present study are meat and meat products, which also contribute substantially to one's intake of sodium and phosphorus, both of which are associated with CVD risk (Tuomilehto et al. 2001, Foley 2007, Dhingra et al 2007). Meat and meat products also constitute the dominant role of the identified traditional dietary pattern.

When examining the diet as a whole and in an isoenergetic design, one must bear in mind that an increase in one element under investigation inevitably entails a decrease in another. Low or high amounts of a particular food or of a particular energy-yielding nutrient cannot be considered an isolated determinant of an observed association. In concrete food choice situations, a food to be avoided will be replaced by another food. The findings of this study suggest that even choices made in a conscious effort to eat a healthy diet, such as avoidance of dietary fat, may lead to unhealthy situations. Although the focus of the general discussion on dietary fat has shifted from the quantity to the quality of fat, there seems to be a firmly established belief among the general public, that all fat should be avoided. One could argue that dietary guidelines need a more detailed definition of the role of distinct food items in a balanced diet, rather than grouping foods into recommended and not recommended according to their macronutrient composition, especially when it comes to different dietary fats.
7 Conclusions and future considerations

The Cardiovascular Risk in Young Finns study represents a comprehensive approach to the relationships between cardiovascular risk and lifestyle factors, including diet. Its strengths include a long follow-up period, a population-based setting from childhood on, a high participation rate and a comprehensive, multidisciplinary approach. In this thesis, comprehensive exploratory dietary pattern analyses proved useful in studying the existing food habits, the stability of food choices from childhood to adulthood, and the relationship between food choices and cardiovascular health among the study population.

In the longitudinal analyses, a general shift towards recommendations was observed in the mean nutrient intakes over 21 years among the study population. Clear associations emerged between childhood and adulthood diets, supporting a childhood role in the development of food preferences. The apparent influence of childhood habits is important to bear in mind when planning educational strategies for the primary prevention of lifestyle-related diseases. However, further studies on food choices and their development over the entire lifespan are needed. Nutrition in early childhood may also influence adulthood health independently.

As an essential part in discovering the broad picture of the development of CVD, new knowledge on the relation between diet and several risk factors, as well as early atherosclerotic vascular changes, is added to the existing body of knowledge. In conclusion, long-term adherence to traditional food choices proved to be a significant determinant of several CVD risk factor levels and to increase the risk for developing CVD, especially among men. Health-conscious food choices, on the contrary, were associated with lower levels of CVD risk factors. In addition, among those with lower-fat diets (either intentionally or unintentionally), a high intake of protein may serve as a marker of increased risk for CVD.

Food choices, especially when expressed as dietary patterns, seem to play a significant role in the development of CVD. These findings offer practical, food-based information on the associations between diet and risk factors for CVD in Finns, and encourage the further use of dietary patterns in epidemiological research.

The Cardiovascular Risk in Young Finns is an ongoing study, and its latest follow-up was carried out in five towns around Finland in 2007. As the subjects approach middle age and thus become more vulnerable to non-communicable diseases, the project will increasingly be able to add to our knowledge of the development of food choices and of
other nutritional factors, their relevance, determinants, tracking and associations with diet-related diseases. In a life-course approach to the study questions, investigating all the relevant phenomena taking into account the effects of age, time, birth cohort and their interactions, will be possible. Variation in health-related outcomes will increase with age, and new endpoints will emerge. Many lifestyle factors may easily cluster in individuals, thus constituting an individual risk profile, just as do some diet-related non-communicable diseases. For example, risk factors for low bone density, a strong risk marker of osteoporosis, are increasingly being linked to the risk factors of CVD in population studies. In the near future, the project will expand to examine more thoroughly the effects of gene-diet interaction on the development of atherosclerosis and osteoporosis in the Young Finns study population. This unique setting offers great analytical challenges as well as far-reaching research opportunities.
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