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DIETARY HABITS AND CARDIOVASCULAR DISEASE:

Evidence from a Swedish cohort of 60-year-old men and women.

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“All things are subject to interpretation whichever interpretation prevails at a given time is a function of power and not truth.”

Friedrich Nietzsche

ABSTRACT

BACKGROUND AND AIM: Diet, in particular dietary fats, and cardiovascular disease (CVD) are closely related. Dietary fats might be captured by measuring blood fatty acid profiles. The role of diet as well as the role of blood fatty acid (FA) levels, in CVD aetiology is still uncertain. Aims of this thesis were to investigate in a large cohort of 60-year-old Swedish men and women: 1) The association between self-reported dietary intake, with a specific focus on foods rich in fat, and selected serum cholesterol ester FAs (Project I); 2) The relation between self-reported intake of specific types of dietary fats (primary aim) and fruit and vegetables (secondary aim) and incident of CVD and all-cause mortality (Project II); 3) The relation between serum cholesterol FAs, with a specific focus on polyunsaturated FAs (PUFA)eicosapentaenoic acid (EPA), docosaesaenoic acid (DHA), linoleic (LA) and linolenic (ALA) acid and incident CVD and all-cause mortality (Project III).

METHODS: Data collected between 1997 and 1998 from 4,232 individuals (2,039 men and 2,139 women) aged 60, randomly selected from Stockholm County were used. The participants were followed regarding incident CVD up to 31st December 2012 using national registers yielding 359 incident CVD cases and 595 deaths. From nutritional data, collected by questionnaires, we created: 1) five diet scores reflecting intake of saturated fats in general, and fats from dairy, fish, processed meat and vegetable oils and margarines (Project I, II) 2) binary variables classifying study participants into exposed and unexposed and evaluating 16 specific dietary factors (Project II). Gas chromatography was used to assess 13 FAs in serum cholesterol esters (Project I, III). Association between each diet score and specific FAs was assessed by percentile differences (PD) with 95% confidence intervals (CI) at the 10th, 25th, 50th, 75th, and 90th percentile of each FA across levels of diet scores using quantile regression (Project I). Crude and adjusted Cox proportional hazards models were used to estimate hazard ratios (HR) with 95% CI in the association between specific self-reported dietary fats (diet scores and single dietary items), fruit and vegetables intake (Project II) and serum PUFA (Project III) and incidence of CVD and all-causes mortality.

RESULTS: In men and women combined, fish intake was associated with high serum proportions of EPA (50^{th}PD=31.41, 95% CI= 27.77; 35.05) and DHA (50^{th}PD=10·51, 95% CI= 9.40; 11.62). Vegetable fat intake was associated with high serum proportion of total PUFA (50^{th}PD 36.34, 95% CI= 22.77;49.92) and low proportion of total SFA (50^{th}PD=−11.33, 95%CI= −14.92;−7.73). (Project I) In women, an increased risk of CVD was related to high consumption of spread butter or margarine (≥10g/day vs <10g/day), HR=1.49, CI=1.02 ; 2.20, and oily potatoes (≥2 times/week vs <2time/week), HR=2.00, CI=1.11;3.60. In men, an increased risk of early death was related to the consumption of butter (vs margarine), HR=1.28, CI=1.01; 1.62, high consumption of spread butter or margarine, HR=1.57, CI=1.23; 2.02 and egg consumption ≥4 times/week (vs <4times/week), HR=1.53, CI=1.15;2.02. In men, daily intake of fruits (vs <1time/day) was inversely related to early death, HR=0.75, CI=0.60; 0.94. (Project II) High serum EPA and DHA proportions were inversely associated with CVD in women (for EPA HR= 0.79, 95% CI 0.64; 0.97; for DHA HR= 0.74 0.61; 0.89) but not in men. Inverse associations with early death were also noted in men for high serum EPA proportion, HR=0.82, 95% CI 0.71;0.95; and DHA proportion, HR= 0.82, 95%CI= 0.71;0.94, and in women for high serum EPA proportion, HR=0.79, 95%CI= 0.65;0.96, and DHA proportion, HR= 0.78, 95% CI= 0.66;0.93. High serum ALA proprotion was associated with moderately increased of CVD incidence, HR= 1.16, 95% CI=1.02;1.32 in women whereas high serum LA proportion was associated with reduced all-cause mortality in men, HR= 0.73 95% CI=0.64;0.83. (Project III).

CONCLUSION: Based on our results, self-reported intake of fish and vegetable fats was clearly associated with serum PUFA. High intake of specific foods and not fats in general may have negative effects on CVD for women and all-causes mortality for men, whereas fruit may reduce mortality only in men. Similarly serum EPA, DHA and LA were protective for CVD and all-causes mortality with gender difference whereas serum ALA might be associated with increased of CVD in only women.

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INTRODUCTION

Cardiovascular disease

Epidemiology

Cardiovascular disease (CVD) is the leading cause of death worldwide[1]. (Fig.1) Among them, coronary heart disease (CHD) - including stable and unstable angina pectoris, myocardial infarction (MI), heart failure and sudden death - and ischemic stroke are the most common manifestation of CVD[2].

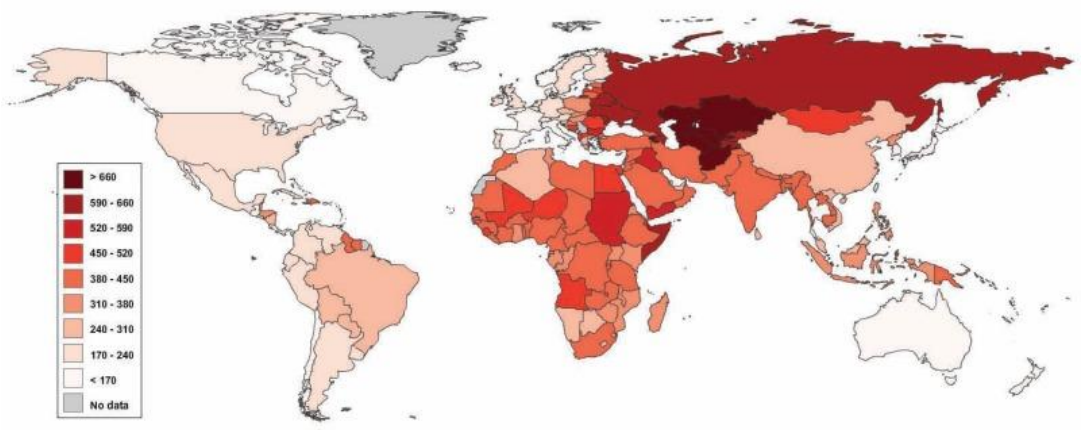

Fig 1: Age-standardized deaths due to cardiovascular disease (rate per 100,000), 2004

According to World Health Organization (WHO) data, 17.5 millions of death each year are for CVD. Of these, 7.6 million are attributed to CHD and 5.7 million to stroke[1]. In Europe nearly half of all death is for CVD (47% - 52% in women and 42% in men): CHD is the main cause of CVD death- 1.8 million of death whereas stroke is the second cause accounting for 1.1 million deaths. Death rate from CHD and stroke is generally higher in Central Europe than in Northern, Southern and Western countries[3]. In Sweden, since 1980s overall mortality rates from CVD have been declining by 53.4 in men and 52% in women in all age group[4]. Specific Swedish data of mortality of CHD and stroke are reported in Appendix, Table 1-2.

Aetiology

Although the aetiology is multifactorial, atherosclerosis is the main cause of CHD and ischemic stroke. Atherosclerosis is an inflammatory disease in which immune mechanism interact with metabolic risk factors to initiate, propagate and activate lesions in the arterial tree[5,6]. Several hypotheses have been

proposed to explain the initiation event in atherogenesis, e.g. the response to injury, response to retention, and oxidation process. These hypothesis are not mutually exclusive and may even be compatible with each other[7]. The oxidation hypothesis empathize the importance of oxidative modification in the atherosclerotic process, because compared to native LDL, oxidized LDL is preferentially taken up in the arterial wall. This hypothesis makes a role of diet and, in general lifestyle in atherogenesis.

Atherosclerotic lesions (atheromata) are asymmetrical focal thickenings of the innermost layer of the arteria, the intima. They consist of cells, connective tissue elements, lipids and debris. Blood-borne inflammatory and immune cells constitute an important part of an atheroma, the remainder being vascular endothelial and smooth-muscle cells[6]. The atheroma is preceded by fatty streak, an accumulation of lipid laden cell beneath the endothelium. Most of the cells in the fatty streak are macrophages, together with some T cells. Fatty streak are prevalent in young people and may progress to atheromata or eventually disappear. In the centre of the atheroma, foam cells and extracellular lipid droplets form a core region, which is surrounded by a cap of smooth-muscle cells and a collagen-rich matrix[6]. T cells, macrophages and mast cells infiltrate the lesions and are particularly abundant in the shoulder region where the atheroma grows. Many of the immune cells exhibit signs of activation and produce inflammatory cytokines[6].

Coronary and cerebrovascular ischemia is for the most cases due to the formation of an occluding thrombus on the surface of the plaque. Plaque rupture and endothelial erosion are the main causes of thrombosis[8,9].

Plaque rupture (60-70% of the case) exposes pro-thrombotic material from the core of the plaque (phospholipids, tissue factors and platelet-adhesive matrix molecular) to the blood. Rupture occurs where the fibrous cap is thin and partly destroyed. In these sites activated immune cells are abundant. They produce several inflammatory molecules and proteolytic enzymes that can weaken the cap and activate cells in the core, transforming the stable plaque into a vulnerable unstable structure that can rupture and induce thrombosis[6]. (Fig 2)

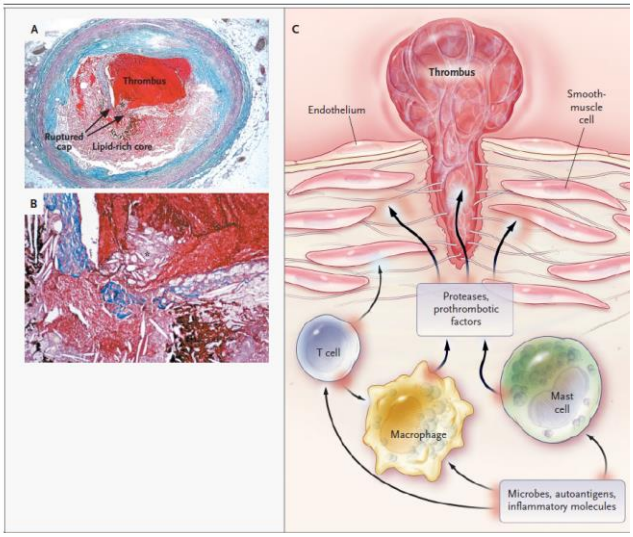

Fig 2:Atherosclerosis Lesion in a Human Artery (source Ross et al.)

Endothelial erosion of the plaque (20-25% of cases) has received less attention than the plaque rupture. The apoptosis of endothelial cells could contribute to their desquamations. Oxidative stress can promote endothelial apoptosis- generally hypochlorous acid. The product of myeloperoxidase, an enzyme released by activated leucocyte associated with atheromata- can initiate the apoptosis and consequently production of pro-coagulant tissue factors. Endothelial cells can also express proteinases that may sever their tethers to the underlying basement membrane-activation of matrix metalloproteinase that can catalyse the initial attack on fibrillar collagen[10].

Risk Factors

The risk factors for CVD, including CHD and stroke are well known. Traditionally these potential risk are divided in non-modifiable - sex, age, family history, genetics- and modifiable - diabetes, dyslipidaemia, hypertension, obesity, diet, smoking, physical activity, psychological factors.

Non-modifiable risk factors

Age is linear related with CHD and stroke. Ethnic origin plays a role: African or Asian are at higher risks of developing cardiovascular disease than other racial groups[11].

Men are at greater risk of coronary heart disease than a pre-menopausal woman. But once past the menopause, a woman's risk is similar to a man's. Risk of stroke is similar for men and women[2]. However, CVD prevalence tends to be higher for men than for women. Yet, women experience their first cardiovascular events later in life, approximately 9 years later than men[12]. Similarly, a recent review of

stroke found that men have their first stroke an average 4.3 earlier than women[13] . The reason most often cited for these gender differences is the protective role of oestrogen on the development of CVD risk factors- blood lipids, blood pressure[14]. Other factors that could contribute to decreased CVD incidence and mortality rate in women than in men could be related to the lower prevalence of smoking in women. However, women are more likely to be sedentary than men and increased prevalence in obesity has been noted among women compare to men. Unique in women is the pregnancy and postpartum period in which women are at increased risk of stroke[14].

Strong predictor of CHD is premature family history of cardiac events, since it has long been observed that coronary atherosclerosis tend to aggregate in families[15]. The National Cholesterol Education Program Third Adult Treatment Panel (NCEP ATP III) recommended that age of onset should be before age 65 in the father and 55 in the mother[16]. In the Cardio200 study, it was observed that family history of CHD was associated with about 4 fold time increased of non-fatal acute coronary event in male and 3 fold increased in female. No gender differences were observed regarding the effect of family history on coronary heart disease. The risk for non-fatal acute coronary event was multiplicative increased when family history of hypertension, diabetes and hypercholesterolemia were also accounted for in the risk models. Moreover, a sibling history has been more strongly associated than parental history with subclinical atherosclerosis[17].

Such predictive power of a positive family history is the hallmark of genetic component in the aetiology of CVD. There are several well characterized single and gene disorder that might be contribute to CVD such as certain forms of familiar hypercholesterolemia linked to mutation of the apolipoprotein B gene and during the past few years, there have been major advance in the identification of genetic risk factors for CHD, stroke risk factors thank to the advent of genome-wide association study (GWAS). GWAS identified multiple genetic variants that increased susceptibility to atherosclerosis and its risk factors (blood lipid and blood pressure in specific). The identification of the genetic loci 9p21.3 associated strongly with CHD and myocardial infarction has led to identified 47 chromosomal loci all of which increase the susceptibility to coronary heart disease. However, variant at these loci explain less than 10% of the heritability of CHD and a common scientific view suggested that genetic of CVD involved a complex interplay of many different genes and much work remains to develop thorough understanding of the complex gene-gene and gene-environmental interaction involved in the developed of CVD[18].

Modifiable risk factor

Smoking

In the Global burden of Disease study, Lopez et al. have estimated that 880,00 death from CHD and 412,00 deaths from stroke were attributable to tobacco - based on the estimates of the relative risk of death among smokers for CHD, stroke and hypertensive heart disease[19]. Smoking cessation has been shown to have significant impacts on reducing CHD as reported in a major review of the evidence where the authors determined that successful smoking cessation reduce CHD mortality risk by up to 36%. Although the specific timeline of reduction depend on the number of years and cigarettes smoked daily, it is conceivable that, over time, former smokers CVD risk can drop to levels similar to that of someone who has never smoked[20] . Consensus in the literature is that CVD risk drops precipitously within the first 2 to 3 years of smoking cessation. WHO 2008 data source has shown that smoking rates are higher among poorest populations and women[21].

Physical activity

Physical activity has been showed to be associated with CVD independently by the effects on weight and obesity. Increasing of physical activity has been shown to decrease the risk of CHD and stroke, the risk of some risk factors related to CVD including diabetes, obesity, hypercholesterolemia and hypertension. As previous published, the general trend of physical activity is decreasing in developing countries. Globally, with the exception of several European Eastern countries women are more likely to be physically inactive than men, adults over 50 years of age were more likely to be inactive then younger adults and city dweller more than the country inhabitants[22].

Obesity/Overweight

A review on the evidence of the relation between obesity and risk of CVD concluded that obesity and overweight confer a significantly elevated risk of CHD and stroke[23] . The association is partly but not completely mediated through hypertension, hypercholesterolemia and diabetes. Abdominal obesity in particular is associated with both CHD and stroke independently of BMI. One of the key contributors to obesity is excess in energy intake for a higher consumption of foods that that can vary across countries. However, in general worldwide trend showed rapid increases in oil use and slow decline in the

consumption of cereal as contributors to calories. Increasing in sugar consumption food and in particular in sugar sweeteners beverage (including soft drinks, juice drink and energy and vitamin water drink)[24].

Hypertension

A review of the global burden of hypertension shows that 54% of stroke and 47% of coronary heart disease are attributable to hypertension. Among the major underlying risk factors for hypertension are daily salt intake, body weight and access to treatment[25].

Dislipidemia

High blood concentrations of low density lipoprotein (LDL), triglycerides and lower of high-density particle (HDL) are associated with increased risk of CVD. The INTERHEARTH study has showed that abnormal lipids were the most important risk factors for myocardial infarction by odd ratio in all global regions[26]. Successful intervention program in a number of countries has further supported the causal link between dyslipidaemia and CVD by demonstrating that reductions in cholesterol lead to decrease CVD morbidity and mortality e.g. Finland.

Modification of LDL to form oxidized LDL has been showed to be important in both initiation and progression of atherosclerosis. Lipoprotein (a) - an LDL like particle- has been independently associated with higher risk of CHD and stroke in a recent meta-analysis[27]. Lipoprotein-associated Phospholipase A2 (Lp-PLA2) - an enzyme bound to LDL that hydrolyses oxidized phospholipids on oxidized LDL with the generation of pro inflammatory markers as lysophosphatidylcholine and oxidized LDL- has been shown to be associated with primary event and recurrences of MI and stroke. Moreover, a recent publication showed an independent association between the functional property of HDL, cholesterol efflux capacity, and atherosclerotic cardiovascular disease in a large population based cohort, free from cardiovascular disease[28].

Diabetes

CVD risk is linearly associated with blood glucose. Thus individual without a diagnosis of diabetes but who are at increased risk of developing diabetes in future, have a higher risk of CVD. People with diabetes have a more than 2 fold greater risk of fatal and non-fatal CVD compared to non-diabetics. In

2001, 1.49 million of death from CHD and 709,000 from stroke were attributable to high blood glucose[29]. The most single risk factors for diabetes 2 is obesity but unhealthy diet, physical inactivity independently raise the population risk for diabetes. The prevalence of diabetes is worldwide growing; according to WHO source around 347 million of people have diabetes.

Psychosocial factors

Psychosocial factors have been consistently associated with both the onset and the progression of CVD in large epidemiological prospective studies in several populations. These factors include depression, anxiety, anger, hostility acute and chronic stress and lack of social support. Depression and personality so called type A are the most recognized factors associated with an increased risk of CVD[30].

Air pollution

Over the past 20 years, there has been an increasing body of evidence linking air pollution to higher risk of CVD. Several epidemiological studies showed that both short and long term exposure to particulate matter air pollution significantly increases cardiovascular events and CVD death.[11] In these studies, the relative risk of CVD mortality increased by approximately 1% for every 10 microgram/m^3 increase in daily concentration of fine particle pollution. Despite the robust epidemiological evidence of air pollution´s negative effect on CVD incidence and mortality, the specific mechanism by which particulate matter increase CVD risk are still unclear. Different mechanism have been proposed, specifically thought the activation sympathetic nervous system, release of pro inflammatory or oxidative stress inducing compounds from the lung and soluble particulate matter entering in the blood stream after inhalation that directly act on the cardiovascular system.

Fatty acids

Fatty acids are the basic structural components of triglycerides (TGs) and are also found in phospholipids and cholesterol esters. Fats provide functions as storage units for energy (9Kcal for each 1g of fat), structural units in membranes and precursors to eicosanoids[31].

Chemically, fatty acids are characterized by methylene group on one side that impart hydrophobic characteristics and carboxyl group on the other side of a carbon backbone. Their nomenclature vary for

the length of the chain, number of double bonds (called unsaturation) and the position of the first double bond on the carbon chain opposite the carboxyl group. The characteristics of the fatty acids are important because may affect the chemical and physical properties of the fatty acids (e. double bond confer liquidity to the fatty acids).

The most known categorization of the fatty acids is based on the double bonds: saturated fatty acids (SFA) -no double bonds-, monounsaturated (MUFA) - one double bond- polyunsaturated fatty acids - more than one double bond-. Moreover, the double bond may be in cis- or trans- configuration when the hydrogen atoms attached to the double bonds are on the same or opposite side respectively. The most common shorthand notation is the following: C:D n- (or omega/ω) where C is the number of carbons atoms, D the number of unsaturation and n- refers to the positions of the first double bound closest to the methyl end of the fatty acid.

Fat digestions, metabolism and physiology

Fatty acids in human body are derived by the dietary fats and in part they are produced endogenously through synthesis, elongation and desaturation process. Fats in food are mainly contained in triglycerides. Since the fatty acids are water insoluble compounds, they can not be transferred to the enterocytes in their intact form. Therefore, the ingested TGs are emulsified and hydrolysed to monoacylglycerols and free fatty acids prior the absorption. The digestive process requires different enzymes secreted in the digestive system. Usually, the entire fat digestive and absorption process lasts for 16-24h. Briefly, the dietary fat is masticated and mixed with lingual lipase, followed by hydrolysis by gastric lipase in the stomach and then by pancreatic lipase in the small intestine[32]. The TGs are first hydrolysed by a gastric acid lipase which predominantly attacks the short-and medium chain fatty acids in the position 3 of the TGs molecule. The gastric lipase is responsible for up to 20% of total TG hydrolysis. The short and medium fatty acids released in this step are absorbed directly into the bloodstream from the stomach. Further, the further triglycerides (around 80%) and the remainder of the partially digestive hydrolysis products formed primarily by gastric lipase are hydrolysed in the upper part of the duodenum to form monoglycerides (generally with PUFA in position 2) where the action of pancreatic lipases together with bile salts, lysophospholipids and unesterified cholesterol form mixed micelles from which the digest lipids are absorbed in the small intestine. These micelles are aggregate in a way that polar (hydrophilic) ends of the

molecules face the water and the non-polar portion (hydrophobic) form the core and are very small particles and easily diffuse the microvilli of the enterocyte of the intestinal wall[32]. In humans, the absorption of most commons dietary fatty acids and fats in general is>95%. However, according some studies some fatty acids e.g. stearic acid might be low[33].

Fatty acids of chain length shorter than C12 which are directly absorbed across the gut wall as individual fatty acids and bounded to albumin and are transported to the liver where they are rapidly oxidized. The other absorbed lipids are esterified to newly TGs and phospholipids in the smooth endoplasmatic reticulum and are used to synthetize chylomicrons, together with apoproteins, which are secreted to the lymph and then to the general blood stream[32]. In the peripheral tissue they are cleaved by lipoprotein lipase –loosing TG and chylomicrons remnants, interchanging components with other lipoproteins - and are taken up by the liver. The other lipoproteins differ in lipids and protein composition and size and are classified according to the density. Briefly, the larger triacylglycerol-rich chylomicrons and the very low density lipoproteins (VLDL) are mainly involved in delivery of triacylglycerols to tissue. The smaller low density lipoproteins (LDL) and the high density lipoproteins (HDL) are more involved in the regulation of the cellular cholesterol content. LDL particles deliver cholesterol to the cells whereas HDL particles remove cholesterol and transport it to the liver for excretion[34].

Saturated fatty acids

Saturated fatty acids are classified according to the chain length in short (2-7 carbons atoms), medium (8-13 carbons) and long (14-20 carbons) and very long (>20 carbons). The most common saturated fatty acids in the diet are in Fig 3.

Common name	Systematic name	Abbre-viation	Typical sources
Butyric	butanoic	C4:0	dairy fat
Caproic	hexaenoic	C6:0	dairy fat
Caprylic	octanoic	C8:0	dairy fat, coconut and palm kernel oils
Capric	decanoic	C10:0	dairy fat, coconut and palm kernel oils
Lauric	dodecanoic	C12:0	coconut oil, palm kernel oil
Myristic	tetradecanoic	C14:0	dairy fat, coconut oil, palm kernel oil
Palmitic	hexadecanoic	C16:0	most fats and oils
Stearic	octadecanoic	C18:0	most fats and oils
Arachidic	eicosanoic	C20:0	peanut oil
Behenic	docosanoic	C22:0	peanut oil
Lignoceric	tetracosanoic	C24:0	peanut oil

Fig. 3: Main common SFA in foods

The majority of saturated fatty acids are transformed through elongation and desaturation in new fatty acids with longer length and in monounsaturated fatty acids. Exception is for the acid pentadecanoic (15:00) and 17:00 heptadecanoic fatty acids. These two fatty acids have an uneven carbossilic chain can not be metabolized and transformed by human enzymes and are only synthetized by bacterial flora in the rumen[35].

Monounsaturated fatty acids

The common MUFA in the diet are described in Fig 4. In general, they have an even number of carbon atoms, between C14-C24 and the double bond is mostly located in position n-9. Oleic acid, palmitoleic acid are the most common MUFA. The oleic acid is the most widely distributed of all the natural fatty acids. As the saturated fatty acids, MUFA might be synthetized endogenously by saturated fatty acids thought specific elongases and desaturases. Moreover, erucic acid is also important in human diet occurring in seed oils of plants in the family of Brassicaceaae, particularly in mustard oil and high erucic rapeseed oil (40-60%). Since in animal model -but not in human- acid erucic has been shown to promote myocardial lesion, it has been removed from rapeseed plants in the most part of the world.

Common name	Systematic name	Abbreviation	Typical sources
Palmitoleic	*cis*-9-hexadecenoic	9c-16:1	marine oils, macadamia oil, most animal and vegetable oils
Oleic	*cis*-9-octadecenoic	9c-18:1 (OA)	all fats and oils, especially olive oil, canola oil and high-oleic sunflower and safflower oil
cis-Vaccenic	*cis*-11-octadecenoic	11c-18:1	most vegetable oils
Gadoleic	*cis*-9-eicosenoic	9c-20:1	marine oils
	cis-11-eicosanoic	11c-20:1	marine oils
Erucic acid	*cis*-13-docosenoic	13c-22:1	mustard seed oil, high erucic rapeseed oil
Nervonic	*cis*-15-tetracosenoic	15c-24:1	marine oils

Fig 4: Main common MUFA in food

Polyunsaturated fatty acids

PUFA are divided in n6 and n3 families. Linoleic (LA) and alfa-linolenic acid (ALA) are the main fatty acids and are the precursors. LA and ALA are also essential fatty acids that must be provided by the diet because the human body lack the enzymes delta 12 and delta 15 desaturases that capable of introducing double-bonds at the n6 and n3 positions. They are synthetized only in plant sources[36]. (Fig.5)

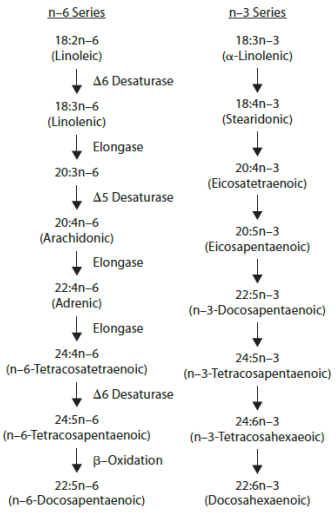

Fig 5: Pathway of n-6 and n-3 fatty acids metabolism

n-6 PUFA

As introduced earlier, the main n-6 PUFA is the linoleic acid. It occurs often in the diet and in particular in some vegetables oils as sunflower, safflower, corn and soybean oils. (Fig.6) Because it is widely distributed in main common dietary fats, many population overconsumed LA and the intake of n3 fatty acids is very often lower than ideal. The metabolism of LA is presented in Fig.5. Physiologically, AA and DGLA are the most important conversion products of LA. Both are substrates for eicosanoids, involved in a variety of physiological actions including modulating inflammation, platelet aggregation, immune response, cell growth and proliferation, contraction and dilatation of smooth muscle cells[33].

Common name	Systematic name	Abbreviation	Typical sources
Linoleic acid	*cis*-9,*cis*-12-octadecadienoic	18:2n–6 (LA)	most vegetable oils
γ-Linolenic acid	*cis*-6,*cis*-9,*cis*-12-octadecatrienoic acid	18:3n–6 (GLA)	evening primrose, borage and blackcurrant seed oils
Homo-γ-linolenic acid	*cis*-8,*cis*-11,*cis*-14-eicosatetrienoic acid	20:3n–6	very minor component in animal tissues
Arachidonic acid	*cis*-5,*cis*-8,*cis*-11,*cis*-14-eicosatetraenoic acid	20:4n–6 (AA)	animal fats, liver, egg lipids, fish
Docosatetraenoic acid	*cis*-7,*cis*-10,*cis*-13,*cis*-16-docosapentaenoic acid	22:4n–6	very minor component in animal tissues
Docosapentaenoic acid	*cis*-4,*cis*-7,*cis*-10,*cis*-13,*cis*-16-docosapentaenoic acid	22:5n–6	very minor component in animal tissues

Fig 6: Main common n-6 in food

n-3 PUFA

ALA is the main n3. It occurs in very high concentrations in flaxseed oil (55%) and perilla oil. Among the common vegetable oils is present in the canola (6-10%)and soybean oils(5-8%). The other important n3 PUFAs are acid eicosapentaenoic (EPA) and docosapentaenoic acid (DHA) which are major components

of marine lipids. Fish as mackerel, salmon, sardine herrings, are excellent source. (Fig.7) EPA and DHA are also precursors of eicosanoids.

Common name	Systematic name	Abbreviation	Typical sources
α-Linolenic	*cis*-9,*cis*-12-*cis*-15-octadecatrienoic acid	18:3n−3 (ALA)	flaxseed oil, perilla oil, canola oil, soybean oil
Stearidonic acid	*cis*-6,*cis*-9,*cis*-12,*cis*-15-octadecatetraenoic acid	18:4n−3 (SA)	fish oils, genetically enhanced soybean oil, blackcurrant seed oil, hemp oil
	cis-8,*cis*-11,*cis*-14,*cis*-17-eicosatetraenoic acid	20:4n−3	very minor component in animal tissues
Eicosapentaenoic acid	*cis*-5,*cis*-8,*cis*-11,*cis*-14,*cis*-17-eicosapentaenoic acid	20:5n−3 (EPA)	fish, especially oily fish (salmon, herring, anchovy, smelt and mackerel)
Docosapentaenoic acid	*cis*-7,*cis*-10,*cis*-13,*cis*-16, *cis*-19-docosapentaenoic acid	22:5n−3 (n−3 DPA)	fish, especially oily fish (salmon, herring, anchovy, smelt and mackerel)
Docosahexaenoic acid	*cis*-4,*cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19-docosapentaenoic acid	22:6n−3 (DHA)	fish, especially oily fish (salmon, herring, anchovy, smelt and mackerel)

Fig 7: Main common n-3 PUFA in food.

Trans fatty acids

Trans fatty acids do not occur in nature excepting for small amounts (2-6%) in ruminants -particularly dairy products. The majority of fatty acids come from technological treatment - as partial hydrogenations of oils - to produce fat blends for margarine, shortening and deep fat frying[33].

Diet

Dietary fats in Sweden

In Sweden, the most important source of dietary fat are 1) spread, butter and oils 2) milk and dairy products 3) meat products 4) fish 5) nuts.

Generally, fat containing dairy product, butter, butter based spread, meat product, sweet, bakery product and confectionary are the main source of SFA, represented by palmitic acid, following by stearic and myristic. Dairy product and meat product are also the main food containing pentadecanoic acid and heptadecanoic acids.

MUFA are derived from several group of food. However, rapeseed oils and liquid margarines are the main source of MUFA and in specific 18:1n9. Soft margarines, vegetables oils, nut and fish are the main source of PUFA. In specific, the n6 linoleic acid is the main PUFA in the diet and it is present mainly in vegetables oils (90%) as sunflower and soybeans oils. The n-3 alfa-linoleic acid is encompassed in high concentration in linseed oil (55%). Fish in particular fatty fish (mackerel, salmons, herrings) are the main source of EPA and DHA[37,38].

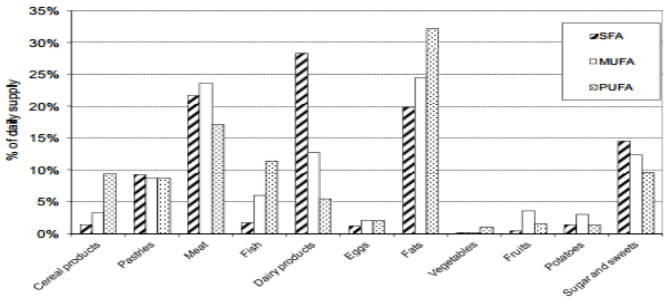

Fig 8: Percentage contribution of SFA, MUFA and PUFA from group foods in Sweden

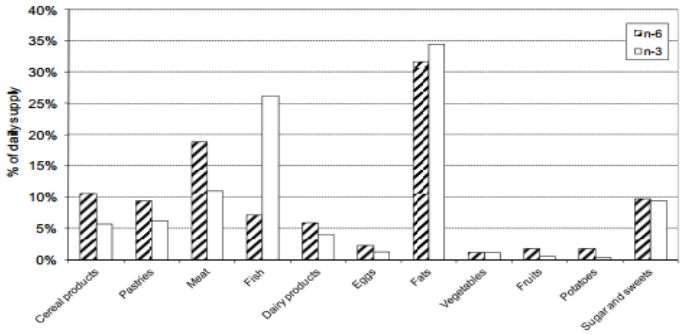

Fig 9: Percentage contribution of n-6 and n-3 PUFA from group foods in Sweden

Trends in diet

In Europe, the amount of fats present in the food supply has remained relatively stable overall since 1990 after a great decline from 1970 when nutritional campaign were adopted to reduce dietary content of saturated fat and cholesterol. Moreover, recent FAO data have shown an increase of fruit and vegetables much of Europe. In the Nordic countries vegetables intake has been shown to increase gradually in recent decade where the consumption of fruit has increased dramatically, now equally the high consumption levels in the Southern Europe[3].

These global results are confirmed in Swedish national data. A Swedish nutritional survey was conducted 1997-98 (Riksmaten) aiming to carry out differences in nutritional habits in the Swedish population in last decades (survey so called Hulk, 1989)[39]. The results indicate that certain changes in dietary habits have occurred since the first survey carried out in 1989. In Riksmaten a more frequent consumption of juice and nectar, pizza, rice, pasta, nuts and snacks and sweets were seen for both men and women. Women also more often consumed vegetables, milk, meat and poultry and alcoholic beverages, and men more

often consumed jam and marmalade, ice cream, and soft drinks. A less frequent consumption was seen for cheese, offal, bread, sweet bakery products, spreads, cream and sugar (as additional foods), and desserts. Men also less frequently consumed eggs, fish, pulses, and porridge. The observed differences in consumption frequency were mostly reflected in consumed amounts. Exceptions were bread and sweet bakery products. A higher consumption was also seen for vegetables (men), sausages (men and women), meat and poultry (men). Part of these differences could be explained by changes in the standard portion sizes used in the record book.

Moreover, results from the market basket study 2010- a Swedish survey performed by the National Food agency with the purpose of obtaining information on level of nutrients in foods- have showed that the proportion of SFA, MUFA, PUFA in the current market basket was similar to that in previously market basket 2005 and 2002. However, the supply of total fat is higher, mainly due to larger contribution from sugar and sweet in which chocolate and cream are high in fat[38].

Swedish recommendations for dietary fats and group of food in general

According to Swedish National Recommendations (SNR 2012), total fat content of the diet should not provide more than around 30% of energy: saturated fat should be restricted to at most 10% energy and the content of PUFA should lie between 5-10% (1% from n3 fatty acids).The mean of total fat intake have remained stable from 1997 to 2011 (34%) and the intake of SFA has slightly decreased from around 14%E to 13%E where the intake of PUFA has increased from a mean of 4.7%E to 5.6%. Although, from 1960 to 1990 the dietary content of trans fatty acids has decreased in all the Nordic countries (primarily through reduced use of partially hydrogenated fats in food production) and the dietary content of PUFA have increased, the proportion of SFA is still above the recommendations and the ratio of unsaturated to saturated fatty acids is below the recommendation in the Nordic countries[40,41].

Meat products

Six to seven portion of meat per week, including sandwiches meat and lean (average of 5%) and fatty meat(15% of fats). Sausage is included once per week. Reindeer and other game are included once a month where three eggs per week are recommended[41].

Fish

Lean and fatty fish and seafood are included twice to three times per week including in the sandwiches[41].

Milk, cheese and other dairy product

The recommendation is that 500 ml of milk per day is sufficient still holds true. Assuming that 10-15 g of cheese are equivalent to 100 ml of milk, low fat milk and cheese (<=17% of fats) are the choices on most occasions. The amount of cheese is the maximum possible and it is equivalent of a cheese sandwich every day. The current consumption of cheese is slightly above the recommendations (28g/day for women and 31g/d for men) and is for cheese with high fat content. The cream included in the food list is cooking cream (15% fat) and equivalent products with a low fat content[41].

Margarine and oil for spreading

Margarine and oil for spreading, cooking and salad dressing contribute around 38% of fat. Around 40% of margarine and oil is intended for spreading and the rest for cooking and salad dressing. One slice of bread is assumed to use 5g if a spread with 60% fat is chosen, it mean that for women is allowed 3-4 slides with spread per day and for men five. Sandwiches with spreadable filling such as liver pate or whey cheese are assumed to not include margarine. Spread fat can be varied regards to the type and quantity of fats that are in the different products. Important factor is to keep the total amount of fat originating from spread constant and to choose a margarine that includes a high content of unsaturated fatty acids. There is no place for butter on bread. Fat for cooking is mainly liquid margarine or oil. Solid margarine is only used for baking; butter can in that case replace solid cooking margarine[41]. However, the consumption of butter results to be doubled from 1998 to 2012[42].

Leeway

Leeway defined as savoury snacks, pastries, cakes, ice cream, jam, sweet and alcoholic drinks should be restricted as most as possible[41].

Bread

From 6 to 8 slices (assuming that one slice of breads is 25g) depending on sex. Two kind of bread are used: fibre rich group consist of bread with a 6g/100 and above e.g. crispy bread and wholemeal rye bread whereas the group with lower fibre content (<6g/100g) include e.g. white bread and refined rye bread. Potatoes, rice and pasta are recommended for every meal. More frequency in the use of potato is recommended than rice and pasta because potatoes are still the most common staple food[41].

Fruit and vegetables

The recommendations for fruit and vegetables intake are 500g per day. Fruits is calculated on 12 type of common fruit and berries. Juice is also included at most 100g per day. Vegetables are divided in 200 groups:1) group with more fibre (>2g/100g) and include e.g. broccoli, white cabbage, beans, peas spinach, carrots and other root vegetables 2) group with less fibre (<2g/100g) e.g. lettuce, tomatoes, cucumber, pepper, onion and mushroom. Concerning pulses (peas and beans) the recommended amount is about 28g and 31g/d for women and men respectively where the real amount consumed is three times smaller[41].

Dietary assessment methodology

In nutritional epidemiology, there are different method to asses self-reported dietary intake:1) dietary record 2) 24-hour dietary recall 3) Food frequency and Brief dietary Assessment Instruments 4) Diet history.

Dietary record

The respondent records the foods and beverages and the amount of each consumed over one or more days. Ideally, the recordings are done at the time of the eating occasion in order to avoid reliance on memory. The amounts consumed may be measured, using a scale or house-hold measures (cup or tablespoon) or estimated, using models, pictures or no aid. If multiple days are recorded, they are usually consecutive and no more than 7 days are included. The dietary record method has the potential for providing quantitatively accurate information on food consumed during the recording period. By recording foods as they are consumed, the problem of omission may be lessened and the foods more fully

described. Measurement of amounts of foods consumed at each occasion should provide more accurate portion size than if the respondents were recalling sizes of foods previously eaten. A potential disadvantage is that is subject to bias both in the selection of the sample and the sample´s compilation of the number of days recorded. Moreover, recording foods as they are being eaten can affect both the type of food chosen and the quantities consumed. Unless dietary records are collected electronically, the data can be burden-some to code and can lead to high personal cost[43].

24-hour dietary

In the 24 hour dietary recall, the respondent is asked to remember and report all the food and beverages consumed in the preceding 24 hours or in the precedent day. The recall is typically conducted by interview- in person or by telephone, although self-electronic administration has recently become available. Ideally, interview would be dieticians with education in foods and nutrition. A quality control system to minimize error and increase reliability of interviewing and coding 24 hours recalls is essential. There are many advantages. When an interviewer administers the tools and records the responses, literacy of the respondent is not requested. For self-administered versions, literacy can be a constraint. Because of the immediacy of the recall period, respondents are generally able to recall most of their dietary intake. There is relatively little burden on the respondents, those who agree to give 24 hours dietary recall are more likely to be representative of the population than are those who agree to keep food records. Thus it can be useful across a wide range of populations. In addition, dietary recall occurs after the food has been consumed, so there is less potential for the assessment method to interfere with dietary behaviour. Main weakness of the 24 hours recall approach is that individuals may not report their food consumption accurately for various reasons (knowledge, memory and interview situation)[43].

Food frequency approach

The food frequency approach asks respondents to report their usual frequency of consumption of each food from a list of food for a specific period. Information is collected on frequency, but little detail is collected on the other characteristics of the foods as eaten, such as the method of cooking, or the combination of foods in meals. Overall nutrient intake estimates are derived by summing, over all foods, the products of the reported frequency of each food by the amount of nutrient in a specified (or assumed)

serving of that food to produce an estimated daily intake of nutrients, dietary constituents and food groups. FFQ approach is inexpensive and the required time to complete it is usually short. The major limitation is that it contains a substantial amount of measurement error. Many detail of dietary intake are not measured and the quantification of intake is not as accurate as with recall or records[43].

A short version of the food frequency is the Brief Dietary Assessment Instruments. It is also called screeners. These instruments might be useful in situations that do not require either assessment of the total diet or quantitative accuracy in dietary estimates. Several limitations: they do not capture information about the entire diet. Most measures are not quantitatively meaningful and the estimate of dietary intake for the population usually may not be made. Even when measures aim to provide estimate of the total intake, the estimate are not precise and have a large measurement error[43].

Diet history

A dietary history is any dietary assessment that asks the respondent to report about past diet. The major strength is its assessment of meal patters and details of food intake rather than intakes for a short period of time or only frequency of food consumption. A weakness of the approach is that respondents are asked to make many judgements about both the usual foods consumed and the amounts of those foods eaten[43].

Measurement of fatty acids in serum

Measurement of fatty acids in blood and or adipose tissue is generally used to capture the dietary intake in particular the fat intake. Fatty acids composition of a wide variety of biological sample has previously measured. However, the lipid pools most often reported in literature are subcutaneous adipose tissue, plasma total phospholipids, cholesterol ester, triacylglycerol (TAG), total plasma lipids, platelet and erythrocytes[44].

Fatty acid composition of serum cholesterol ester (CEs) has been assessed in association dietary intake in several epidemiological studies. Cholesterol can exit free or esterified to a single fatty acid as CE. In blood, approximately two thirds of cholesterol is in the esterified form[45]. The intracellular enzyme responsible for the synthesis of CE from cholesterol and acyl-CoA is acyl-CoA-choelsterol acyl transferase (ACAT), which has specificity for 18:1 n9[46]. So the majority of absorbed cholesterol is esterified in the enterocyte either by reverse of cholesteryl esterase or via CAT. These enzymes are also

responsible for the formation of CE for storage within other cells and for providing CEs for VLDL secretion liver. CEs may also be formed in the blood and in humans the esterification largely takes place in plasma by the transfer of fatty acids from sn-2 position of PC in particles containing apoA1 (such as HDL) under the influence of lecithin-cholesterol acyl transferase (LCAT). The specificity for human LCAT is reported to be in the order 18:2 n-6>18:1 n-9<20:4 n-6>SFA which accounts for the high proportion of 18:2n6 in CEs. Moreover, selectivity for 20:5n-3 over 22:6 n-3 have been shown for LCAT. Since in the serum there are higher proportion of 18:2 n-6 than 18:1 n-9 in CE, that means that the majority of plasma CE must derive from LCAT, rather than ACAT[47]. Over half of CE fatty acids are 18:2 n-6 (52(47.1-54.6) %), 16:0 (13.6(10.9-18.3) %), 18:1 n-9 (19.3 (15-23) %). The abundance of 16:1 n-7(4(2.8-6.8) %) and 20:4 n-6 (5.1(2.5-7.7) %) are similar. Serum fatty acids reflects intake of the last few days or hours.

Diet and blood fatty acids

Several studies have investigated the relation between diet and blood fatty acid profiles. The most consistent findings are for fish intake. In these studies, fish intake was associated with blood 20:5n-3 (eicosapentaenoic acid, EPA) and 22:6n-3 (docosaesaenoic acid, DHA) [48-56]. Studies that investigated whether fats from intake of dairy products associate with blood fatty acid composition have identified a relation between fats from dairy products and blood levels of odd-chained saturated 15:0 (pentadecanoic acid) and 17:0 (heptadecanoic acid), particularly in Swedish populations[57-60]. Few studies have investigated how blood or tissue fatty acid profile relates to intake of fats from processed meat and eggs[56,61,62]. Studies that investigate foods containing a high polyunsaturated/saturated fat ratio report varying results, depending on population characteristics such as ethnicity[56,62,63]. Studies that examine how foods rich in saturated fats relate to blood levels of saturated fatty acids (SFA) and/or monounsaturated fatty acids (MUFA) have shown discrepant results: some investigations report clear relations, others do not[51,54,56,63-72].

Diet in relation to CVD

Diet might play a prominent role in the onset of CVD. The relation between diet and CVD has been studied extensively. The prominent role of diet on CVD is related to the atherosclerosis process. In the

1950, controlled feeding studies have showed an increase of serum cholesterol ester in relation to saturated fatty acid intake[73]. Meanwhile, epidemiological studies found that increased serum cholesterol predicted risk of CVD in human population. These discoveries set the base for the diet-heart hypothesis, which postulated a primary role of dietary saturated fat and cholesterol in the cause of atherosclerosis and CHD in human[74]. This hypothesis gained further support from ecological associations relating saturated fats and cholesterol to rates of CHD in cohort from different countries and from studies of migrants from low to high risk countries. However, the original hypothesis was overly simplistic (correlation does not imply causation) because the effect of diet on CHD can be mediated through multiple biological pathway other than serum cholesterol and LDL cholesterol [75]. In these past two decades, understanding of food and nutrients likely to promote the effect on CVD has grown substantially owing to studies of mechanism of atherosclerosis and the metabolic effect of foods. Although several epidemiological and dietary interventional studies have been performed, evidence of the association of dietary foods and in particular dietary fats intake on CVD is still controversial[76,77]. Research in this complex field is still necessary in order to improve the understanding of this relationship and to prevent CVD.

Results from a recent meta-analysis of prospective cohort studies showed that saturated fats consumption was not associated with risk of CVD including as endpoint CHD and stroke[78]. However earlier single prospective and interventional studies have showed a positive and or inverse association between saturated fats intake and CVD[78-80]. However, diet source of saturated fatty acids might be important. Yet, different prediction in relation to CVD depend on the complex of food matrix and not only from the single fatty saturated fatty acids that encompassed in the foods[81,82].

Studies that examined the association between dairy consumption (milk, cheese, butter, cream, yogurt, ice cream) and risk for CVD including CHD and stroke, as well all causes or CVD death showed controversial results[83]. Recent meta-analyses and prospective cohort studies showed a decrease, increase and no risk of CVD (and all cause of mortality) and intake of dairy product (See Rice review). In general, cheese intake is associated with reduced risk where a positive or no association has been showed for milk and butter. (Ref) Still some association between dairy intake and risk of CVD and total mortality have been observed only in women and not for men or vice versa. Same contrast results come from studies that

evaluating whether low dairy products were associated to reduction on the risk of CVD (or total mortality) compared to high fats dairy intake[83].

Studies that have investigated whether meat consumption was related to CVD (and total mortality) have showed no protective or harmful association between red meat intake and different chronic disease whereas processed meat intake has been noted to increase the risk of CVD and all-causes mortality in several study[84,85]. Regarding egg consumption and risk of cardiovascular disease, the results remains controversial. A recent meta-analysis have noted that a consumption of eggs up to one per day was not associated with increased risk of CHD or stroke whereas other studies have found a reduced risk in CHD and stroke in relation a low consumption of eggs (less than 2 time per week)[86]. Few studies have investigated the role of eggs on total mortality and the most have been performed in men[87-89].

Few epidemiological studies have been investigated the relation between vegetables oils and risk of CVD and total mortality[90]. Evidence from interventional studies have shown a reduced CHD risk (and total mortality) when fats from animal original riched in saturated fatty acids were replace for vegetables oils[77,91].

On the other hand, fat intake from fish, mainly EPA and DHA, have been shown to be protective on CVD through anti-inflammatory, anti-thrombotic, anti-arrhythmic hypotrygliceridemic effect and involved in mechanism of vascular relaxation and plaque stability[92]. However, a recent meta-analysis noted an inverse association between fish consumption and the risk of CHD and stroke, single prospective studies are inconsistent showing a positive association or no association[93]. Moreover, few studies have evaluated association between fat fish intake and CVD in men and women separately[93].

Beside fats, other specific foods have been found associated with CVD. Generally, fruit and vegetables have been shown to reduce the risk of CVD and the evidence is stronger for vegetables than for fruits[80]. However, the effect of fruit and vegetable on total mortality is still uncertain[94].

Few studies have focused whether other specific food as French fried, pastry, chocolate, are associated to the risk of CVD. Yet the results are still poor of evidence.

Blood fatty acids in relation to CVD

Blood and tissue fatty acids might capture self-reported dietary intake and in particular fats intake[44]. Previous studies have investigated the association between fatty acids and CVD using blood fatty acids

instead of the dietary fats intake to predict the risk of CVD[95]. Fatty acids might be involved in the onset of CVD, and in specific atherosclerosis, thought different mechanisms (e.g. pro-atherogenic, pro-inflammatory, anti-inflammatory)[91,96]. It has been shown that the different effects of fatty acids on the metabolism depend on type of fatty acids[34,97]. Moreover, discrepant evidence came from studies relating dietary fats and CVD and blood fatty acids and CVD[95]. In general, prospective studies showed no clear association between dietary intake of saturated, MUFA, PUFA, n6 and n3, and CVD whereas intake of alfa-linolenic acid have been shown to be protective for CVD[95]. A recent meta-analysis investigating the association between circulating fatty acid and risk of CVD has showed no clear association between blood total saturated, total monounsaturated, total n-3 and n-6 fatty acids and the risk of CVD[95]. However, considering individual fatty acids, 17:00, EPA and DHA, and arachidonic acid have been showed to be associated with a reduce risk of CVD[95]. Yet, few studies have considered sex-specific association between fatty acids and risk of CVD[90].

Rational for this thesis

Diet, in particular dietary fats and CVD are closely related. Dietary fats might be captured in blood fatty acids profile[44]. Investigations in this field are still useful to understand possible underlying biological mechanism involved in CVD. Furthermore, several previous studies have shown as both dietary and blood fats might affect a variety of metabolic factors -inflammatory, lipids, glucose- involved in the atherosclerotic process and generally in the onset of CVD[97]. However, the role of dietary fatty acids and blood fatty acids, in specific saturated fatty acids and polyunsaturated fatty acids, in the association with incidence of CVD is still uncertain[95]. Thus, clearly further investigations are needed. The rational of this thesis was to improve knowledge how specific self-reported dietary habits and fats relate to blood or adipose tissue composition of fatty acids and clarify the role of dietary self- reported habits and blood fatty acids in relation to CVD.

AIMS

The overall aim of this project thesis was to study the association between dietary fats with specific focus on food rich in fats and risk of cardiovascular disease (CVD) in a large cohort of 60-year-old Swedish men and women living in Stockholm County, Sweden.

The specific aims of this thesis were to investigate in a large cohort of 60-year-old Swedish men and women:

1) The association between self-reported dietary intake with a specific focus on foods rich in fat and selected fatty acid cholesterol esters measured in serum (Project I).

2) The relation between specific kind of fats intake and incident of CVD -including angina, myocardial infarction and stroke, and all-cause mortality in men and in women separately. A secondary aim was then to investigate the association between fruit and vegetables intake and incidence of CVD and all-causes of mortality. (Project II).

3) The relation between serum cholesterol fatty acids with a specific focus on serum polyunsaturated fatty acids (PUFA) including acid linoleic, alfa-linolenic, eicosapentaenoic and docosaesaenoic and incident of CVD and all-causes mortality in men and women separately. (Project III)

SUBJECTS AND METHODS (Project I, II,III)

The projects of this thesis are based on the data from the population-based cohort of 60 years old men and women residing in Stockholm County, Sweden. The design of the projects is described in the table below.

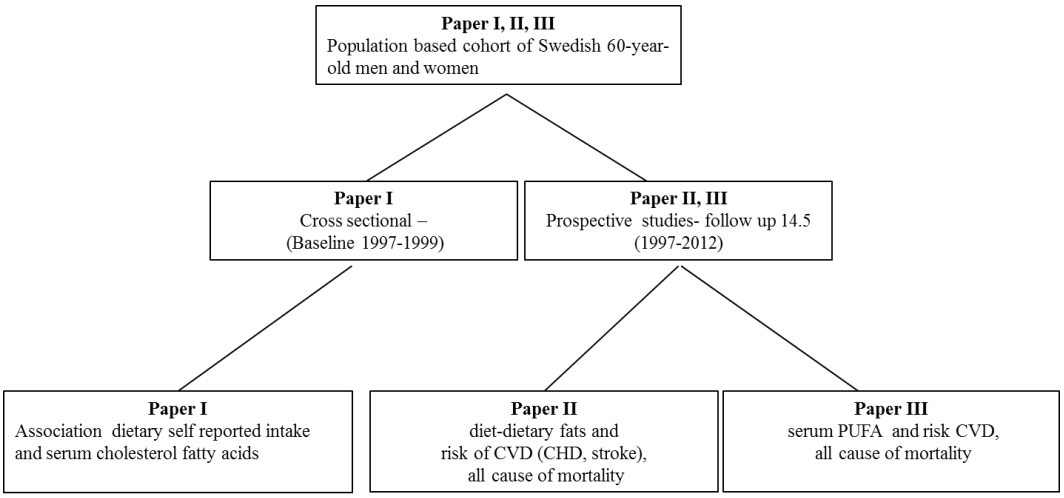

Fig.10: Study design of Project I-III

Study population

Project I, II, III are based on data collected from men and women who participated in a screening of cardiovascular disease risk factors between August 1997 and March 1999. Every third man and woman living in Stockholm County who was born between 1 July 1937 and 31 June 1938 (60 years old) was invited to participate. Of these (5,460), 4,232 (78% response rate), 2,039 men and 2,193 women, agreed to participate. The participants underwent a physical examination that included anthropometric measurements (height, weight, waist, hip, and sagittal abdominal diameter). Blood pressure was also measured and blood samples were drawn after overnight fasting. The participants completed an extensive questionnaire that included providing information about their disease history, health status, medication therapy, lifestyle, and nutritional habits.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethical Committee at Karolinska Institutet (Institutional review board), registration number 96-398. Verbal informed consent was obtained from all participants.

Dietary assessment

Dietary assessment was considered as exposure in Project I and Project II. The questionnaire included 16 questions on current habits regarding the consumption of 16 specific food items: 1) sandwiches, 2) type of spread butter or margarines, 3) quantity of butter and margarine, 4) cheese, 5) milk or yoghurt, 6) fruits, 7) vegetables, 8) lean fish, 9) fatty fish, 10) sausages or bacon, 11) visible fat of meat, 12) potato dishes, 13) eggs, 14) type of oil or spread butter or margarine used to panfry, 15) cream or sour cream ("crème fraiche"), and 16) desserts or snacks. Each question had either three or four possible answers. (Appendix, Table 3) By the three or four alternative answers binary variables were created classifying study participants into exposed and unexposed to 16 specific dietary factors for Project II.

In addition, we created five diet scores: dairy score (Dairyscore); meat score (Meatscore); saturated fat score (Satscore); vegetable oil score (Vegoilscore); and fish score (Fishscore). As the names suggest, the five diet scores represented the intake of specific foods: Dairyscore reflects intake of dairy products (butter, cheese, milk, yoghurt, and cream); Meatscore reflects intake of sausages, bacon, visible meat fats, and eggs; Satscore reflects intake of dairy products, sausages, bacon, and eggs; Vegoilscore reflects intake of margarines and vegetable oils; and Fishscore reflects intake of fatty and lean fish. Because questions about intake of sandwiches, fruits, vegetables, potatoes dishes, and pastries/desserts were not clearly associated with specific fatty acids, they were not included in any scores. For each question, we gave score points based on three categories previous created –low, intermediate, high intake–. (data not shown) The alternative answer "zero", reflecting a zero quantity of fats, generated 0 points, the intermediate level of fats generated 1 point, and high intake generated 2 points. (Appendix, Table 4) Each diet score was based on the individual sum of points generated by the question(s).

We used diet scores (continuous) for Project I and both diet scores and the single dietary questions (binary) for Project II.

Measurement of serum cholesterol fatty acids

Serum cholesterol ester fatty acids were used for Project I as outcome and for Paper III as exposure. Fatty acids in serum cholesterol were analysed as described in detail by Boberg et al.[98] Briefly, serum samples were stored at -80°C until the analyses were performed in 2012. The percentage composition of methylated fatty acids was determined by gas chromatography (GC) with a flame ionization detector

(FIO) and helium as the carrier gas. The GC system used for the analysis consisted of 30-m glass capillary column coated with Thermo TR-FAME (Thermo Electron Corporation, USA), an Agilent Technologies system consisting of model GLC 6890N, an autosampler 7683, and Agilent ChemStation. The temperature was programmed to 150°C - 260°C.

Thirteen fatty acids were identified using standards from Nu Check Prep (Elysian, MN, USA). Total SFA were calculated as sum of all measured SFA, and total MUFA and total PUFA were calculated as the sum of all measured MUFA and all measured PUFA, respectively. The ratio 16 : 1n-7/16 : 0 (SCD16) was calculated as ratio of 16 : 0 to 16 : 1n-7. The SCD16 reflects the activity of the rate-limiting enzyme stearoyl-COA desaturases, which transforms saturated fatty acids 16 : 0 into the MUFA 16 : 1n-7. SCD16 is often used to estimate the proportion of conversion from SFA to MUFA.

Measured fatty acids were expressed as the percentage of the sum of the total fatty acids (%) that were analysed. In our population, the assessments of fatty acid profile failed in 15 samples. In 67 subjects, the fatty acid profile is missing because the available amount of the serum was too small. Overall, 4,150 samples were available for further analysis.

Percentile of serum fatty acids at 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} were used as outcome in Project I where continuous values and quartile of serum fatty acids were used as exposure in Project III.

Endpoint definition

The endpoint was incidence of CVD and all-causes mortality in Project II-III. CVD and all-causes mortality were defined using the Hospital Care Register and the Cause of death register in Sweden[99,100]. Total CVD incidence was first time ischemic CVD event including fatal and non-fatal myocardial infarction, fatal and non-fatal ischemic stroke and hospitalization due to angina pectoris - International classification of Disease 10^{th} revision (ICD-10) codes: I20, I21, I25, I46, and I63-I66-. All-cause mortality included all causes of deaths -ICD-10, codes I1-I99-. All participants were followed regarding incident of CVD and death up to 31^{st} December 2012. During the follow-up (median 14.5 y), 306 men and 185 women suffered from their first CVD event. By then end of the follow-up time, 359 men and 236 women had died. Participants that had a prior event of CVD at the baseline were excluded by analysis in Project II-III.

Confounding factors

To be a confounder, a variable must be related to the exposure and independently associated with the outcome (Fig.11)

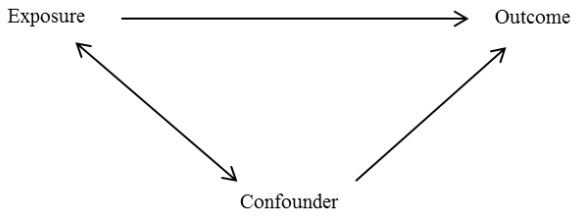

Fig 11: Directed Acyclic Graph (DAG) of the concept of confounder

In the analysis, possible confounding factors were taken in consideration and adjusted for. In Project I we adjusted for sex, smoking status and physical activity, in Project II smoking status, physical activity and education, in Paper III smoking status, physical activity, education, alcohol intake diabetes, hypercholesterolemia, hypertension, BMI, alcohol intake.

Smoking status was categorized as never smokers and ever smokers (current smokers plus former smokers). Physical activity during leisure time in the past year was categorized as either inactive (inactive and light activity at least two hours/week) or active (moderate activity 1-2 times/week or intensive activity ≥3 times/week). Three categories of education level were formed: 1) <9 years of school (compulsory school); 2) 9-12 years of school (secondary); and 3) >12 years of school (university or college). Pre-diabetes was defined as fasting glucose levels between ≥6.1 mmol/l and ≤6.9 mmol/l, a range found in fasting glucose (FG) diagnostic criteria. Diabetes was defined as fasting serum glucose level ≥7.0 mmol/l (WHO cut-off) or self-reported diagnosis of diabetes or use of anti-diabetics medication. Hypertension was determined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or self-reported diagnosis of hypertension or use of anti-hypertensive medication. Hypercholesterolemia was defined as total cholesterol ≥6.45 mmol/l or self-reported ypercholesterolemia or use of lipid-lowering drugs. Hypertriglyceridemia was defined as triglycerides >2.3 mmol/l. Alcohol intake was classified as <1 drink (=14 g ethanol) per wk, <1 drink/d, <2 drink/d, and ≥2 drink/d (Project III)

Statistical methods

Baseline demographic characteristics were summarized by their median values and interquartile range (IQR) for continuous variables and proportions for categorical variables. (Project I, II, III) The 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentile values were used to describe the distributions of serum cholesterol fatty acids (Project I-III). Analyses were performed in the entire sample and then in men and women separately. Sex differences in the distribution of serum fatty acids were tested using the Wilcoxon's rank sum test (Mann-Whitney's test) for continuous variables. Differences across sex were considered significant at 0.05. All statistical analyses were performed using STATA version 12.1 (Stata Corp, College Station, TX, USA).

Project I

Diet score were the exposure of interest and they were treated as continuous variable. The outcome was percentile of serum cholesterol fatty acids at 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th}.

Each diet score was associated to specific serum cholesterol fatty acids selected according to current knowledge about what fatty acids are encompassed in different dietary items, and based on the current literature. Dairyscore – 14 : 0, 15 : 0, 16 : 0, 18 : 0, 16 : 1n-7, 18 : 1n-9, SCD16, Total SFA and Total MUFA; Meatscore – 14 : 0, 15 : 0, 16 : 0, 18 : 0, 16 : 1n-7, 18 : 1n-9, and SCD16; Satscore – 14 : 0, 15 : 0, 16 : 0, 18 : 0, 18 : 1n-9, SCD16, Total SFA, and Total MUFA; Vegoilscore – 18 : 2n-6, 18 : 3n-3, Total PUFA, Total SFA; and Fishscore – 20 : 5n-3, and 22 : 6n-3.

Quantile regression was used to estimate the association between diet score and fatty acids. Percentile differences (PD) at the 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentile of each fatty acid were calculated. The PD's were estimated with 95% confidence intervals and were interpreted as increase (positive regression coefficient) or decrease (negative regression coefficient) at the specified percentile of the proportion of serum fatty acids for each point's increase of the specific diet scores. Quantile regression is a regression method in which instead of modelling the mean, we model the percentiles of the distribution. Interpretation of the results is the same of the linear regression but applied to the percentiles of the distribution and not only mean. Quantile regression is useful in particular for those variables (as diet or

serum fatty acids) that might have a different distribution at different percentiles of the population distribution and can be skewed. Thus, quantile regression gives a more detailed description of the considered association and allows analysis to the entire distribution (where the linear regression estimates only the mean of the distribution)[101]. Subanalysis were performed using diet score as categorical variable. Sex specific stratified analyses were performed to investigate possible differences in the relation dietary score and fatty acids.

Project II

The exposure of interest were diet score treated as continuous variable and single questions treated as binary (0=risky factor,1=protective) where the outcome was binary (yes/no) either for incidence case of CVD and all-causes mortality. Cox proportional hazard model (HR) and 95% of confidence interval (CI) were used to estimate the relation between self-reported dietary intake- scores and single questions - and incidence of CVD and all-causes mortality in men and women separately. In addition to crude model adjustment for possible confounder smoke, physical activity and education were run.

Subanalysis was also performed to investigate specific relation between exposure and MI and stroke.

In sensitive analysis, to exclude possible effect of other confounders and/or intermediate factors that could explain the results of the estimate, we also adjust for country of birth, hypercholesterolemia, hypertriglyceridemia, hypertension, obesity (BMI>30), diabetes, family history- binary variables (yes/no) - duration of menstruation and total SFA, MUFA, PUFA, n-3 and n-6 serum cholesterol fatty acids - continuous variables.

Project III

The exposure of interest was serum cholesterol fatty acids (continuous and quartile) and the outcome was categorized as binary of incidence case of CVD and all-causes mortality. To estimate the relation between serum specific PUFA fatty acids- acid linoleic, alfa-linolenic, eicosapentaenoic and docosaeaenoic -and CVD and all-causes mortality we used Cox proportional Hazard model with 95% of confidence interval. Analyses were run separately in men and women. To avoid possible effect of potential confounders we

adjust for potential confounders including smoke, physical activity, education, obesity, alcohol intake, diabetes, hypertension and hypercholesterolemia.

RESULTS

Baseline characteristics of all participants, in men and women separately, are shown in Table 1 where Table 2 shows demographic, anthropometric, risk and metabolic factors between CVD and not CVD individuals.

The percentile values (at 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th}) of serum cholesterol fatty acids proportion (%) are shown in Table 3. As expected, linoleic acid (18:2 n6; n-6 PUFA) was the major fatty acids in serum cholesterol followed by 18:1 (oleic acid, MUFA) and 16:00 (palmitic acid, SFA) fatty acids whereas the least common fatty acid was pentadecanoic acid (15:0, SFA). Sex-specific differences in fatty acids distribution were found in: all specific SFA, in total MUFA including palmitoleic acid (16:1), total n6 with gamma– linoleic acid (18:3n6) and total n3 including alfa–linolenic acid (18:3 n-3) and DHA. No sex differences were observed for oleic acid, linoleic acid, diomo–gamma linolenic (20:3 n-6) and total PUFA. (Appendix, Table 6)

The distributions of the diet score points were as follow (median, IQR; range): Dairyscore 5, (4−7; 1−12) (n=4107); Meatscore 1, (0–2; 0–6) (n=4105); Satscore 7, (5−9;1−17) (n=4105); Vegoilscore 2, (1−2; 0–4) (n=4105); Fishscore 2, (1−2; 0–4) (n=4105). Lower points of Satscore, Dairyscore and Meatscore were noted in women than men whereas no differences for Fishscore and Vegoilscore. (Appendix, Table 5)

Project I

Dietary fats and serum cholesterol fatty acids relation

Estimates from quantile regression modelling of each diet score, in relation to the percentiles (10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th}) of serum cholesterol of fatty acids are shown in Table 4. Each point of increase of Fishscore associate with an increase of serum proportion of 20:5n3 (EPA) and 22:6n3 (DHA) in crude models and models adjusted for sex, smoke and physical activity (model 1) (Table 4, a). The increases of these fatty acids for each unit´s increase of the Fishscore were greater in the upper part (90^{th} percentile) than in the lower part (10^{th} percentile) of the distribution (Fig 9a–9b). EPA showed greater percentiles differences than DHA. Moreover, in sex stratified analysis a clearer increase of EPA, DHA in relation to each unit´s increase of Fishscore was noted in men compared to women.

A high Vegoilscore was associated to increase of serum proportion of total polyunsaturated fatty acid and decrease of total proportion of saturated Table 4, b. The change of PUFA in relation to each unit´s increase of the Vegoilscore was higher in the lower side of the PUFA distribution (10^{th} percentile) than in the upper (90^{th} percentile) (Fig 1c). The absolute coefficient of total SFA increased with the order of quantile (from 10^{th} to 90^{th} percentiles) whereas the decrease of the total SFA was observed to be similar across percentile (Fig 1d). Moreover, a higher intake of vegetable oil associated with an increase of linoleic acid serum proportion (18:2n6) in the crude model but not in the upper part of the distribution (90^{th}) after adjustment. The coefficient of linoleic acid decreased, with the order of the quantile (from 10^{th} to 90^{th} percentiles). In sex stratified analysis, a similar pattern of association between Vegoilscore and specific fatty acids was showed.

A slight increase of 14:00, 15:00 and 18:00 serum cholesterol fatty acids was associated with Dairyscore, Satscore whereas Meatscore was not clearly associated with any specific fatty acid. (Table 4c−e). The estimated coefficients of these diet scores were not generally monotone functions of the order of the quantile (from 10^{th} to 90^{th} percentiles). In sex stratified analysis (Appendix, Table 7) we observed no clear different association between Dairyscore, Meatscore, Satscore and the selected fatty acids.

A clear association has been found between the five single questions and serum cholesterol fatty acids. In particular, we noted: 1) a decrease of 20:4n6 (arachidonic acid) and increase of serum 18:3n3 (linoleic acid) for each unit increase of intake of sandwiches; 2) a decrease of serum proportion of EPA and DHA for each unit increase of intake of potatoes dishes; 3) increase of EPA and DHA for each unit increase of fruits, 4) decrease of MUFA mainly oleic acid, increase of serum PUFA, in particular EPA and DHA with each unit increase in the intake of vegetables; 5) decrease of serum 16:00 and 16:1n7 and increase of linoleic acid, PUFA and n6 for each unit increase of intake of dessert or snacks. (Appendix, Table 8a-e)

Project II

Dietary fats intake in relation to incidence of CVD

We found no association between general fat intake consumption (diet scores) and incidence of CVD in both men and women (Table 5). However, single specific dietary fatty foods were associated to incidence of CVD in women but not in men. Consumption of more than 10g/day of butter and margarine (vs<than

10g/day) and more than 2 times per week of "oily" potato -pan-fried potatoes, French fries or potato au gratin- (vs <than 2 times per week) increased the risk of CVD in women. (Table 6) These results have no explanation in lifestyle factors (smoking and physical activity) and education. No potential intermediate factors – country of birth, hypercholesterolemia, hypertrygliceridemia, hypertension, obesity, diabetes and total SFA, MUFA, PUFA, n-3 and n-6 serum cholesterol fatty acids – showed to affect the estimates described above (data not shown).

We also looked at the specific associations with CVD, MI and stroke. We noted the following adjusted associations in women when studying MI as an outcome: Dairyscore (HR 1.24, CI 1.06-1.44), Satscore (HR 1.15, CI 1.03-1.30), intake of potato (HR 3.12, CI 1.11-8.84) and >10g/d butter or margarine (vs <10g) (HR 2.30, CI 1.12-4.71). In men, in adjusted model, Fishscore was associated with MI (HR 0.73, CI 0.55-0.98) whereas the single question about visible fat in meat was related to stroke (HR 1.85, CI 1.15-2.97). (Data not shown)

Dietary fat intake in relation to all-causes mortality

Specific groups of fats intake (Dairyscore, Satscore, Vegoilscore, Meatscore) were associated with overall mortality risk in crude model but these results were explained by lifestyle and education factors. (Table 5). For each point´s increase of Dairyscore and Satscore we observed an increased risk of overall mortality in both men and women (Table 2). Yet, in men the risk of early death decreased for each point´s increase of Vegoilscore whereas increased for each point´s increase of Meatscore.

Consumption of spread butter instead of margarines, intake of margarine or butter more than 10g/day and eggs more than 4 times per week increased total mortality in men. (Table 6) Adjustment for smoking, physical activity and levels of education attenuated the estimates, in particular for consumption of spread butter – where lower confidence interval bound was near to 1.00. But still these factors not explained these findings (Table 2). We noted no evidence that country of birth and metabolic factors (hypercholesterolemia, hypertrygliceridemia, hypertension, obesity, diabetes) could explain these associations in men. However, increased mortality risk associated with intake of butter was no longer significant after adjustment for total MUFA (HR 1.22, CI 0.96-1.54), PUFA (HR 1.21, CI 0.96-1.53) and n–6 (HR 1.23, CI 0.97-1.55) serum fatty acids (data not shown).

We found other sex-specific associations between specific intake of single foods and all-causes mortality that were not anymore longer after adjustment. (Table 6)

Fruit and vegetables consumption in relation to incidence of CVD and all-causes mortality

Independently by smoking, physical activity and education, intake of fruits more than 1 time per day was protective for all-causes mortality in men (Table 7). In crude models, we found that consumption of fruit more or equal to 1 times/day was protective for CVD in both men and women and for all-causes mortality in women(Table 2) where intake of vegetables, roots, tubers more than 1 time per day decreased mortality in both men and women. However, these results were partly explained by smoking, physical activity and education since the upper confidence interval bound exceeds 1.00. (Table 7)

Paper III

Serum cholesterol PUFA in relation to incidence of CVD

High serum EPA and DHA proportion were associated with CVD incidence in women. After adjustments for potential confounders, a 1-SD increase in EPA and DHA among women resulted in 21% and 26% reduced risks CVD, respectively. (Table 8) Women in the highest quartile of EPA and DHA had 40 % lower risk of CVD, respectively. (Appendix, Table 9a) However, after adjustment for serum DHA, EPA was not associated with CVD: HRs 0.97 (95% CI 0.74-1.28) per 1-SD increment (data not shown). ALA was associated with increased CVD incidence among women (Table 8) and women in the highest ALA quartile had higher risk (HR 1.69, 95% CI 1.04-2.73) than those in the lowest quartile (HR 1.69, 95% 1.04-2.73). (Appendix, Table 9b) No clear association were observed between serum PUFA and CVD in men.

Serum cholesterol PUFA in relation to all-causes mortality

EPA and DHA serum proportion were inversely associated with all-cause mortality in both men and women separately. LA was associated with decreased of all-cause mortality in men, but not in women (Table 8). Men in the highest LA quintile had 41 % lower mortality risk (HR 0.59; 95% CI 0.41-0.85) compared to those in the lowest quintile. (Appendix, Table 9a) ALA was not associated with all-cause mortality in both men and women. (Table 8)

Table 1: Baseline characteristics of 4,232 participants of the cohort of 60-year-old men and women.

Characteristic	All	Men (n=2,039)	Women (n=2,193)
Physical activity[a] (%) [(m182)]	30·7	34·6	27·04
Smoking (%) [(m182)]:			
Never smoker	39·8	33·2	45·98
Ever smoker	60·2	66·8	54·02
Education (%) [(m164)]:			
≤9years	59·2	56·6	61·68
9-12 years	13·2	15·4	11·12
> 12 years	27·6	28·0	27·20
Country of birth (%):			
Nordic[b]	89·4	88·3	90·6
Non Nordic	10·6	11·7	9·53
Anthropometric data			
BMI (Kg/m^2) median (IQR)	26·32 (23·91-29·09)	26·65 (24·42-29·07)	25·96 (23·43-29·12)
Waist circumference (cm) median (IQR) [(m3)]	92·0 (83·0-100·0)	97·0 (91·0-104·0)	85·0 (78·0-93·0)
WHR [(m4)] median (IQR)	0·89 (0·81-0·95)	0·94 (0·91-0·99)	0·82 (0·78-0·87)
SAD (cm) median (IQR) [(m7)]	20·4 (18·5-22·5)	21·3 (19·5-23·0)	19·5 (17·9-21·4)
Biological markers and blood pressure			
Insulin (µU/L) median (IQR) [(m4)]	8·9 (6·6-12·4)	9·3 (6·8-13·5)	8·6 (6·4-11·7)
Total cholesterol (mmol/L) median (IQR) [(m3)]	5·9 (5·3-6·6)	5·7 (5·1 -6·4)	6·1 (5·4-6·8)
HDL cholesterol (mmol/L) median (IQR) [(m3)]	1·44 (1·19-1·73)	1·27 (1·08-1·5)	1·61 (1·36-1·88)
LDL cholesterol (mmol/L) [(m64)]	3·8 (3·2-4·5)	3·8 (3·2-4·4)	3·9 (3·3-4·5)
Triglycerides (mmol/L) median (IQR) [(m3)]	1·1 (0·8-1·6)	1·2 (0·9-1·7)	1·1 (0·8-1·5)
Systolic BP (mmHg) median (IQR) [(m5)]	136 (123-152)	141 (128-155)	132 (118-148)
Diastolic BP median (IQR) (mmHg) [(m5)]	84 (77-91)	87 (80-94)	81 (75-87)
Metabolic factors			
Hypertension[c] (%) [(m5)]	38·2	46·2	31·0
Hypercholesterolemia[d] (%) [(m3)]	35·1	30·7	39·2
Hypertriglyceridemia[e] (%) [(m3)]	8·4	11·2	5·8
Prediabetes[f] (%)[(m3)]	6·1	8·2	4·1
Diabetes[g] (%) [(m3)]	7·5	10·1	5·2
Obesity (BMI≥30) (%)	19·6	18·9	20·2

BMI, Body Mass Index; WHR, Waist to hip ratio; SAD, Sagittal abdominal diameter; BP, Blood pressure; m, missing value; IQR, interquartile range; [a]Regular physical activity in the leisure time; [b]Nordic: Swedish and Finnish born; [c]SBP ≥140 mmHg, DBP≥90 mmHg, self-reported hypertension or antihypertensive medication; [d]Total cholesterol ≥6·45 mmol/l, self-reported hyperlipedemia or use of lipid lowering drugs; [e]Triglycerides ≥2·3 mmol/l; [f]According to WHO diagnostic criteria defined as fasting glucose levels in the range ≥6·1 and ≤6·9; [g]Fasting serum glucose level ≥7·0 mmol/l (WHO cut-off), self-reported diabetes or anti-diabetic medication.

Table 2: Baseline characteristics between CVD and not CVD participants of the of 60-year-old men and women.

	Men		Women	
Characteristic	**CVD** (n=306)	**NO CVD** (n=1.445)	**CVD** (n=185)	**NO CVD** (n=1.805)
Physical activity[a] (%) (m48)	32.4	35.8	20.56	28.56
Smoking (%) (m48):				
Never smoker	72	81	64.32	79.04
Ever smoker	27.8	18.6	35.68	20.96
Education (%) (m32):				
≤9years	60.8	55.4	73.09	60.34
9-12 years	16.0	15.2	7.17	11.58
> 12 years	23.2	29.3	19.73	28.08
Country of birth (%):				
Nordic[b] (m5)	89.2	88.4	94.05	91.18
Non Nordic	10.8	11.6	5.95	8.82
Anthropometric data				
BMI (Kg/m^2) median (IQR)	26.9 (24.6-29.4)	26.4 (24.3-28.8)	27.1 (24.1-30)	26 (23-29)
Waist circumference median (IQR)	98 (91-103.5)	96.5 (90.5-103)	88.5 (80.5-96.5)	84.5 (77-92)
WHR (m1) median (IQR)	0.96 (0.92-0.99)	0.94 (0.91-0.98)	0.84 (0.80-0.90)	0.82 (0.77-0.86)
SAD (cm) median (IQR) (m3)	21.5 (19.8-23)	21 (19.5-23)	20.4 (18.5-22.5)	19.5 (17.8-21)
Biological markers and blood pressure				
ApoA1 median (IQR) (m7)	1.38 (1.24-1.55)	1.42 (1.27-1.58)	1.6 (1.42-1.76)	1.62 (1.46-1.81)
ApoB median (IQR) (m6)	1.09(0.95-1.27)	1.04 (0.9-1.2)	1.1 (-0.94-1.28)	1.03 (0.89-1.2)
ApoB/ApoA1 median (IQR) (m7)	1.25(1.02-1.5)	1.36 (1.12-1.65)	1.44 (1.14-1.72)	1.5 (1.3-1.9)
Lp(a) median (IQR) (m2)	0.13 (0.06-0.32)	0.11 (0.05-0.31)	0.13 (0.05-.38)	0.13 (0.06-0.35)
Fibrinogen median (IQR) (m16)	3.03(2.56-3.59)	2.8 (2.4-3.3)	3.14 (2.70-3.7)	2.9 (2.5-3.5)
Uric acid(µmol/L) median (IQR) (m2)	325 (287-370)	321 (284-366)	269 (232-307.5)	259 (224-299)
Glucose median (IQR) (m2)	5.4(5-6)	5.3 (5-5.8)	5.02 (4.8-5.8)	5.1 (4.7-5.5)
Insulin (µU/L) median (IQR) (m3)	9.4 (7.3-14.4)	8.8 (6.5-12.8)	9.4 (6.6-12.7)	8.3 (6.2-11.3)
Total cholesterol (mmol/L) median (IQR) (m2)	5.9 (5.2-6.6)	5.8 (5.1-6.4)	6.3 (5.5-6.9)	6.1 (5.4-6.8)
HDL cholesterol (mmol/L) median (IQR) (m2)	1.23 (1.02-1.5)	1.31 (1.1-1.53)	1.5 (1.26-1.78)	1.63 (1.39-1.9)
LDL cholesterol (mmol/L) median (IQR) (m48)	4 (3.4-4.6)	3.8 (3.2-4.4)	4 (3.3-4.6)	3.8 (3.2-4.5)
Triglycerides (mmol/L) median (IQR) (m32)	1.3 (0.9-1.8)	1.2 (0.8-1.6)	1.2 (0.9-1.7)	1.1 (0.8-1.4)

Systolic BP (mmHg) median (IQR) [(m3)]	145.2(134-160.5)	140 (127-153.5)	141 (125.5-159)	130.5 (117-146)
Diastolic BP (mmHg) median (IQR) [(m3)]	89.5 (83.5-96)	86 (79.5-93)	84.5 (78-91)	80 (74-87)
Metabolic factors				
Hypertension[c] (%) [(m1)]	54.6	40.2	41.62	28.14
Hypercholesterolemia[d] (%) [(m2)]	35	28.3	46.20	37.97
Hypertriglyceridemia[e] (%) [(m2)]	14.4	8.7	13.04	4.66
Prediabetes[f] (%)[(m32)]	13.4	7.3	9.24	3.44
Diabetes[g] (%) [(m2)]	14.38	7.5	11.41	3.88
Obesity (BMI≥30) (%)	20.9	16.6	25.41	18.28

BMI, Body Mass Index; WHR, Waist to hip ratio; SAD, Sagittal abdominal diameter; BP, Blood pressure; m, missing value; IQR, interquartile range; [a]Regular physical activity in the leisure time; [b]Nordic: Swedish and Finnish born; [c]SBP ≥140 mmHg, DBP≥90 mmHg, self-reported hypertension or antihypertensive medication; [d]Total cholesterol ≥6·45 mmol/l, self-reported hyperlipedemia or use of lipid lowering drugs; [e]Triglycerides ≥2·3 mmol/l; [f]According to WHO diagnostic criteria defined as fasting glucose levels in the range ≥6·1 and ≤6·9; [g]Fasting serum glucose level ≥7·0 mmol/l (WHO cut-off), self-reported diabetes or anti-diabetic medication.

Table 3: Percentiles (10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th}) of serum cholesterol ester fatty acid distributions, expressed as proportions of the total fatty acid amount, in 4,150 participants of the cohort of 60-year-old men and women.

	Percentile values of fatty acids (%)				
Fatty acid	10^{th}	25^{th}	50^{th}	75^{th}	90^{th}
14 : 0	0·65	0·76	0·89	1·02	1·25
15 : 0	0·17	0·19	0·22	0·25	0·30
16 : 0	10·54	10·92	11·36	11·86	12·34
18: 0	0·62	0·69	0·76	0·86	0·96
Tot. SFA[a]	12·25	12·70	13·25	13·86	14·47
16 : 1n-7	2·26	2·72	3·40	4·34	5·54
18 : 1	20·52	21·54	22·59	23·80	25·05
Tot. MUFA[b]	23·15	24·49	26·09	28·00	30·15
18 : 2n-6	43·04	45·90	48·76	51·21	53·50
18 : n-6	0·53	0·67	0·86	1·09	1·33
20 : 3n-6	0·54	0·62	0·71	0·82	0·92
20 : 4n-6	4·94	5·52	6·23	7·01	7·81
18 : 3n-3	0·64	0·75	0·86	1·00	1·13
20 : 5n-3	1·12	1·44	1·87	2·46	3·25
22 : 6n-3	0·62	0·74	0·89	1·06	1·24
20 : 5n-3+22 : 6n-3	1·81	2·22	2·75	3·48	4·42
Tot. PUFA [c]	55·98	58·36	60·61	62·49	64·10
16 : 1n-7/16 : 0	0·20	0·24	0·30	0·38	0·47

[a]Total SFA calculated as sum of all saturated fatty acids; [b]Total MUFA calculated as sum of all monounsaturated fatty acids; [c]Total PUFA calculated as sum of all polyunsaturated fatty acids.

Table 4(a-e): Percentile differences with 95% confidence intervals, expressed as serum fatty acid proportions, at the 10th, 25th, 50th, 75th, and 90th percentile of serum cholesterol ester fatty acid distributions, across points of increase of diet score (Fishscore, Vegoilscore, Dairyscore, Satscore, Meatscore). Results based on 4,030 participants of the cohort of 60-year-old men and women.

Fatty acid		Percentile differences of fatty acids (%)				
		10th	25th	50th	75th	90th
(a) Fishscore						
20 : 5n-3 (EPA)	Crude	22·52 (19·66, 25·39)	27·73 (24·78, 30·68)	33·52 (29·99, 37·05)	45·22 (39·97, 50·48)	57·74 (47·04, 68·43)
	Adjusted[a]	21·92 (18·59, 25·25)	27·23 (24·13, 30·32)	33·01 (29·34, 36·63)	44·05 (38·14, 49·95)	54·25 (44·38, 64·12)
22 : 6n-3 (DHA)	Crude	9·33 (8·16, 10·50)	10·72 (9·63, 11·81)	11·57 (10·49, 12·66)	12·36 (10·94, 13·78)	14·61 (12·60, 16·62)
	Adjusted[a]	8·70 (7·43, 9·98)	10·32 (9·19, 11·44)	11·11(9·98, 12·24)	11·58 (10·18, 12·98)	14·58 (12·51, 16·65)
(b) Vegoilscore						
18 : 2n-6	Crude	65·27 (37·82, 92·72)	40·04 (21·38, 58·71)	36·30 (20·30, 52·30)	26·43 (9·87, 42·98)	21·13 (1·29, 40·97)
	Adjusted[a]	59·88 (33·58, 86·18)	42·46 (23·96, 60·96)	27·60 (12·55, 42·64)	19·61 (3·21, 3·60)	13·98 (–3·08, 31·04)
18 : 3n-3	Crude	0·31 (–1·00, 1·13)	–0·35(–1·11, 0·41)	–0·14 (–0·90, 0·61)	–0·28 (–1·29, 0·73)	0·63(–0·80, 2·06)
	Adjusted[a]	–0·31 (–1·35, 0·73)	–0·31(–1·09, 0·47)	–0·18 (–0·98, 0·61)	–0·22 (–1·17, 0·73)	0·31(–1·00, 1·62)
Tot. SFA	Crude	–6·87 (–11·50, –2·24)	–7·60 (–10·96, –4·25)	–7·61 (–10·88, –4·35)	–9·80 (–14·06, –5·55)	–14·08 (–19·85, –8·31)
	Adjusted[a]	–6·67 (–11·11, –2·23)	–8·13 (–11·80, –4·45)	–8·65 (–12·15, –5·17)	–11·37 (–15·58, –7·15)	–12·64 (–18·60 , –6·67)
Tot. PUFA	Crude	61·02 (34·98, 87·05)	46·05 (29·02, 63·09)	28·61 (17·13, 40·09)	28·68 (16·41, 40·94)	26·94 (11·62, 42·26)
	Adjusted[a]	57·78 (35·07, 80·50)	48·56 (33·79, 63·34)	28·35 (15·63, 41·08)	20·42 (8·44, 32·39)	22·98 (8·20, 37·76)
(c) Dairyscore						
14 : 0	Crude	1·54 (1·15, 1·91)	1· 37 (1·01, 1·72)	1·09 (0·76, 1·42)	1·10 (0·73, 1·47)	1·34 (0·80, 1·99)
	Adjusted[a]	1·33 (0·91, 0·18)	1·31 (0·95, 1·68)	1·10 (0·74, 1·46)	1·13 (0·73, 1·53)	1·29 (0·67, 1·92)
15 : 0	Crude	0·28 (0·19, 0·37)	0·30 (0·22, 0·37)	0·26 (0·19, 0·34)	0·29 (0·20, 0·39)	0·35 (0·21, 0·48)
	Adjusted[a]	0·28 (0·17, 0·38)	0·31 (0·25, 0·38)	0·29 (0·21, 0·36)	0·34 (0·25, 0·42)	0·38 (0·24, 0·52)
16 : 0	Crude	–0·87 (–2·48, 0·74)	–1·84 (–3·16, –0·52)	–1·47 (–2·73, –0·21)	–2·78 (–4·33, –1·23)	–3·80 (–5·80, –1·80)
	Adjusted[a]	–1·53 (–2·93, –0·12)	–3·03 (–4·33, –1·73)	–2·84 (–3·94, –1·73)	–3·44 (–4·98, –1·89)	–4·21 (– 6, –2·43)
18 : 0	Crude	0·50 (0· 28, 0·71)	0·49 (0· 29, 0·70)	0·42 (0·19, 0·65)	0·46 (0·16, 0·76)	0·67 (0·19, 1·15)
	Adjusted[a]	0·59 (0· 34, 0·83)	0·40 (0· 20, 0·60)	0·33 (0·11, 0·56)	0·34 (0·03, 0·64)	0·54 (0·08, 1·00)
16 : 1n-7	Crude	–0·33 (–2·01, 1·34)	–0·44 (–2·35, 1·46)	–2·85 (–5·00, –0·70)	–2·79 (–5·87, 0·27)	–4·74 (–10·09, 0·60)
	Adjusted[a]	–0·19 (–1·68, 1·30)	–1·10 (–2·79, 0·60)	–4·07 (–6·18, –1·96)	–2·39 (–5·53, 0·73)	–4·03 (–9·17, 1·11)
18 : 1	Crude	7·11 (2·68, 11·54)	4·69 (1·69, 7·68)	3·21 (0·12, 6·29)	5·34 (1·69, 9·00)	5·95 (0·17, 11·72)
	Adjusted[a]	5·48 (1·45, 9·51)	4·28 (1·03, 7·53)	3·77 (0·57, 6·98)	1·63 (–1·97, 5·23)	1·01 (–4·22, 6·24)
16 : 1n-7/16 : 0	Crude	–0·01 (–0·13, 0·15)	–0·04 (–0·11, 0·19)	–0·18 (–0·36, 0·00)	–0·26 (–0·52, 0·00)	–0·16 (–0·61, 0·28)
	Adjusted[a]	–0·04 (–0·10, 0·18)	–0·01 (–0·16, 0·14)	–0·24 (–0·42, –0·06)	–0·11 (–0·38, 0·14)	–0·30 (–0·74, 0·14)
Tot. SFA	Crude	1·40 (–0·70, 3·49)	0·12 (–1·33, 1·58)	0·08 (–1·38, 1·54)	0·04 (–1·82, 1·90)	–2·03 (–4·50, 0·44)
	Adjusted[a]	0·44 (–1·52, 2·41)	–0·46 (–2·17, 1·25)	– 1·38 (–2·88, 0·11)	– 1·39 (–3·34, 0·57)	–3·11 (–5·91, –0·32)
Tot. MUFA	Crude	2·68 (·2·42, 7·78)	3·20 (–0·95, 7·36)	2·09 (–2·61, 6·80)	1·64 (–7·12, 14·08)	3·48 (–7·12, 14·08)
	Adjusted[a]	1·26 (–4·09, 6·60)	2·60 (–2·21, 7·42)	0·54 (–3·89, 4·98)	0·38 (–5·63, 6·40)	–5·17 (–14·62, 4·28)
(d) Satscore						
14 : 0	Crude	0·80 (0·48, 1·12)	0·64 (0·37, 0·92)	0·38 (0·13, 0·64)	0·38 (0·13, 0·67)	0·65 (0·21, 1·10)
	Adjusted[a]	0·64 (0·35, 0·93)	0·57 (0·29, 0·86)	0·31 (0·05, 0·58)	0·36 (0·04, 0·68)	0·55 (0·06, 1·04)
15 : 0	Crude	0·10 (0·04, 0·17)	0·13 (0·07, 0·19)	0·13 (0·07, 0·18)	0·17 (0·09, 0·24)	0·16 (0·06, 0·27)
	Adjusted[a]	0·10 (0·03, 0·17)	0·13 (0·08, 0·19)	0·13 (0·07, 0·20)	0·19 (0·12, 0·26)	0·20 (0·09, 0·31)
16 : 0	Crude	0·08 (–1·29, 1·29)	–0·47 (–1·49, 0·53)	–0·69 (–1·64, 0·26)	–1·75 (–2·91, –0·58)	–2·26 (–3·83, –0·90)
	Adjusted[a]	–0·91 (–2·05, 0·23)	–2·13 (–3·11, –1·14)	–2·15 (–3·06, –1·24)	–2·76 (–3·98, –1·55)	–3·30 (–4·67, –1·93)

18 : 0	Crude	0·40 (0·24, 0·56)	0·46 (0·30, 0·61)	0·43 (0·26, 0·60)	0·49 (0·26, 0·73)	0·57 (0·19, 0·95)
	Adjusted[a]	0·39 (0·20, 0·57)	0·33 (0·18, 0·48)	0·25 (0·08, 0·43)	0·31 (0·08, 0·55)	0·45 (0·08, 0·82)
16 : 1n-7	Crude	–0·79 (–2·08, 0·49)	–1·52 (–2·95, –0·10)	–3·30 (–4·88, –1·73)	–2·89 (–5·24, –0·54)	–3·56 (–7·66, 0·53)
	Adjusted[a]	–0·31 (–1·47, 0·85)	–1·25 (–2·59, 0·09)	–3·33 (–4·92, –1·74)	–1·76 (–4·21, 0·68)	–3·30 (–7·42, 0·82)
18 : 1	Crude	5·10 (1·70, 8·50)	3·22 (0·97, 5·47)	2·21 (–0·17, 4·60)	4·56 (1·76, 7·37)	4·85 (0·43, 9·26)
	Adjusted[a]	3·35 (0·21, 6·49)	2·92 (0·35, 5·50)	2·33 (–0·80, 4·83)	1·15 (–2·97, 5·27)	–12·97 (–22·07, –3·87)
16 : 1n-7/16 : 0	Crude	–0·05 (–0·15, 0·06)	– 0·08 (–0·19, 0·04)	–0·25 (–0·38, 0·11)	–0·22 (–0·4 2, –0·02)	–0·22 (–0·57, 0·11)
	Adjusted[a]	–0·00 (–0·11, 0·10)	–0·04 (–0·15, 0·09)	–0·22 (–0·37, –0·08)	–0·09 (-0·29, 0·11)	–0·21 (–0·55, 0·14)
Tot. SFA	Crude	1·78 (0·20, 3·26)	0·30 (–0·80, 1·40)	0·21 (–1·14, 1·56)	1·23 (–3·04, 0·58)	1·23 (–3·04, 0·59)
	Adjusted[a]	0·24 (–1·24, 1·71)	–0·65 (–1·94, 0·64)	–1·33 (–2·51, –0·15)	–1·95 (–3·45, –0·45)	–3·48 (–5·67, –1·30)
Tot. MUFA	Crude	1·64 (–2·21, 5·49)	1·37 (–1·75, 4·50)	–0·06 (–3·57, 3·45)	–0·44 (–5·02, 4·14)	2·13 (–5·76, 10·03)
	Adjusted[a]	–0·01 (–3·97, –3·95)	0·44 (–3·20, 4·09)	–1·34 (–4·67, 2·00)	–0·93 (–5·46, 3·59)	–4·75 (–12·13, 2·64)
e) Meatscore						
14 : 0	Crude	–0·63 (–1·30, 0·03)	–0·79 (–1·38, –0·20)	–0·90 (–0·15, –0·32)	–0·94 (–1·59, –0·29)	–0·86 (–1·96, 0·24)
	Adjusted[a]	–0·94 (–1·61, –0·26)	–1·13 (–1·78, –0·48)	–1·31 (–1·88, –0·74)	–1·42 (–2·10, –0·74)	–1·34 (–2·40, –0·28)
15 : 0	Crude	–0·17 (–0·32, –0·02)	–0·20 (–0·32, –0·08)	–0·17 (–0·29, –0·05)	–0·09 (–0·25, 0·07)	–0·01 (–0·26, 0·23)
	Adjusted[a]	–0·20 (–0·35, –0·06)	–0·23 (–0·37, –0·10)	–0·22 (–0·35, –0·08)	–0·17 (–0·34, 0·02)	–0·15 (–0·38, 0·08)
16 : 0	Crude	3·56 (1·07, 6·04)	2·36 (0·13, 4·59)	2·04 (–0·13, 4·20)	0·18 (–2·39, 2·74)	–1·40 (–5·04, 2·23)
	Adjusted[a]	0·37 (–2·30, 3·04)	–1·73 (–4·04, 0·57)	–0·19 (–3·87, 0·10)	–4·31 (–7·01, –1·61)	–3·69 (–6·67, –0·72)
18 : 0	Crude	0·68 (0·31, 1·04)	0·85 (0·51, 1·18)	0·83 (–0·46, 1·20)	1·11 (0·60, 1·61)	0·82 (–0·00, 1·64)
	Adjusted[a]	0·46 (0·01, 0·91)	0·44 (0·11, 0·78)	0·36 (–0·03, 0·75)	0·54 (0·02, 1·06)	0·39 (– 0·48, 1·27)
16 : 1n-7	Crude	–2·53 (–5·30, 0·23)	–5·23 (–8·36, –2·1)	–7·34 (–10·91, –3·77)	–6·51 (–11·79, –1·23)	–8·30 (–17·85, 1·25)
	Adjusted[a]	–0·95 (–3·50, 1·60)	–2·76 (–5·70, 0·18)	–5·29 (–8·90, –1·67)	–2·83 (–8·12, 2·46)	–6·55 (–15·42, 2·33)
18 : 1	Crude	2·95 (–4·89, 10·79)	3·49 (–1·51, 8·48)	3·70 (–1·55, 8·95)	6·19 (0·12, 12·26)	4·00 (–5·16, 13·16)
	Adjusted[a]	4·91 (–1·68, 11·51)	3·61 (–2·24, 9·46)	1·52 (–4·10, 7·14)	2·89 (–3·55, 9·34)	2·97 (–6·26, 12·22)
16 : 1n-7/16 : 0	Crude	–0·27 (–0·52, –0·02)	–0·45 (–0·71, –0·19)	–0·66 (–0·95, –0·37)	–0·57 (–0·98, –0·15)	–0·70 (–1·42, 0·01)
	Adjusted[a]	–0·10 (–0·35, 0·14)	–0·17 (–0·43, –0·08)	–0·35 (–0·67, –0·03)	–0·16 (–0·61, 0·29)	– 0·24 (–1·03, 0·05)
Tot. SFA	Crude	3·75 (0·43, 7·08)	1·32 (–1·12, 3·76)	0·83 (–1·65, 3·30)	0·97 (–2·05, 3·98)	– 1·32 (–5·54, 2·90)
	Adjusted[a]	–0·68 (–3·97, 2·61)	–2·30 (–5·15, 0·54)	–2·84 (–5·50, –0·19)	–4·75 (–8·18, –1·32)	–7·63 (–12·34, –2·92)
Tot. MUFA	Crude	1·17 (–7·41, 9·76)	– 3·07 (–9·87, 3·73)	– 4·67 (–12·24, 2·90)	–4·47 (–14·62, 5·68)	3·14 (–13·86, 20·15)
	Adjusted[a]	0·02 (–8·94, 8·99)	–0·06 (–8·12, 8·00)	–5·18 (–13·46, 3·10)	–2·47 (–13·03, 8·09)	–3·75 (–20·06, 12·56)

To improve the readability of percentile differences presented, values were multiplied by 100. [a]Model adjusted for sex, physical activity, and smoking.

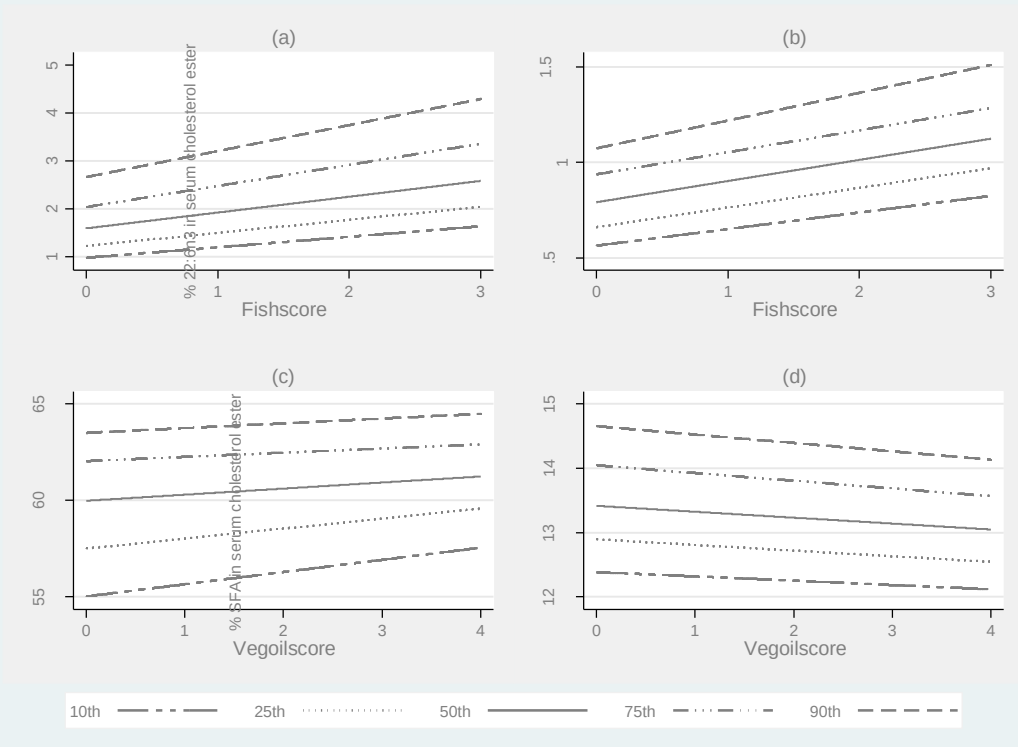

Fig. 12 (a) (b) (c) (d): The lines describe the predicted increase or decrease of serum cholesterol fatty acids proportion at the 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} fatty acid percentile, based on a model adjusted for sex, physical activity, and smoking status using the median of each covariate for each point's increase of the diet score. **(a)** Predicted increase of serum cholesterol 20:5 n-3 proportion for each point's increase of Fishscore; **(b)** Predicted increase of serum cholesterol 22:6 n-3 proportion for each point's increase of Fishscore; **(c)** Predicted increase of serum cholesterol total PUFA proportion for each point's increase of Vegoilscore; **(d)** Predicted decrease of serum cholesterol total SFA proportion for each point's increase of Vegoilscore.

Table 5: Hazard ratios (HR) and 95% confidence intervals (CI) for incidence of cardiovascular disease (CVD) and all-causes of mortality in relation to diet scores. Results based on participants of the cohort of 60-year-old men and women.

	Men		Women	
	Incident CVD	**All-cause mortality**	**Incident CVD**	**All-cause mortality**
Dietscore	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Fishscore cont[1]				
Crude	0.95 (0.81-1.10)	1.05 (0.92-1.21)	0.90 (0.74-1.09)	0.86 (0.72-1.03)
Adjusted[2]	0.96 (0.82-1.12)	1.10 (0.95-1.28)	1.02 (0.84-1.25)	0.95 (0.79 - 1.14)
Vegoilscore cont[1]				
Crude	0.99 (0.88 - 1.12)	0.87(0.78-0.97)	1.09 (0.93 - 1.27)	1.04 (0.90 - 1.19)
Adjusted[2]	1.03 (0.91 - 1.16)	0.93 (0.82 - 1.04)	1.12 (0.96 - 1.31)	1.06 (0.92 - 1.23)
Dairyscore cont[1]				
Crude	1.01 (0.96 - 1.06)	1.08 (1.08-1.14)	1.05 (0.98 - 1.12)	1.08 (1.02-1.15)
Adjusted[1]	0.97 (0.92 - 1.03)	1.03 (0.97 - 1.08)	1.00 (0.93 - 1.08)	1.02 (0.95 - 1.09)
Satscore cont[1]				
Crude	1.01 (0.97-1.05)	1.07 (1.03-1.11)	1.05 (0.99 - 1.11)	1.06 (1.01-1.11)
Adjusted[2]	0.98 (0.94 - 1.02)	1.03 (0.99-1.07)	1.01 (0.95 - 1.07)	1.01 (0.96 - 1.06)
Meatscore cont[1]				
Crude	1.03 (0.95 - 1.12)	1.11 (1.03 - 1.20)	1.11 (0.98 - 1.25)	1.04 (0.93 - 1.17)
Adjusted[2]	0.98 (0.90 - 1.07)	1.05 (0.97 - 1.14)	1.05 (0.92 - 1.19)	0.99 (0.88 - 1.11)

[1]diet score used as continuous variable [2]model adjusted for smoking, physical activity and education

Table 6: Hazard ratios (HR) 95% confidence intervals (CI) for incidence of cardiovascular disease (CVD) and all-cause mortality in relation to specific foods intake. Results based on participants of the cohort of 60-year-old men and women.

	Men		Women	
	Incident CVD	**All-cause mortality**	**Incident CVD**	**All-cause mortality**
Variables	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
<1/week lean **fish**	Ref	Ref	Ref	Ref
≥1/week	0.88 (0.70-1.11)	1.03 (0.82-1.29)	0.84 (0.62-1.14)	0.75 (0.57-0.98)
Adjusted[1]	0.85 (0.67-1.07)	1.06 (0.84-1.33)	1.00 (0.73-1.37)	0.85 (0.64-1.12)
<1/week fatty **fish**	Ref	Ref	Ref	Ref
≥1/week	1.00 (0.77-1.29)	1.10 (0.87-1.40)	0.96 (0.68-1.34)	0.88 (0.65-1.21)
Adjusted[1]	1.06 (0.81-1.39)	1.19 (0.93-1.53)	1.11 (0.79-1.56)	1.02 (0.74-1.40)
≤2 **slices of bread**/day	Ref.	Ref.	Ref.	Ref.
≥3/day	0.85 (0.68-1.07)	1.04 (0.83-1.29)	0.90 (0.67-1.20)	1.02 (0.78-1.33)
Adjusted[1]	0.88 (0.70-1.11)	1.13 (0.90-1.41)	0.92 (0.68-1.24)	1.08 (0.82-1.41)

Margarines (for sandwiches)	Ref.	Ref.	Ref.	Ref.
Butter	1.07 (0.84-1.37)	1.36 (1.09 - 1.71)	0.85 (0.61-1.19)	1.05 (0.79-1.40)
Adjusted[1]	1.05 (0.81-1.35)	1.28 (1.01-1.62)	0.83 (0.59-1.16)	1.05 (0.79-1.41)
<10g **Butter/Margarine**	Ref.	Ref.	Ref.	Ref.
≥10g Butter/Margarine	1.14 (0.87-1.50)	1.85 (1.47-2.34)	1.72 (1.18-2.49)	1.11 (0.77-1.61)
Adjusted[1]	1.04 (0.78-1.38)	1.57(1.23-2.02)	1.49 (1.02-2.20)	0.98 (0.67-1.43)
≤1 **Cheese slices**	Ref.	Ref.	Ref.	Ref.
≥2	1.02 (0.81-1.30)	1.11 (0.88-1.39)	0.87 (0.65-1.16)	1.05 (0.80-1.38)
Adjusted[1]	0.98 (0.76-1.24)	1.00 (0.79-1.26)	0.86 (0.64-1.16)	0.94 (0.72-1.24)
Light milkfat- or no milk products	Ref.	Ref.	Ref.	Ref.
Standard milk (3%)	1.03 (0.82-1.29)	1.10 (0.88-1.36)	1.05 (0.79-1.40)	1.31 (1.01-1.71)
Adjusted[1]	0.97 (0.77-1.23)	0.95 (0.77-1.19)	0.96 (0.71-1.29)	1.17 (0.90-1.53)
<1times/week **sausages/bacon**	Ref.	Ref.	Ref.	Ref.
≥1times/week	1.07 (0.85-1.34)	1.29 (1.04-1.60)	1.30 (0.96-1.77)	1.21 (0.91-1.60)
Adjusted[1]	1.01 (0.80-1.28)	1.18 (0.94-1.47)	1.15 (0.83-1.57)	1.11 (0.83-1.48)
No meatfat	Ref.	Ref.	Ref.	Ref.
Meatfat	1.09 (0.87-1.36)	1.07 (0.86-1.32)	1.23 (0.89-1.71)	1.20 (0.89-1.61)
Adjusted[1]	1.03 (0.82-1.30)	0.97 (0.78-1.21)	1.15 (0.82-1.60)	1.11 (0.81-1.49)
≤1times/week **Oily potatoes**[2]	Ref	Ref.	Ref.	Ref.
≥2times/week	1.03 (0.74-1.42)	1.27 (0.95-1.69)	2.00 (1.11-3.59)	1.51 (0.85-2.71)
Adjusted[1]	1.02 (0.73-1.42)	1.14 (0.85-1.54)	2.00 (1.11-3.60)	1.49 (0.84-2.68)
<4 **eggs** /week	Ref.	Ref.	Ref.	Ref.
≥4times/week	0.98 (0.69-1.38)	1.66 (1.27-2.18)	0.97 (0.59-1.60)	1.03 (0.66-1.60)
Adjusted[1]	0.90 (0.63-1.30)	1.53 (1.15-2.02)	0.84 (0.50-1.41)	0.90 (0.57-1.41)
Panfry margarines or oil	Ref.	Ref.	Ref.	Ref.
Butter	1.09 (0.82-1.46)	1.31 (1.00-1.70)	0.96 (0.65-1.40)	0.84 (0.58-1.22)
Adjusted[1]	1.01 (0.75-1.37)	1.19 (0.91-1.57)	0.89 (0.60-1.32)	0.79 (0.54-1.15)
≤1times/week **Cream**[3]	Ref.	Ref.	Ref.	Ref.
≥2times/week	0.75 (0.51-1.10)	1.12 (0.81-1.54)	1.09 (0.69-1.74)	0.75 (0.46-1.24)
Adjusted[1]	0.69 (0.47-1.03)	0.99 (0.71-1.38)	1.04 (0.64-1.67)	0.68 (0.41-1.13)
≤1times/week **Desserts**[4]	Ref.	Ref.	Ref.	Ref.
≥2times/week	0.88 (0.64-1.21)	0.96 (0.71-1.29)	0.88 (0.58-1.34)	0.97 (0.66-1.40)
Adjusted[1]	0.91 (0.65-1.26)	1.03 (0.76-1.40)	1.00 (0.65-1.51)	1.01 (0.69 - 1.48)

[1] Adjusted for smoking, physical activity and education [2]including pan-fried potato, French fries and potatoes au gratin; [3]including sour cream, crème fraiche and sauces; [4]including potato chips, chocolate, croissants, Danish pastries, cakes and cheeses.

Table 7: Hazard ratios (HR) 95% confidence intervals (CI) for incidence of cardiovascular disease (CVD) and all-cause mortality in relation to self-reported intake of vegetables and fruits. Results based on participants of the cohort of 60-year-old men and women.

Diet Variables	**Incident CVD**	**All-cause mortality**	**Incident CVD**	**All-cause mortality**

	Men		Women	
	HR (95% CI)	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
<1/day **fruits/berries**	Ref	Ref	Ref	Ref
≥1times/day	0.73 (0.58-0.91)	0.63 (0.50-0.78)	0.66 (0.48-0.90)	0.62 (0.47-0.82)
Adjusted[1]	0.83(0.66-1.05)	0.75 (0.60-0.94)	0.80 (0.58-1.11)	0.83 (0.62-1.11)
<1/day **vegetables[2]/roots/tubers**	Ref	Ref	Ref	Ref
≥1times/day	0.83 (0.66-1.04)	0.70 (0.57-0.87)	0.83 (0.61-1.13)	0.73 (0.55-0.96)
Adjusted[1]	0.94 (0.74-1.19)	0.81(0.64-1.01)	0.98 (0.71-1.35)	0.90 (0.67-1.19)

[1]Model adjusted for education, smoking and physical activity; [2] except for lettuce, cucumber and tomatoes

Table 8: Hazard ratios and 95% of confidence interval for incident of cardiovascular disease (CVD) and all-cause mortality in relation to serum PUFA. Results based on participants of the cohort of 60-year-old men and women.

				Men (n=1733)	Women (n=1938)
Incident CVD	Events (person-years)			304 (22,274)	180 (26,469)
	Hazard ratio (95% CI)[1]	EPA	Crude	0.99 (0.88, 1.12)	0.78 (0.64, 0.96)
			Adjusted[2]	1.02 (0.90, 1.16)	0.79 (0.64, 0.97)
		DHA	Crude	0.93 (0.83, 1.05)	0.70 (0.59, 0.84)
			Adjusted	0.96 (0.85, 1.09)	0.74 (0.61, 0.89)
		LA	Crude	0.88 (0.78, 0.99)	0.94 (0.80, 1.09)
			Adjusted[1]	0.90 (0.79, 1.03)	0.99 (0.84, 1.18)
		ALA	Crude	0.99 (0.88, 1.11)	1.15 (1.01, 1.31)
			Adjusted[1]	1.02 (0.91, 1.14)	1.16 (1.02, 1.32)
Total mortality	Deaths (person-years)			265 (23,917)	191 (27,418)
	Hazard ratio (95% CI)	EPA	Crude	0.79 (0.69, 0.91)	0.78 (0.65, 0.94)
			Adjusted[1]	0.82 (0.71, 0.95)	0.79 (0.65, 0.96)
		DHA	Crude	0.78 (0.68, 0.89)	0.75 (0.63, 0.89)
			Adjusted[1]	0.82 (0.71, 0.94)	0.78 (0.66, 0.93)
		LA	Crude	0.73 (0.65, 0.83)	0.93 (0.80, 1.07)
			Adjusted[1]	0.73 (0.64, 0.83)	0.95 (0.81, 1.12)
		ALA	Crude	1.10 (0.98, 1.23)	0.99 (0.87, 1.13)
			Adjusted[1]	1.10 (0.99, 1.22)	0.98 (0.86, 1.12)

ALA, α-linolenic acid; EPA, ;DHA, ; LA, ; CVD, cardiovascular disease;
[1]Per 1 SD increment: LA (women, 4.09%; men, 4.20%; total, 4.14%), ALA (women, 0.20%; men, 0.20%; total, 0.20%), EPA (women, 1.02%; men, 0.95%; total, 0.99%), DHA (women, 0.24%; men, 0.25%; total, 0.25%).
[2]Adjusted for sex (only in analyses of the total study population), BMI, smoking, physical activity, education, alcohol intake, diabetes, hypertension, hypercholesterolemia.

DISCUSSION

Paper I

Relation self-reported dietary fat and serum cholesterol fatty acids

In this large population-based cohort of 60-year-old Swedish men and women, high intake of lean and fatty fish (Fishscore), was associated with high serum proportions of EPA and DHA. Moreover, a high intake of vegetable oils and margarines (Vegoilscore) was associated positively to serum proportions of total PUFA and negatively to serum proportions of total SFA. Intake of fats from dairy products (Dairyscore) and intake of saturated fats in general (Satscore) was associated slightly with specific SFA, but intake of processed meat and eggs (Meatscore) was not.

As in previous studies, a clear relation between fish intake and EPA and DHA was noted[50,54,56,62,70,102,103]. Only in a few investigations, all of them with a sample size less than 1,320 subjects, the observed associations were less clear [49,103-105]. To the best of our knowledge, our study is the first study on the relation between fish intake and fatty acids performed in a Nordic general population of more than 4,000 subjects. Our results suggest that for 60-year-old individuals, regardless of their individual fatty acid profile in terms of proportion for EPA and DHA, the amount of fish intake may affect serum levels of EPA and DHA, fatty acids that are associated with CVD risk and other chronic diseases[106]. We found that the association between Fishscore and EPA and DHA was more pronounced in men than in women. The results of an earlier study on a cohort of 4,949 British 64-year-old men and women, however, showed the opposite[107]. In both studies, the frequencies of fish intake were similar in men and women, and the overall proportions of serum EPA and DHA were slightly higher in women than in men. The discrepancy of the results might be related to differences in cooking methods or different distributions of unmeasured factors in the two populations that affect the biological response to fish intake.

Our findings of an association between Vegoilscore and increased total PUFA and decreased total SFA in serum are in line with the results reported from a Finnish study that included 1,033 men and which used a similar approach as we did to categorize fat intake. The Finnish study also observed that spread and cooking fats with high content of PUFA were negatively associated with myristic acid and positively associated with linoleic acid measured in erythrocytes[56]. Our finding that there is no clear association between Vegoilscore and linoleic acids in the upper percentile of the linoleic acid distribution after

adjustments needs confirmation. The Finnish study observed a positive association between spread and cooking fats and alfa-linolenic acid, but our study found no such association. The different results may relate to the fact that different populations may use different amounts of spread and cooking fats (i.e., alfa-linolenic acid intake may differ). Studies that considered margarines and vegetable oils separately have shown positive associations between margarines and linoleic acids[63] but a negative association between vegetable oils and alfa-linolenic acid[62].

The slight positive associations between Dairyscore and myristic acid and pentadecanoic acid found in our study are consistent with findings from previous investigations performed in Scandinavian countries[56,57,59,60,102], and in three large cohorts in Europe and Australia (each including around 4,000 subjects)[62,63,69]. Our finding of a slight positive association between Dairyscore and serum proportion of stearic acid disagrees with the findings reported in two large European cohort studies, where the associations tended to be reversed[62,69].

Our results of slightly higher serum proportion of myristic acid and stearic acid in relation to Satscore agree with results from two studies performed in Finland[54,56]. One of these studies, which included 84 men and women, observed an association with serum proportion of palmitic acid as well[54]. The other study, including 1,033 men, studied only myristic acid (measured in erythrocytes) and found an association[56]. No previous study has investigated the intake of saturated fat in general in relation to pentadecanoic acid, a relation that our study found.

In our study the lack of association observed between Meatscore and specific serum fatty acids might be related to the endogenous synthesis of the even-numbered carbon chain of SFA and MUFA.

Project II

Relation self-reported dietary fat and risk of incidence of CVD and all-causes mortality

In this cohort of 60 years old men and women, high self-reported intake of specific group of fats - fats from food rich in saturated, fats from dairy, fish, animal products and vegetables oils and margarine - was not predictive of incidence of CVD and all-causes mortality. However, with regard to sex-specific relation single specific foods rich in fat were better predictors of incidence of CVD, including MI, stoke, and total mortality. Consumption of more than 10g/day of butter and margarine increase CVD incidence in women and all-causes mortality in men. Intake of more than 2 times per week of “oily” potato (pan-fried potatoes, French fries or potato au gratin) increased CVD incidence in women where consumption

of spread butter (instead of margarine) and intake of eggs more than 4 times per week were associated with increase total mortality in men. Daily intake of fruits but not vegetables was protective on all-causes mortality in only men.

Our finding of no association of self-reported intake of food rich in saturated fats (Satscore) and incidence of CVD, including stroke and MI, and all-causes mortality in both men and women is generally consistent with findings reported in other epidemiological studies[78,80,108,109]. However, our findings are in contrast with those earlier prospective studies reporting clear increased or decreased risk of CVD and total mortality in relation to intake of saturated fat intake[78]. Compare to our investigation, these different results might be related to the fact that most of these studies found an association between saturated fat intake and CVD and all-causes mortality in specific subset of the study populations with a small number of cases -i.e younger versus older, women v men and for some CHD endpoint-[78,80].

Concerning our findings of no clear association of saturated fat from dairy (Dairyscore) and meat product (Meatscore) and incidence of CVD and all-causes mortality, earlier epidemiological studies reported no association where some other studies reported a protection or harmful effect on CVD and total mortality[83,84]. However, these different results might be referred to several factors: wide spectrum of dairy and meat products available on the market with diverse nutritional characteristics, unrepresentative population and different nutritional methods used for the assessment, amount of fats diverse across populations.

Our no significant protection of fats from fish and CVD and all-causes mortality in both men and women are confirmed in some previous large prospective studies but in contrast with results from meta-analysis and other epidemiological studies across different countries[93,110,111]. These discrepancies might be explained by differences across populations in cooking methods fish, kind of fish, presence of contaminants as mercury or PBC that might increase the incidence of CVD and mortality. These differences might also explained the reason because in other Nordic studies a lack of clear protection of fish intake and incidence of CVD and all-causes mortality have been shown[112-114].

Our results of no clear association between high intake of vegetables oils, margarines and CVD and all-causes mortality is consistence with some epidemiological studies but not with others[115,116]. Differences in the results might be related to different source of PUFA across populations as well foods that goes with these vegetables oils and margarine.

Only few studies have considered the association between fat intake from specific foods intake and incidence of CVD and all-causes mortality, considering also sex-specific relations[82]. Our finding about association between a specific consumption of more than 10g/day of butter and margarine and CVD in women is in line to some extent with one previous large Swedish cohort study of only women[117]. As in our study, in this investigation the authors found a specific association between butter used to spread (and not for cooking) and MI in women. Opposite to our results of increased total mortality in relation to intake of butter, in a large Dutch cohort of men and women no association were noted in men for intake of butter and total mortality where a slight increase risk of CHD was noted for women[108].

To our knowledge, no previous studies have reported a specific association between "oily potato" including French fries or potato au gratin and incidence of CVD. However, evidences from studies investigating the association between fried food and cream and CVD (and total mortality) are controversial reporting no association and increased risk[83,118] .

Several studies have investigated the relation between eggs intake and incidence of CVD, MI and stroke, and CVD mortality whereas the association with all-causes mortality is limited to few studies[86-89]. Our finding of increased total mortality and intake of eggs more than 4 times per week in only men agree with the results found in a large cohort study observing physician men (21,327) [87]. But in two other large cohorts of men no association between intake of 1 eggs per day and MI, CVD mortality and all-causes mortality have been reported[88,89]. However, these results should be interpret with caution and need further investigations to understand how is the relation with specific cause of mortality.

Our results of a protective association of daily fruit intake for total mortality in men are generally consistent with other previous studies[94,119,120]. As in our study, in the Kuopio Ischemic Heart Disease risk factors (KIHD) study, the middle-aged Finnish men were noted to have reduced risk of mortality with daily fruits, berries and vegetables[120]. On the other hand, our finding about no clear protection for daily intake of vegetables and CVD and all-causes mortality might be depend on the process and the method of cooking vegetables that it might affect healthy components of vegetables - antioxidant and vitamins-. This might be strength by studies that found a lower risk in CVD and mortality in relation to intake of raw vegetables compared to the cooked[121].

Findings from this cohort study suggest that considering fats, and in specific saturated fats as heterogeneous group might hide some specific associations between diet and incidence of CVD and all-causes mortality.

If the sex-specific associations between single questions were to be causal we can speculate that it might have been for sex-specific effects of some protective or harmful components in butter, oily potatoes, fruits on the potential intermediate factors (e.g. blood lipids, fatty acids, glucose, hormones) involved in the atherosclerotic process of CVD, and in the induction of all-causes mortality[122]. For instance, as shown in our Project III, sex specific association between serum PUFA and CVD and total mortality was noted. Moreover, our results of an association of single specific food and CVD in women and total mortality in men might suggest that maybe similar foods might have a non-fatal prediction for women and fatal for men. Also the interactions between nutritional components, environmental factors and genes may explain the gender differences[123].

Dairy products are important contributors to saturated fatty acids in Sweden[38,41]. Butter, cream (potatoes au gratin) are rich in saturated fatty acids, fried food (French fries) in trans fatty acids and margarine are important source of PUFA, linoleic and alfa linolenic acid nowadays (where 15-20 years ago were rich in trans fatty acids). Generally, there is evidence that saturated and trans fatty acids are related to CVD and mortality intake through an increase in LDL cholesterol where LA and ALA might reduce LDL cholesterol, blood pressure and promote insulin sensitivity[96,97]. In our study we did not find a significant association between reported quantity of butter or oily potatoes with cholesterol lipids; at the baseline, among those reporting a high intake of butter we noted a modification in triglycerides in women (data not shown). Moreover, we can not exclude that these associations regarding spread butter and margarine, "oily potato" and CVD might be confounded by intake of carbohydrate source as bread for spread butter and margarine and potato for the oily potato (that it was not possible to calculate).

Eggs are the major source of cholesterol but even other nutrient, mineral and folate, B vitamins, proteins[124]. Previous studies have reported an increased mortality in diabetic subjects in relation to intake of eggs[86]. However, adjustment for metabolic factors including diabetes does not explain these

associations. We might think that high eggs intake affect metabolic factors that might enhance the induction of tumors more than the atherosclerosis process[125].

Fruits and vegetable are composed by different protective components such as potassium, folate, vitamins, fibers and other phenolic compounds[126]. These nutrients act through a variety of potential mechanisms, such as reduction of antioxidant stress, improvement of lipoprotein profile, lowering of blood pressure, increase of insulin sensitivity and improvement of hemostasis regulation[127,128].

Still our findings suggest that the associations between diet and CVD are not independent. But as shown for the most our results the association is dependent by lifestyle factors -smoking and physical activity- and education. Some previous study have showed the importance to change lifestyle –smoke cessation or increasing physical activity- is better prevention than only have an healthy diet based on reduced saturated fat intake.

Project III

Relation serum cholesterol PUFA and risk of incidence of CVD and all-causes mortality

In our study, we found a sex-specific association between selected serum PUFA and incidence of CVD and total mortality. N-3 serum EPA and DHA were associated with decreased risk of incidence of CVD in women and mortality in both gender. Serum ALA was associated with increased risk of incidence of CVD in only women where serum LA was associated to decreased risk total mortality in men. To our knowledge, this is one of the largest population-based prospective cohort study with mortality data and equal proportions of men and women, addressing incidence of CVD and all-causes mortality in relation to selected serum FA.

The observed discrepancies between men and women regarding protective effects of EPA and DHA on CVD, are confirmed in a recent meta-analysis where the authors have reported no associations between n-3 PUFA and incidence CVD in men[90]. This sex-specific differences in the investigated association might not explained by differences in fish intake, as found in Project I in the same population. However, a greater proportion of serum DHA among women have been found in our study and previous studies have reported higher DHA concentrations in women compared to men independently by intake[54,129]. DHA may have sex-specific effects on the development of CVD, (e.g., platelet aggregation have ben shown to be

inhibited by DHA supplementation in women compared to men)[130,131]. Our findings of protection of serum EPA and DHA and all-cause mortality in both men and women is in line with what reported in previous studies [132].

The moderate increased of CVD incidence in women in relation to high serum concentration of ALA is difficult to interpret. In a randomized trial- Lyon Diet Heart Study- the authors have suggested protective effects of ALA intake when consumed as margarines on CVD [133]. However, epidemiological evidence of CHD-protective effects of circulating ALA is somewhat inconclusive[134,135] [136]. Some studies reported no clear association where others have reported cardioprotective effects of ALA[134]. Moreover, no clear assocciation between serum ALA and intake of vegetable oils and margarine (major dietary source of ALA) were observed in the Project I. As previously suggested, circulating ALA concentration is affected by factors not related to ALA intake and a high proportion of circulating ALA may be an indicator of poor conversion of ALA to longer chained n-3 PUFA[135]. In our study, ALA was only associated with an increased CVD risk among women with the lower EPA and DHA concentrations (data not shown). In addition, the mean ALA concentrations in women (0.90 %) and men (0.87%) in the present study were above previously reported mean or median serum CE ALA concentrations in free-living individuals (0.40-0.84%) [90,136-138].

In line with our results of seum EPA and DHA and total mortality, a recent investigation in elderly American men and women reported that plasma phospholipid long-chain n-3 PUFA were inversely associated with total mortality[106].

Our results of a protection of serum LA and total mortality are consistent with previous other studies where plasma phospholipid LA was inversely associated and total and CVD mortality among middle-aged/older men in Northern Europe[136,139]. Such data are well in line with the robust LDL-cholesterol lowering effects when compared with saturated fats, and is a likely contributing mechanism behind the inverse associations between LA and CHD and CVD mortality observed in several cohorts [90,136,140].

Strengths

Strength of the present thesis is the fact that it is based on a large, representative, and thoroughly characterized sample of 60-year-old men and women residing in Stockholm County, Sweden.

The study's age homogeneity means we may exclude bias related to age.

The studies included in this thesis is one of the largest globally to relate self-reported fat intake to fatty acids in serum cholesterol esters (Project I), and to relate serum PUFA-reflecting the corresponding dietary intake-to incidence of CVD and all-causes mortality(Project III).

Another strength is related to our use of quantile regression modelling of associations that may be give a more detailed picture of the distribution of the fatty acids. As it may identify in our results, the association may vary across percentiles of the fatty acid distribution (Project I)

Long follow up (14.5y) and the use of Swedish register for the determination of incident of CVD and all-causes mortality might reduce possible non differential misclassification of the outcome[141]. (Project II-III)

Limitations

A potential limitation of our study is the fact that the information about diet was gathered using a self-reported questionnaire that included relatively crude questions about dietary intake. We could not fully assess the exact fat intake from our dietary questionnaires as the questions do not specify the serving size and allow no detailed calculation of nutrients and energy intake. For this reason, some observed associations between diet scores and specific serum cholesterol fatty acids may be diluted, assuming that intakes of different dietary items are equally underreported and overreported[142-145]. In addition, results based on questionnaires composed of simple questions such as ours, as opposed to more detailed nutritional information that food frequency questionnaires offer, require less exact recall and are easier to fill out accurately, perhaps making it more subject to smaller measurement error (Project I-II). Another study limitation was that serum cholesterol fatty acids were expressed as percentages of the total amount and not as absolute values. An increase in the proportion may not be relevant as the corresponding absolute increase. Furthermore, fatty acid proportions are inherently an imperfect reflection of dietary intake fat because to some extent the amount depends on endogenous synthesized SFA and MUFA. Moreover, the chosen method of measurement does not include measurements of trans fatty acids. The long-term storage of our sample before analysis may also be a weakness of the present study, although the samples were not previously defrosted and it has been shown that storage for 12 years at -80°C does not change the composition of the polyunsaturated fatty acids[146,147] (Project I, III).

Dietary intake and serum fatty acids were measured only at baseline (14.5 years earlier) without repeated measurements (Paper I-III). Thus, dietary habits and consequently serum fatty acids might be changed

over the follow-up period and affect the true estimates. However, we might speculate that elderly people are less prone to change their habits and in particular dietary habits (Paper II). Although we adjusted for several possible confounding and intermediate factors, we can not exclude possible residual confounding from other possible factors (Project I-III). We might not exclude competitive risk from other disease (Project II-III).

CONCLUSIONS

In this PhD thesis the associations between diet and in specific dietary fats and CVD (and all-causes mortality) have been investigated. The results from this large Swedish cohort of 60 years old men and women suggested that:

- independently from sex, physical activity, and smoking status, fats from fish intake strongly associate with serum cholesterol fatty acids EPA and DHA, whereas intake of margarines and vegetable oils associate with PUFA. The intake of fats from foods containing saturated fat in general, specifically dairy products, associates only slightly with myristic acid, pentadecanoic acid, and stearic acids, and intake of processed meat, visible fat and eggs does not show any relation with serum cholesterol fatty acids.

- independently from physical activity, smoking status and education, the association between self-reported dietary intake and incidence of CVD and all-causes of mortality is sex- and food specific. In women excess intake of spread butter or margarine and oily potatoes may have more negative effects on incidence of CVD where in men high intake of butter, eggs are harmful whereas intake of fruits is protective for all-causes mortality. In general, no association between fats from dairy, meat, fish product and vegetable oils and margarine and CVD and all-causes mortality have been shown in both men and women.

- associations between specific serum PUFA and incidence of CVD are to some extent sex-specific. Serum EPA, DHA are protective for CVD incidence in women only and for all-causes mortality in both sex. ALA has a negative effect on CVD incidence in women where LA is protective for all-causes mortality in men.

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APPENDIX

Table 1: Age Standardized death rates from CHD, adults aged under 65, by sex,1980 to 2010,Europe (deaths per 100,000)

Men	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Albania								36	37	42			38	34	30	35	42	36	37	40	40	39	41	45	48							
Armenia		104	108			107	113	100	119	127	137	143	145	145	130	143	134	125	120	123	115	114	124	120			115		120	117	
Austria	83	85	85	84	79	77	74	73	67	64	61	63	61	58	58	57	55	55	51	48	47	43	38	33	30	30	30	30	27	28	29
Azerbaijan		151	153			152	150	161	171	161	188	209	197	219	191	203	199	190	179	173	175	158	155	156	139			55			
Belarus		137	139			153	136	141	137	146	150	159	164	198	201	216		207	215	219	207	222	233	234				199	207	213	
Belgium	77	73	73	70	65	62	57	52	47	43	40	38	38	40	37	38	37	36	34	34					27	27					
Bosnia and Herzegovina						67	70	71	73	70	70	73																			
Bulgaria	77	71	78	80	83	83	84	83	83	85	89	91	91	105	100	105	94	101	95	87	84	83	84	82	80	81	74	70	64		
Croatia						70	69	66	65	63	65	70	68	68	65	65	68	73	74	69	65	65	62	65	57	58	56	52	55	53	
Cyprus																				11	10				40	41	41	40	41	40	
Czech Republic							143	138	135	135	143	132	127	116	109	103	97	87	81	78	73	68	65	60	58	55	54	54	51	49	
Denmark															55	51	43	41	38	35	31	30	26	26	24	20	21				
Estonia		170	158			175	171	163	156	164	174	173	179	203	214	195	161	140	167	137	137	136	136	126	123	104	102	91	85	75	
Finland								126	121	118	110	102	96	87	82	78	74	68	65	63	65	54	52	54	51	48	46	44	41	39	
France	39	38	38	36	37	37	36	33	32	29	28	27	26	25	24	23	23	22	22	21	21	20	19	19	17	17	16	16	15		
Georgia		141	132			128	120	134	153	150	150	161	174		167	176	152	145	141	132	132	130				73	62	60		48	
Germany											61	62	60	58	55	53	50	47	45	42	39	38	36	35	33	32	31	30	28	27	26
Greece	54	52	55	54	53	56	53	56	54	53	53	52	52	50	50	51	52	54	53	54	51	53	50	52	55	50	49	52	47	48	
Hungary	130	141	143	141	141	139	140	136	135	133	136	139	141	143	131	132	123	117	118	119	108	99	94	97	97	105	97	94	87	88	
Iceland		117	97	137	107	100	78	83	87	57	60	63	51	64	62	51	42	45	34	48	39	31	34	33	39	30	21	29	26	21	
Ireland	142	140	136	143	135	132	127	126	113	110	103	101	88	94	86	83	78	71	71	62	60	53	52	46	44	39	35	40	34	34	
Israel	89	89	90	79	77	69	76	71	61	53	50	52	51	47	44	43	33	31	34	30	28	27	25	21	20	19	19	20	18		
Italy	62	60	58	58	54	53	51	49	45	44	41	41	39	38	36	34	32	31	31	28	27	25	24	24			21	19	19		
Kazakhstan		145	144			146	129	135	132	134	143	143	150	178	187	215	220	207	209	194	207	200	204	202	188	193	182	176	157	126	
Kyrgyzstan		111	112			103	98	107	105	105	103	104	109	135	158	152	153	136	132	119	123	127	124	130	128	127	139	136	121	144	
Latvia	187	184	169	180	187	191	159	169	166	172	191	191	225	264	285	246	194	174	177	156	156	164	162	148	145	156	161	157	137	129	
Lithuania		126	133			150	137	145	140	149	167	168	181	198	195	179	155	131	122	125	116	130	126	128	132	146	145	142	134	116	
Luxembourg	85	93	111	86	91	78	74	73	58	56	54	47	52	51	48	47	54	37	36	29	35	33	35	28	31	28	36	22	29		
Malta	130	107	108	117	88	78	92	80	91	77	72	57	71	66	58	49	46	53	54	50	44	46	44	42	38	45	36	32	40	26	32
Netherlands	92	89	86	83	80	81	78	71	66	64	58	55	50	48	46	44	43	39	38	35	34	28	27	28	22	22	20	18	17	16	15
Norway							103	97	94	85	82	73	72	62	57	59	49	47	47	40	35	36	32	27	27	27	25	24	22	21	
Poland	93	88	90	89	97	102	107	110	106	112	112	116	109	99	90	88	85			85	78	72	66	63	61	57	56	54	51	46	
Portugal	48	45	40	41	45	41	39	40	41	37	38	41	38	39	33	33	33	30	31	29	26	26	29	29				19	18	16	
Republic of Moldova		123	127			129	112	108	97	95	108	113	105	116	140	148	136	136	120	139	134	124	126	130	132	146	134	130	125	128	138
Romania	62	67	66	72	72	76	76	80	76	75	85	91	102	111	110	113	117	115	106	100	96	97	97	96	90	86	86	82	81	78	75
Russian Federation	169	163	163	167	173	162	146	150	147	152	161	161	178	231	267	239	214	192	188	208	224	232	244	251	243	242	215	198	197	186	
San Marino																0	18	0	0	17	8					7					
Serbia																				69	64	69	63	62	64	61	66	66	60	59	54	
Slovakia													129	122	122	120	115	108	97	96	92	86	85	79	76	74	72	78	78	78	
Slovenia						71	68	63	55	49	54	57	59	62	54	46	42	49	47	43	40	42	35	39	34	33	30	31	33	29	
Spain	44	43	42	42	42	41	41	39	38	35	35	36	36	34	33	33	34	32	32	31	30	28	27	27	25	24	24	23	22		
Sweden								78	75	65	63	61	55	54	51	50	44	43	39	38	37	36	33	31	29	28	26	27	25	23	23
Switzerland																35	29	31	30	29	26	23	23	22	20	21	20	20			
TFYR Macedonia												68	76	72	81	70	75	70	73	70	71	72	70	62							
Tajikistan		99	91			81	80	85	86	93	90	89	89	107	107	114	98	83	84	90	87	79	85	85	86						
Turkmenistan		148	143			145	148	151	156	180	171	171	166	220	199	195	203	189	175												
Ukraine		149	150			145	125	129	123	128	136	147	160	175	190	208	207	201	186	198	208	205	216	218	225	234	219		217	186	
United Kingdom	143	137	132	132	128	126	122	117	110	102	97	93	88	86	76	74	69	65	62	58		52	49	47	44	41	39	37	35	33	
Uzbekistan		124	123			119	113	108	119	122	125	124	125	139	142	141	141	121	126	121	122	115	129	127	118	121					

Women	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Albania								10	11	10			12	8	11	11	12	12	12	13	15	13	15	17	16							
Armenia		33	35			32	37	43	33	35	38	37	42	41	34	38	35	34	32	30	28	28	29	26			30		26	27	
Austria	20	18	18	19	17	17	16	16	14	14	14	15	14	16	13	14	15	13	13	14	10	11	10	8	7	7	7	6	6	7	6
Azerbaijan		52	51			53	58	60	62	59	71	78	83	90	71	73	75	70	66	65	66	59	60	56	44			18			
Belarus		42	41			43	37	40	36	36	39	39	42	51	50	55		55	55	59	54	59	61	59				46	49	48	
Belgium	18	17	16	16	16	14	13	14	11	10	9	9	9	10	10	10	8	9	9	8					7	7					
Bosnia and Herzegovina						22	25	24	26	23	23	24																			
Bulgaria	22	19	22	22	24	24	22	20	22	22	24	25	25	27	24	26	25	26	25	24	23	22	24	22	21	18	18	15	15		
Croatia						15	16	17	17	16	15	18	19	18	16	16	17	17	17	16	17	16	15	15	14	14	12	11	11	11	
Cyprus																				2	2				7	8	8	7	9	5	
Czech Republic							35	34	31	33	34	33	30	29	28	28	25	22	20	19	18	17	16	16	14	13	12	13	12	11	
Denmark															14	16	13	11	10	9	9	9	7	7	6	6	6				
Estonia		39	34			40	39	35	35	37	39	36	40	42	48	44	36	37	35	32	30	28	28	28	22	20	19	15	16	13	
Finland								25	22	19	18	16	15	14	13	12	11	10	12	10	11	9	9	8	9	7	7	7	7	6	
France	7	6	7	6	6	6	6	6	5	5	5	5	5	4	4	4	4	4	4	4	3	4	3	3	3	3	3	3	3		
Georgia		48	46			47	44	47	52	51	50	53	59		57	55	47	49	43	43	41	41				22	14	14		13	
Germany											14	15	14	14	13	13	12	12	11	10	9	9	9	8	8	8	8	7	7	6	6
Greece	10	11	11	12	11	11	10	12	11	11	10	10	10	10	10	11	10	11	10	11	9	10	10	11	11	10	10	11	10	10	
Hungary	35	39	37	38	37	40	38	37	37	37	38	38	39	40	38	37	34	33	34	33	30	29	28	27	28	28	25	24	24	23	
Iceland		13	23	20	22	19	12	13	23	13	18	9	13	8	15	7	8	8	7	15	14	9	13	11	6	3	4	4	3	1	
Ireland	41	38	40	37	39	35	35	34	31	30	27	21	24	24	21	21	17	19	17	14	12	11	11	10	9	9	8	9	7	9	
Israel	30	32	31	26	24	22	23	22	21	18	17	17	15	15	17	14	10	8	9	7	7	6	5	5	4	4	4	3	4		
Italy	13	12	11	12	11	10	10	9	9	9	8	8	8	7	7	7	7	6	6	6	5	5	5	5			4	4	4		
Kazakhstan		46	44			46	38	40	37	38	39	43	43	54	56	66	67	63	61	59	62	59	56	58	53	54	53	50	43	33	
Kyrgyzstan		45	42			42	40	39	41	38	39	38	39	46	55	60	55	51	46	41	48	45	48	47	48	47	51	47	46	53	
Latvia	47	47	45	45	47	46	40	39	41	40	43	47	52	61	71	58	44	40	40	34	34	38	33	34	34	34	37	33	29	26	
Lithuania		33	35			34	29	34	34	37	37	42	38	43	44	43	38	27	28	24	23	25	25	25	27	27	31	29	26	24	
Luxembourg	23	18	17	15	21	16	13	18	12	14	10	12	9	13	10	8	7	7	8	6	5	8	10	7	10	3	13	8	5		
Malta	49	37	22	30	24	14	34	35	20	26	31	22	26	16	18	14	16	17	15	17	17	15	17	14	6	11	7	6	12	10	6
Netherlands	19	20	18	18	18	18	17	16	16	14	13	15	13	14	13	12	12	11	10	10	9	9	8	7	6	6	5	5	5	5	
Norway							20	19	17	17	18	16	16	14	14	13	10	9	10	11	10	9	8	7	6	6	6	6	4	5	
Poland	18	17	17	18	20	21	21	22	22	24	24	24	24	22	20	20	18			19	17	16	13	13	13	12	12	11	10	10	
Portugal	12	13	12	13	11	11	11	11	11	10	10	11	9	11	9	9	8	8	7	8	7	7	7	7			4	4	4		
Republic of Moldova		73	73			72	61	56	51	50	52	58	53	59	68	73	73	67	59	62	65	58	63	63	56	60	58	53	50	49	51
Romania	24	24	24	26	25	27	26	28	27	27	27	29	31	35	34	35	38	37	36	34	32	33	32	31	28	28	26	25	24	23	21
Russian Federation	47	44	43	44	46	44	37	37	36	37	38	39	43	58	67	60	53	48	45	50	54	56	61	63	59	60	53	47	47	44	
San Marino																0	0	18	0	8	8					0					
Serbia																				22	21	21	20	21	20	20	21	20	18	17	16
Slovakia													34	32	34	33	32	33	29	26	27	21	21	21	21	19	18	21	19	20	
Slovenia						16	12	12	12	11	12	14	12	15	13	10	11	8	11	8	8	8	6	5	7	5	5	4	5	5	
Spain	9	8	8	8	8	7	7	7	7	7	7	7	6	6	6	6	6	6	5	6	5	5	5	5	4	4	4	4	4		
Sweden								16	16	14	15	15	14	12	12	12	10	10	9	10	10	9	9	9	7	7	7	7	7	7	6
Switzerland																6	6	7	7	6	5	4	5	5	4	4	4	3			
TFYR Macedonia												22	23	24	24	23	26	25	25	22	22	23	19	25							
Tajikistan		48	47			39	39	39	43	43	45	45	42	52	61	60	55	41	36	44	43	53	44	41	41						
Turkmenistan		62	55			62	61	61	72	80	74	76	72	100	85	87	98	82	68												
Ukraine		52	52			50	41	41	39	38	41	44	47	55	60	66	66	65	58	63	64	65	68	70	70	74	68		64	57	
United Kingdom	36	34	34	34	34	33	32	31	30	28	28	26	24	23	21	20	19	18	17	15		14	13	12	11	10	10	9	9	8	
Uzbekistan		57	52			57	52	49	52	57	54	60	60	67	72	70	64	59	65	58	60	56	63	58	54	59					

Source: WHO Mortality Database http://www.who.int/healthinfo/morttables/en/index.html
Notes: Blank cells indicate data were not available

Table 2: Age Standardized death rates from stroke, adults aged under 65, by sex,1980 to 2010,Europe (deaths per 100,000)

Men	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Albania								33	31	29			32	25	21	25	24	27	26	27	22	26	22	23	20						
Armenia		27	24			27	28	27	30	32	33	37	37	45	40	39	35	34	30	31	29	27	37	35			30		28	28	
Austria	27	27	25	27	26	23	21	20	20	18	18	16	16	15	15	16	15	14	13	12	11	11	9	8	7	7	6	6	6	7	5
Azerbaijan		58	58			56	53	57	53	44	45	44	50	54	50	49	53	47	41	39	41	34	36	38	39			45			
Belarus		59	55			59	51	50	52	55	56	61	62	73	73	77		79	79	80	74	81	82	79				64	62	62	
Belgium	19	18	18	18	17	15	15	13	13	12	13	13	12	11	11	10	11	11	9	10					9	8					
Bosnia and Herzegovina						30	31	31	34	34	35	42																			
Bulgaria	61	61	62	61	65	66	64	62	66	64	68	68	75	83	78	69	66	70	69	59	63	59	60	55	55	55	57	50	49		
Croatia						48	50	47	47	48	46	45	43	43	42	40	46	40	38	41	38	33	34	29	29	30	29	28	27	22	
Cyprus																				9	7				10	8	7	6	6	5	
Czech Republic							46	44	42	39	44	39	36	33	31	30	27	25	24	24	23	21	20	20	17	17	16	14	14	13	
Denmark															13	13	14	12	12	15	11	12	12	13	12	9	11				
Estonia		54	49			62	54	47	59	53	63	60	68	66	68	66	60	52	53	52	55	50	45	48	41	41	41	37	30	26	22	
Finland								24	23	22	26	22	21	21	18	18	18	17	16	17	13	15	14	14	14	11	12	12	11	10	
France	20	19	19	18	18	17	17	15	14	13	13	12	11	12	10	10	10	10	9	10	8	8	8	8	7	7	7	7	6		
Georgia		70	77			80	73	65	69	69	69	74	72		66	69	62	62	63	67	69	64				61	57	64		48	
Germany											14	16	14	14	14	13	13	12	11	10	10	9	9	8	8	8	8	7	7	7	6
Greece	22	21	20	20	21	20	18	18	18	19	17	19	17	17	17	17	18	17	16	15	17	15	15	14	15	14	14	12	12	12	
Hungary	61	59	62	66	68	67	63	60	59	61	62	61	61	62	58	57	55	52	51	51	49	45	45	43	39	34	34	32	32	29	
Iceland		16	12	19	10	8	15	19	9	8	6	12	7	8	7	9	8	9	6	7	4	5	8	4	6	4	6	5	3	7	
Ireland	24	23	22	21	21	18	17	17	15	15	15	13	11	14	12	12	11	11	11	11	10	10	8	8	8	7	7	5	7	6	
Israel	23	17	18	17	16	17	16	16	12	11	12	11	13	13	12	13	13	10	10	8	9	9	7	7	8	8	6	6	5		
Italy	25	24	23	22	22	21	20	19	17	16	15	15	15	13	13	12	11	11	10	10	9	9	8	8				7	6	6	
Kazakhstan		69	67			65	61	58	58	64	66	65	65	73	82	88	88	88	91	87	94	81	83	93	88	91	84	84	76	74	
Kyrgyzstan		84	80			77	70	68	67	71	75	79	84	98	114	126	106	107	104	104	110	110	110	105	107	108	107	112	94	105	
Latvia	66	65	61	61	62	63	55	51	55	58	68	64	67	81	86	78	72	65	62	56	56	60	62	55	55	55	53	48	42	40	
Lithuania		41	39			41	38	40	33	40	41	41	42	47	45	43	37	38	34	31	30	36	33	35	36	36	38	38	32	36	
Luxembourg	25	28	20	29	31	30	25	24	21	20	21	17	15	12	18	13	17	10	12	8	10	16	13	16	9	7	8	4	10		
Malta	32	26	32	33	19	22	29	38	16	15	10	20	16	16	10	12	14	8	8	8	7	13	12	8	6	8	8	6	7	6	7
Netherlands	15	14	14	12	14	12	12	11	11	11	11	10	10	11	11	10	10	10	9	9	9	9	9	8	7	7	6	6	6	5	5
Norway							12	15	12	12	13	10	11	13	10	10	11	10	9	9	8	8	7	6	7	6	6	6	7	5	
Poland	28	24	24	25	27	29	30	30	30	30	31	32	32	32	31	31	29			35	32	32	30	29	30	28	27	27	26	25	
Portugal	53	50	45	45	43	41	41	36	37	34	35	37	35	34	30	29	29	28	26	24	25	24	22	20				14	14	14	
Republic of Moldova		52	52			69	58	56	54	54	65	68	63	71	80	85	83	84	74	81	83	82	77	79	77	80	71	68	66	66	66
Romania	44	45	44	46	47	48	47	52	49	48	50	51	54	70	73	74	77	79	73	66	62	64	65	62	57	56	53	49	46	45	42
Russian Federation	72	73	71	72	75	72	65	64	64	64	66	67	71	87	103	97	90	85	82	90	98	100	103	104	101	99	87	78	76	71	
San Marino																9	0	0	0	0	6					0					
Serbia																			47	45	49	44	43	42	42	38	36	35	30	32	
Slovakia													35	31	32	30	28	29	31	25	24	21	24	22	19	19	18	24	28	27	
Slovenia						34	27	36	37	32	32	35	33	30	34	26	27	25	25	20	22	18	18	19	18	13	16	12	13	12	
Spain	24	24	22	21	21	21	19	18	18	16	15	15	14	14	13	13	12	12	11	11	10	10	10	9	9	9	8	8			
Sweden								12	12	11	12	12	12	12	11	10	11	10	10	10	9	8	8	8	8	7	7	6	7	6	5
Switzerland																6	6	7	6	5	6	4	4	4	5	4	4	4			
TFYR Macedonia												39	44	45	45	47	51	44	43	46	40	46	44	45							
Tajikistan		49	48			40	39	42	40	40	40	41	46	45	38	35	28	23	16	20	19	19	30	31	25						
Turkmenistan		86	94			93	90	86	90	49	56	76	66	76	66	59	49	30	30												
Ukraine		58	59			63	53	54	54	55	59	65	69	73	77	86	82	79	71	72	74	70	68	67	67	69	66		64	57	
United Kingdom	23	21	20	20	19	19	19	17	16	15	15	15	14	13	13	13	13	12	12	11		11	10	10	9	9	8	8	8	7	
Uzbekistan		65	68			66	62	62	61	63	64	65	62	61	75	72	73	69	58	52	55	57	58	54	48	56					

Women	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Albania								20	22	19			21	20	14	18	15	16	17	16	19	13	14	15	16						
Armenia			17	23			28	23	24	22	27	27	25	32	32	28	27	30	23	23	24	20	21	22	24			18		18	11	
Austria		14	15	13	14	14	12	12	12	10	8	9	10	8	9	8	8	9	8	9	8	7	7	6	6	5	4	5	4	4	4
Azerbaijan			37	40			41	43	42	39	34	29	28	35	38	35	37	36	33	33	32	30	28	27	31	26			29		
Belarus			35	34			38	34	34	32	32	33	35	36	42	42	44		44	42	43	39	41	42	40				30	27	26
Belgium		12	13	13	12	11	10	11	10	9	9	8	7	8	8	7	7	8	8	6	7					6	6				
Bosnia and Herzegovina							20	24	24	27	26	26	27																		
Bulgaria		38	38	42	39	39	37	37	36	35	35	36	38	39	39	36	35	33	35	35	32	30	29	28	26	26	26	25	25	22	
Croatia							30	26	29	25	26	25	25	24	23	23	22	25	21	19	22	19	17	16	17	14	14	12	13	12	12
Cyprus																					1	6				5	4	4	4	7	3
Czech Republic								25	24	22	21	22	18	18	17	15	15	14	12	13	10	11	11	10	9	9	8	7	7	6	6
Denmark																10	8	8	8	8	9	8	7	7	8	8	7	7			
Estonia			35	32			34	32	28	30	33	27	30	40	37	38	33	30	27	25	25	23	21	19	20	15	16	13	15	11	8
Finland								14	13	13	12	11	11	12	10	10	9	9	9	8	10	7	8	7	8	8	6	8	6	5	6
France		10	10	9	9	9	8	8	7	7	6	6	6	6	5	5	5	5	5	5	5	5	4	4	4	4	4	4	3	4	
Georgia			39	40			44	44	38	40	39	40	38	42		35	31	28	34	31	31	31	29				28	23	25		21
Germany												8	9	8	8	8	8	7	7	7	6	6	6	6	5	5	5	5	4	4	4
Greece		16	17	15	15	14	13	13	13	12	12	11	12	12	10	10	10	9	8	10	9	8	8	8	7	7	7	7	6	6	5
Hungary		36	36	34	36	36	35	34	32	30	31	30	30	28	28	26	27	25	24	24	24	22	21	20	18	18	15	14	13	14	12
Iceland			13	13	6	9	9	8	7	8	9	11	7	5	4	5	8	11	8	4	5	10	3	3	4	3	5	5	4	6	1
Ireland		22	19	18	18	16	16	15	11	11	11	11	11	10	9	10	10	10	9	8	10	10	7	6	7	6	4	6	6	5	5
Israel		18	19	15	16	15	12	11	11	9	9	9	8	9	9	8	7	7	5	5	5	5	5	4	4	4	4	3	2	3	
Italy		15	15	14	14	13	12	12	11	10	10	9	9	8	8	8	7	7	7	6	6	6	5	5	5			4	4	4	
Kazakhstan			42	41			42	43	40	38	42	41	40	40	46	53	56	55	57	58	55	55	50	48	53	49	49	45	42	41	41
Kyrgyzstan			56	55			60	55	48	51	50	48	58	60	64	79	84	78	66	72	72	69	71	73	71	67	72	68	58	55	64
Latvia		33	40	34	35	37	38	33	33	28	35	33	32	38	43	42	39	40	33	34	30	31	30	31	27	27	25	24	22	19	18
Lithuania			26	23			25	22	24	22	24	23	23	23	23	25	24	22	23	21	18	16	17	19	17	15	16	18	17	16	14
Luxembourg		20	14	9	19	16	15	18	14	12	13	13	11	13	13	9	12	8	7	13	8	13	10	12	7	8	7	7	7	5	
Malta		16	17	19	18	13	8	7	12	12	15	15	10	12	7	6	5	6	6	3	5	7	4	7	5	5	5	6	5	6	5
Netherlands		11	9	10	9	9	9	9	8	7	8	8	8	8	8	7	8	7	8	8	7	7	7	8	7	6	6	5	5	5	4
Norway								9	9	9	8	7	6	7	8	8	6	6	5	6	5	5	6	5	4	5	4	4	4	3	
Poland		17	17	17	15	17	18	17	18	17	17	17	17	17	16	16	16	16		18	17	16	15	14	14	13	12	12	11	11	
Portugal		30	29	26	26	25	24	22	23	20	19	20	20	18	18	16	15	16	15	14	14	12	12	11	10				7	7	7
Republic of Moldova			38	40			60	52	46	47	46	48	54	47	51	56	61	58	60	55	53	60	60	56	53	55	55	52	45	40	39
Romania		33	33	32	32	34	33	34	37	35	33	33	34	33	43	42	44	44	43	42	38	36	36	36	35	33	31	28	25	24	22
Russian Federation		41	42	42	43	45	44	41	40	39	38	38	38	41	48	54	51	48	46	45	47	50	50	50	50	47	46	40	35	34	31
San Marino																	0	9	0	0	9	15					0				
Serbia																				34	34	34	32	30	30	27	26	25	24	21	19
Slovakia													17	16	16	15	15	15	14	10	8	10	7	8	9	7	8	9	11	11	
Slovenia							17	15	14	15	18	15	18	16	13	15	14	11	10	11	9	9	11	9	7	8	7	7	5	6	6
Spain		15	15	13	12	13	11	11	10	9	8	8	8	7	7	6	6	6	5	6	5	5	5	5	4	4	4	4	4		
Sweden								7	8	7	7	7	7	7	7	6	7	7	6	6	6	6	6	5	5	4	5	4	4	4	4
Switzerland																4	4	4	4	4	4	3	3	3	3	3	3	3			
TFYR Macedonia													30	33	34	34	35	32	33	32	33	34	30	34	30						
Tajikistan			43	40			37	33	34	33	38	33	35	37	36	37	28	23	23	16	21	17	21	30	32	26					
Turkmenistan			64	64			68	76	64	69	39	43	46	47	55	46	42	34	23	21											
Ukraine			35	36			37	35	35	34	34	34	36	39	43	45	47	46	43	39	40	39	38	35	35	33	32	31		28	26
United Kingdom		18	17	17	16	15	15	14	14	13	12	11	11	11	11	10	10	10	9	9	9		8	8	8	7	7	6	6	6	5
Uzbekistan			43	44			47	45	48	43	42	45	44	45	43	51	50	50	44	41	37	37	40	37	35	30	35					

Source: WHO Mortality Database http://www.who.int/healthinfo/morttables/en/index.html
Notes: Blank cells indicate data were not available

Table 3: Dietary questionnaire used in the cohort of 60-year-old men and women.

1)	How many sandwiches do you eat per day? [a]	None 1-2 3-5 at least 6
2)	What do you use as "butter"?	Nothing Butter/Bregott margarine/ low-fat margarine Nytta (Becel)
3)	How much "butter" do you usually spread per slice of bread?	Nothing Less than ½ serving ½ serving (5g) At least 1 serving (10g)
4)	How much cheese do you use per slice if you put cheese on your slice?	I never put cheese on my bread 1 slice or less 2 slices 3 slices or more
5)	What sort of milk or yoghurt do you prefer?	I avoid low–fat products Low-fat products or nothing I do not care Standard (3% fat)
6)	How often do you eat fruit and/or berries? [a]	Seldom A couple of times per week Daily Several times per day
7)	How often do you eat vegetables or roots/tubers? [a]	Never <1 time per day Almost daily or daily >1 time per day
8)	How often do you eat lean fish (cod, flounder)?	Seldom/never 1-2 times per week ≥3 times per week
9)	How often do you eat fatty fish (salmon, herring, mackerel)?	Seldom/neve 1-2 times per week ≥3 times per week
10)	How often do you eat bacon or sausages as a main dish?	Never/almost never 2 times per month Once per week 1-2 times per week
11)	Do you cut off visible fat before you eat your meat?	Yes Sometimes No
12)	How often do you eat pan-fried potatoes, French fries, or potato au gratin? [a]	Almost never A couple times per month 1 time per week 2 times per week
13)	How many eggs do you eat?	≤1 time per week 4-5 times per week 2-3 times per week ≥1 per day
14)	What kind of fat/oil do you use to panfry?	I do not eat pan fried foods Liquid margarine or oil Butter or Bregott Margarine
15)	How often do you use cream, or sour cream ("crème fraiche", sauces)?	Seldom =0 ≥2 times per month=0 Once a week=1 ≥2 times per week=2
16)	How often do you eat potato chips, chocolate, croissants, Danish pastries, cakes, or cheeses? [a]	Almost never =0 ≥ 2 times per month=0 Once a week=1 ≥2 times per week =2

Table 4: Score points according to questionnaire answers in the cohort of 60-year-old men and women.

1)	What do you use as "butter"?	Set of points contributing to Dairyscore and Satscore: Nothing=0 Margarine/ low-fat margarine=1 Nytta (Becel)=1 Butter/Bregott =2 Set of points contributing to Vegoilscore: Nothing=0 Butter/Bregott=0 Margarine/ low-fat margarine=1 Nytta (Becel)=2
2)	How much "butter" do you usually spread per slice of bread?	Set of points contributing to Dairyscore and Satscore: Nothing=0 Less than ½ serving=0 ½ serving (5g)=1 At least 1 serving (10g)=2
3)	How much cheese do you use per slice if you put cheese on your slice?	Set of points contributing to Dairyscore and Satscore: Nothing=0 1 slice or less=0 2 slices=1 3 slices or more=2
4)	What sort of milk or yoghurt do you prefer?	Set of points contributing to Dairyscore and Satscore: Nothing=0 Low-fat products or nothing=0 I do not care=1 Standard (3% fat)=2
5)	How often do you eat lean fish (cod, flounder)?	Set of points contributing to Fishscore: Seldom/never=0 1-2 times per week=1 ≥3 times per week =2
6)	How often do you eat fatty fish (salmon, herring, mackerel)?	Set of points contributing to Fishscore: Seldom/never=0 1-2 times per week=1 ≥3 times per week =2
7)	How often do you eat bacon, or sausages as a main dish?	Set of points contributing to Meatscore: Never/almost never=0 2 times per month=0 Once per week=1 1-2 times per week=2
8)	Do you cut off visible fat before you eat your meat?	Set of points contributing to Meatscore: Yes=0 Sometimes=1 No=2
9)	How many eggs do you eat?	Set of points contributing to Meatscore: ≤1 time per week=0 4-5 times per week=1 2-3 times per week=1 ≥1 per day=2
10)	What kind of fat/oil do you use to panfry?	Set of points contributing to Dairyscore and Satscore: I do not eat pan fried food =0 Liquid margarine or oil=1 Butter or Bregott=2 Margarine=2 Set of points contributing to Vegoilscore: I do not eat pan fried foods=0 Butter or Bregott=0 Margarine=1 Liquid margarine or oil=2
11)	How often do you use cream, or sour cream ("crème fraiche", sauces)?	Set of points contributing to Dairyscore and Satscore: Seldom=0 ≥2 times per month=0 Once a week=1 ≥2 times per week=2

Table 5: Distribution of diet score points (25th, 50th, 75 th) in 2,132 women (W) and 1,975 men (M) included in the cohort of 60-year-old men and women.

	Sex	25th	50th	75th
	W	0	1	1
Fishscore	M	0	1	1
	W	1	2	2
Vegoilscore	M	1	2	2
	W	4	5	7
Dairyscore	M	4	6	7
	W	5	6	8
Satscore	M	5	8	10
	W	0	1	2
Meatscore	M	1	2	3

Table 6 : Percentiles (10th, 25th, 50th, 75th, and 90th) of serum cholesterol ester fatty acid distribution, expressed as percentage of the total amount, in 2,017 women (W) and 2,133 men (M) included in the cohort of 60-year-old men and women.

	Percentile value of fatty acids (%)						
	Sex	10th	25th	50th	75th	90th	P value[a]
	W	0·646	0·751	0·885	1·013	1·134	
14 : 0	M	0·661	0·763	0·895	1·034	1·186	<0·01
	W	0·166	0·187	0·212	0·242	0·272	
15 : 0	M	0·168	0·193	0·221	0·252	0·284	<0·001
	W	10·397	10·777	11·189	11·635	12·108	
16 : 0	M	10·709	11·099	11·565	12·035	12·492	<0·001
	W	0·609	0·669	0·744	0·833	0·944	
18 : 0	M	0·641	0·704	0·784	0·876	0·981	<0·001
	W	2·484	2·955	3·606	4·521	5·645	
16 : 1n-7	M	2·103	2·509	3·156	4·134	5·408	<0·001
	W	20·527	21·522	22·607	23·785	24·995	
18 : 1n-9	M	20·515	21·559	22·581	23·805	25·144	0·60
	W	43·081	45·879	48·663	51·187	53·546	
18 : 2n-6	M	42·946	45·891	48·863	51·244	53·388	0·60
	W	0·527	0·685	0·884	1·116	1·369	
18 : 3n-6	M	0·527	0·664	0·841	1·059	1·304	<0·001
	W	0·546	0·624	0·716	0·825	0·926	
20 : 3n-6	M	0·550	0·621	0·708	0·810	0·922	0·11
	W	4·939	5·520	6·219	7·041	7·835	
20 : 4n-6	M	4·930	5·528	6·265	6·986	7·805	0·70
	W	0·659	0·759	0·874	1·015	1·145	
18 : 3n-3	M	0·627	0·738	0·850	0·978	1·112	<0·001
	W	1·168	1·465	1·880	2·467	3·226	
20 : 3n-6	M	1·086	1·416	1·856	2·440	3·272	0·11
	W	0·646	0·758	0·897	1·062	1·236	
22 : 6n-3	M	0·601	0·728	0·877	1·050	1·234	<0·01

Tot. SFA	W	12·045	12·558	13·046	13·614	14·207	<0·001
	M	12·448	12·918	13·460	14·070	14·666	
Tot. MUFA	W	23·336	24·661	26·331	28·138	30·178	<0·001
	M	22·962	24·346	25·827	27·739	30·141	
Tot. PUFA	W	56·047	58·407	60·591	62·508	64·137	0·61
	M	55·883	58·293	60·624	62·464	64·072	
16 : 1n-7/16 : 0	W	0·226	0·267	0·321	0·398	0·487	<0·001
	M	0·187	0·220	0·272	0·354	0·451	

[a]Differences across sex were tested using the Wilcoxon's rank sum test (Mann-Whitney's test), at the 50th percentile only. P-values < 0·05 were considered significant.

Table 7 (a–e): Percentile differences with 95% confidence intervals, expressed as serum fatty acid proportions, at the 10th, 25th, 50th, 75th and 90th percentile of serum fatty acids distribution, across points of increase of diet scores (Fishscore, Vegoilscore, Dairyscore, Satscore, Meatscore). Sex specific results based on 4,030 participants of the cohort of 60-year-old men and women.

		Percentile differences of fatty acids (%)				
Fatty Acid		10th	25th	50th	75th	90th
		(a) Fishscore				
	Crude W	21·99 (17·91, 26·07)	24·91 (21·41, 28·41)	29·98 (25·08, 34·88)	41·63 (34·05, 49·21)	56·77 (40·38, 73·16)
20 : 5n-3 (EPA)	Crude M	23·01 (18·89, 27·13)	30·99 (25·94, 36·03)	37·32 (31·99, 42·65)	50·64 (42·78, 58·50)	60·98 (47·03, 74·94)
	Adjusted[a] W	22·81 (18·47, 27·16)	24·96 (21·17, 28·74)	28·82 (23·86, 33·78)	38·36 (30·33, 46·40)	54·09 (38·04, 70·14)
	Adjusted[a] M	20·25 (15·62, 24·87)	29·88 (24·96, 34·80)	35·76 (30·24, 41·29)	48·51 (40·16, 56·86)	55·22 (41·67, 68·76)
	Crude W	9·63 (8·14, 11·11)	9·73 (8·30, 11·17)	10·30 (8·78, 11·83)	11·43 (9·55, 13·30)	13·94 (11·29, 16·58)
22 : 6n-3 (DHA)	Crude M	9·05 (7·12, 10·99)	12·10 (10·61, 13·60)	12·76 (11·17, 14·35)	13·83 (11·66, 16)	14·14 (11·21, 17·06)
	Adjusted[a] W	9·35 (7·66, 11·03)	9·22 (7·77, 10·67)	10·09 (8·52, 11·66)	10·62 (8·64, 12·61)	14·05 (11·27, 16·84)
	Adjusted[a] M	8·85 (6·83, 10·86)	12·01 (10·35, 13·67)	12·19 (10·52, 13·85)	12·72 (10·76, 14·69)	14·96 (11·72, 18·20)
		(b) Vegoilscore				
	Crude W	67·03 (29·70, 104·36)	42·15 (16·52, 67·77)	30·54 (7·94, 53·15)	29·01 (4·80, 53·22)	10·98 (–17·22, 39·19)
18 : 2n-6	Crude M	81·38 (37·78, 124·98)	46·11 (19·35, 72·88)	49·90 (27·38, 72·41)	27·22 (5·75, 48·68)	34·33 (5·17, 63·50)
	Adjusted[a] W	56·72 (18·80, 94·65)	49·51 (25·12, 73·89)	26·46 (5·65, 47·26)	19·11 (–4·39, 42·61)	1·32 (–22·10, 24·74)
	Adjusted[a] M	73·47 (32·11, 114·82)	36·91 (7·80, 66·01)	34·17 (11·78, 56·55)	27·19 (6·37, 48·01)	22·16 (–1·60, 45·93)
	Crude W	0·66 (–0·79, 2·10)	0·25 (–0·88, 1·38)	0·08 (–1·01, 1·17)	0·37 (–1·02, 1·77)	0·86 (–1·11, 2·83)
18 : 3n-3	Crude M	–0·91 (–2·46, 0·65)	–0·97 (–2·09, 0·16)	–0·55 (–1·63, 0·52)	–0·72(–1·99, 0·55)	–0·11 (–1·92, 1·70)
	Adjusted[a] W	0·09 (–1·29, 1·48)	0·05 (–1·03, 1·12)	0·04 (–1·06, 1·15)	0·22 (–1·16, 1·59)	0·71 (–1·35, 2·78)
	Adjusted[a] M	–0·81 (–2·29, 0·68)	–0·97 (–2·10, 0·15)	–0·61 (–1·66, 0·43)	–0·68 (–1·97, 0·62)	0·02 (–1·74, 1·79)
	Crude W	–6·55 (–12·62, –0·47)	–8·08 (–12·58, –3·58)	–7·71 (–12·12, –3·30)	–11·02 (–16·28, –5·75)	–11·48 (–19·89, –3·07)
Tot. SFA	Crude M	–6·99 (–11·89, –2·08)	–9·98 (–15·27, –4·68)	–10·40 (–15·58, –5·22)	–11·36 (–17·57, –5·14)	–15·94 (–23·91, –7·96)
	Adjusted[a] W	–7·98 (–14·23, –1·73)	–6·97 (–11·32, –2·59)	–7·88 (–12·31, –3·46)	–11·78 (–16·86, –6·71)	–11·34 (–20·10, –2·58)
	Adjusted[a] M	–6·79 (–12·09, –1·49)	–9·51 (–14·85, –4·17)	–10·31 (15·60, –5·02)	–12·63 (–18·79, –6·47)	–14·87 (–22·62, –7·12)
	Crude W	62·13 (30·26, 94·00)	38·91 (15·91, 61·93)	31·14 (14·64, 47·64)	25·37 (9·47, 41·27)	21·47 (0·09, 42·84)
Tot. PUFA	Crude M	75·25 (37·08, 113·43)	66·89 (42·57, 91·22)	35·19 (19·00, 51·38)	39·14 (21·10, 57·18)	30·49 (10·14, 50·83)
	Adjusted[a] W	62·39 (29·25, 95·53)	41·36 (22·77, 59·96)	33·52 (16·17, 50·86)	17·60 (1·13, 24·06)	21·01 (0·77, 41·26)
	Adjusted[a] M	56·89 (13·32, 100·45)	65·13 (42·31, 87·94)	32·42 (14·97, 49·89)	24·54 (8·45, 40·63)	23·11 (1·33, 44·89)

(c) Dairyscore

14 : 0	Crude W	1·65 (1·14, 2·15)	1·34 (0·79, 1·89)	0·96 (0·47, 1·46)	1·27 (0·75, 1·80)	1·04 (0·18, 1·90)
	Crude M	1·33 (0·7, 1·94)	1·37 (0·88, 0·19)	1·09 (0·60, 1·59)	0·77 (0·22, 1·33)	1·20 (0·37, 2·01)
	Adjusted[a] W	1·62 (1·07, 2·16)	1·35 (0·79, 1·91)	1·11 (0·60, 1·63)	1·42 (0·90, 1·94)	1·39 (0·46, 2·32)
	Adjusted[a] M	1·17 (0·57, 1·77)	1·25 (0·77, 1·73)	1·10 (0·58, 1·63)	0·81 (0·21, 1·42)	1·26 (0·42, 2·10)
15 : 0	Crude W	0·27 (0·15, 0·39)	0·31 (0·21, 0·40)	0·28 (0·18, 0·39)	0·32 (0·18, 0·45)	0·27 (0·05, 0·48)
	Crude M	0·26 (0·13, 0·40)	0·28 (0·18, 0·39)	0·20 (0·10, 0·30)	0·27 (0·13, 0·41)	0·36 (0·17, 0·55)
	Adjusted[a] W	0·26 (0·11, 0·41)	0·31 (0·21, 0·41)	0·35 (0·24, 0·45)	0·33 (0·20, 0·45)	0·37 (0·15, 0·59)
	Adjusted[a] M	0·35 (0·23, 0·47)	0·33 (0·23, 0·43)	0·24 (013, 036)	0·35 (0·22, 0·49)	0·43 (0·23, 0·62)
16 : 0	Crude W	–1·18 (–3·44, 1·08)	–3·75 (–5·62, –1·88)	–3·39 (–4·85, –1·92)	–4·72 (–6·92, –2·51)	–5·51 (–8·47, –2·55)
	Crude M	–0·53 (–2·49, 1·44)	–1·83 (–3·51, –0·16)	–1·69 (–3·41, –0·03)	–2·50 (–4·61, –0·38)	–2·99 (–5·47, –0·51)
	Adjusted[a] W	–1·70 (–3·95, 0·55)	–3·80 (–5·67, –1·94)	–3·32 (–4·79, –1·86)	–4·13 (–6·36, –1·90)	–5·19 (–7·93, –2·45)
	Adjusted[a]M	–0·92 (–2·75, 0·90)	–2·09 (–3·89, –0·30)	–2·85 (–4·62, –1·08)	–2·75 (–4·80, –0·70)	–3·71 (–6·09, –1·33)
18 : 0	Crude W	0·41 (0·10, 0·71)	0·34 (0·04, 0·64)	0·51 (0·22, 0·79)	0·46 (–0·01, 0·94)	0·57 (–0·13, 1·28)
	Crude M	0·50 (0·12, 0·89)	0·45 (0·17, 0·73)	0·28 (–0·02, 0·58)	0·33 (–0·06, 0·71)	0·70 (0·03, 1·37)
	Adjusted[a] W	0·43 (0·08, 0·78)	0·34 (0·03, 0·65)	0·38 (0·08, 0·68)	0·45 (–0·02, 0·92)	0·33 (–0·39, 1·05)
	Adjusted[a] M	0·64 (0·27, 1·00)	0·47 (0·19, 0·75)	0·26 (–0·06, 0·59)	0·24 (–017, 0·64)	0·64 (–0·09, 1·37)
16 : 1n-7/16 : 0	Crude W	0·02 (–0·17, 0·21)	–0·11 (–0·33, 0·10)	–0·40 (–0·65, –0·14)	–0·27 (–0·62, –0·08)	–0·11 (–0·65, 0·42)
	Crude M	0·05 (–0·13, 0·22)	0·19 (–0·01, 0·39)	0·17 (–0·05, 0·39)	0·37 (–0·02, 0·75)	–0·26 (–0·92, 0·41)
	Adjusted[a] W	0·02 (–0·20, 0·23)	–0·12 (–0·36, 0·12)	–0·61 (–0·88, –0·35)	–0·36 (–0·67, –0·04)	–0·34 (–0·98, 0·30)
	Adjusted[a] M	0·00 (–0·17, 0·17)	0·08 (–0·10, 0·26)	0·10 (–0·12, 0·33)	0·10 (–0·28, 0·48)	–0·15 (–0·73, 0·43)
Tot. SFA	Crude W	0·54 (–2·52, 3·60)	–1·05 (–3·33, 1·24)	–2·20 (–4·10, –0·30)	–2·11 (–4·77, 0·55)	–4·71 (–8·18, –1·24)
	Crude M	1·40 (–0·76, 3·56)	0·59 (–1·89, 3·07)	–0·33 (–2·65, 1·98)	–1·08 (–3·84, 1·69)	–0·32 (–4·48, 3·84)
	Adjusted[a] W	0·32 (–2·62, 3·25)	–1·98 (–4·05, 0·08)	–1·96 (–3·89, –0·02)	–2·15 (–4·81, 0·51)	–4·53 (–7·99, –1·06)
	Adjusted[a] M	0·84 (–1·47, 3·15)	0·76 (–1·74, 3·25)	–0·44 (–2·73, 1·86)	–1·58 (–4·33, 1·18)	0·87 (–3·43, 5·18)
16 : 1n-7	Crude W	–0·19 (–2·17, 1·79)	–2·16 (–4·78, 0·46)	–5·60 (–8·62, –2·58)	–4·51 (–8·86, –0·17)	–6·09 (–12·71, 0·52)
	Crude M	1·39 (–0·46, 3·23)	1·55 (–0·58, 3·68)	0·75 (–2·05, 3·55)	2·98 (–1·43, 7·28)	–4·80 (–13·45, 3·85)
	Adjusted[a] W	–0·75 (–3·03, 1·53)	–3·11 (–5·76, –0·46)	–7·52 (–10·43, –4·61)	–7·22 (–11·43, –3·01)	–4·65 (–12·06, 2·76)
	Adjusted[a] M	0·72 (–1·22, 2·65)	0·00 (–2·21, 2·21)	–0·15 (–3·10, 2·79)	0·36 (–4·20, 4·93)	–1·78 (–9·86, 6·31)
18 : 1n-9	Crude W	9·15 (3·59, 14·71)	4·25 (–0·57, 9·07)	2·40 (–0·20, 6·79)	3·03 (–2·24, 8·30)	0·17 (–7·83, 8·16)
	Crude M	0·83 (–6·12, 7·79)	4·80 (0·88, 8·72)	3·84 (–0·60, 8·29)	9·80 (4·44, 15·16)	10·07 (1·53, 18·60)
	Adjusted[a] W	6·85 (0·90, 12·81)	2·63 (·2·40, 7·67)	2·04 (–2·09, 6·17)	–1·29 (–6·44, 3·85)	–2·37 (–9·48, 4·74)
	Adjusted[a] M	1·06 (–5·60, 7·71)	5·30 (1·26, 9·35)	5·95 (1·28, 10·62)	5·99 (0·41, 11·58)	6·02 (–2·48, 14·53)

(d) Satscore

14 : 0	Crude W	0·71 (0·21, 1·22)	0·46 (0·02, 0·89)	0·15 (–0·23, 0·53)	0·64 (0·21, 1·07)	0·36 (–0·36, 1·08)
	Crude M	0·74 (0·33, 1·15)	0·69 (0·33, 1·06)	0·49 (0·12, 0·86)	0·13 (–0·32, 0·58)	0·52 (–0·08, 1·13)
	Adjusted[a] W	0·51 (0·10, 0·92)	0·41 (–0·02, 0·85)	0·14 (–0·25, 0·52)	0·57 (0·14, 1·01)	0·68 (–0·03, 1·39)
	Adjusted[a] M	0·65 (0·20, 1·10)	0·63 (0·27, 0·99)	0·46 (0·07, 0·84)	0·20 (–0·26, 0·65)	0·50 (–0·14, 1·15)
15 : 0	Crude W	0·08 (–0·02, 0·17)	0·10 (0·02, 0·18)	0·13 (0·05, 0·27)	0·13 (–0·04, 0·30)	0·08 (–0·04, 0·20)
	Crude M	0·12 (0·02, 0·02)	0·13 (0·05, 0·22)	0·06 (–0·01, 0·14)	0·12 (0·02, 0·02)	0·05 (–0·14, 1·15)
	Adjusted[a] W	0·08 (–0·04, 0·20)	0·14 (0·07, 0·22)	0·16 (0·07, 0·25)	0·19 (0·09, 0·29)	0·18 (0·01, 0·35)
	Adjusted[a] M	0·12 (0·02, 0·21)	0·13 (0·05, 0·22)	0·06 (–0·01, 0·14)	0·12 (0·02, 0·22)	0·16 (0·02, 0·31)
16 : 0	Crude W	–0·53 (–2·35, 1·29)	–2·48 (–4·00, –0·96)	–2·57 (–3·74, –1·40)	–4·19 (–5·89, –2·50)	–4·54 (–6·83, –2·24)
	Crude M	–0·06 (–1·51, 1·40)	–1·50 (–2·84, –0·16)	–1·33 (–2·66, –0·01)	–1·87 (–3·50, –0·24)	–2·17 (–3·86, –0·48)
	Adjusted[a] W	–0·72 (–2·50, 1·07)	–2·56 (–4·13, –1·00)	–2·50 (–3·68, –1·32)	–3·88 (–5·66, –1·32)	–4·71 (–7·06, –2·37)
	Adjusted[a] M	–0·66 (–2·05, 0·73)	–2·04 (–3·33, –0·74)	–1·99 (–3·36, –0·62)	–2·26 (–3·85, –0·67)	–2·51 (–4·31, –0·71)
18 : 0	Crude W	0·29 (0·04, 0·55)	0·31 (0·09, 0·54)	0·38 (0·16, 0·61)	0·44 (0·06, 0·81)	0·31 (–0·27, 0·90)
	Crude M	0·39 (0·12, 0·65)	0·32 (0·11, ·53)	0·21 (–0·03, 0·45)	0·30 (0·02, 0·59)	0·69 (0·19, 1·19)
	Adjusted[a] W	0·32 (0·05, 0·59)	0·34 (0·11, 0·57)	0·32 (0·08, 0·57)	0·42 (0·06, 0·78)	0·21 (–0·35, 0·76)
	Adjusted[a] M	0·36 (0·11, 0·60)	0·31 (0·10, 0·53)	0·20 (–0·04, 0·45)	0·28 (–0·01, 0·58)	0·64 (0·09, 1·19)
16 : 1n-7/16 : 0	Crude W	0·04 (–0·20, 0·12)	–0·17 (–0·34, 0·00)	–0·41 (–0·60, –0·22)	–0·34 (–0·63, –0·06)	–0·22 (–0·66, 0·21)
	Crude M	0·04 (–0·09, 0·18)	0·16 (0·00, 0·32)	0·32 (0·03, 0·60)	0·32 (0·03, 0·60)	–0·12 (–0·64, 0·40)
	Adjusted[a] W	–0·02 (–0·19, 0·15)	–0·23 (–0·42, –0·04)	–0·60 (–0·81, –0·39)	–0·41 (–0·68, –0·14)	–0·34 (–0·83, 0·15)
	Adjusted[a] M	–0·03 (–0·16, 0·10)	0·08 (–0·06, 0·22)	0·09 (–0·07, 0·26)	0·15 (–0·14, 0·44)	–0·00 (–0·42, 0·42)
Tot. SFA	Crude W	0·65 (–1·73, 3·04)	–0·74 (–2·55, 1·08)	–1·99 (–3·53, –0·46)	–3·24 (–5·42, –1·06)	–4·91 (–7·65, –2·18)
	Crude M	0·57 (–1·02, 2·15)	–0·22 (–2·11, 1·67)	–0·57 (–2·33, 1·19)	–1·19 (–3·23, 0·84)	–0·77 (–4·04, 2·48)
	Adjusted[a] W	0·14 (–2·11, 2·38)	–1·63 (–3·23, –0·02)	–1·68 (–3·22, –0·14)	–2·52 (–4·70, –0·34)	–4·78 (–7·51, –2·04)
	Adjusted[a] M	0·36 (–1·33, 2·06)	–0·48 (–2·33, 1·37)	–0·78 (–2·52, 0·97)	–1·49 (–3·59, 0·61)	–0·81 (–4·00, 2·37)
16 : 1n-7	Crude W	–0·62 (–2·19, 0·93)	–2·92 (–4·98, –0·87)	–5·53 (–7·92, –3·14)	–4·41 (–8·06, –0·76)	–5·36 (–10·36, –0·35)
	Crude M	1·26 (–0·15, 2·68)	1·44 (–0·17, 3·06)	1·05 (–1·03, 3·13)	2·46 (–0·91, 5·84)	–1·45 (–8·31, 5·40)
	Adjusted[a] W	–1·32 (–3·00, 0·35)	–3·92 (–6·04, –1·81)	–6·48 (–8·80, –4·17)	–6·90 (–10·28, –3·53)	–4·66 (–10·08, 0·77)
	Adjusted[a] M	0·50 (–0·94, 1·95)	0·000 (–1·66, 1·66)	–0·11 (–2·34, 2·11)	0·85 (–2·57, 4·27)	–0·69 (–6·77, 5·38)
18 : 1n-9	Crude W	5·90 (1·53, 10·27)	0·99 (–2·88, 4·85)	1·08 (–2·47, 4·64)	1·74 (–2·37, 5·85)	–0·67 (–6·83, 5·47)
	Crude M	2·83 (–2·07, 7·73)	4·82 (1·94, 7·69)	8·85 (4·99, 1·27)	7·90 (1·46, 14·33)	7·89 (1·46, 14·33)
	Adjusted[a] W	4·88 (0·56, 9·20)	1·92 (–2·12, 5·96)	–0·15 (–3·43, 3·14)	–2·24 (–6·24, 1·75)	–1·94 (–7·87, 3·99)
	Adjusted[a] M	1·32 (–3·60, 6·24)	4·20 (0·99, 7·40)	5·53 (1·92, 9·13)	5·64 (1·59, 9·69)	4·39 (–1·98, 10·76)
	Crude W	2·22 (–3·20, 7·65)	–2·69 (–7·77, 2·39)	–2·49 (–7·72, 2·39)	–2·49 (–7·71, 2·74)	–5·90 (–17·53, 5·72)

	Crude M	–0·43 (–5·91, 5·04)	7·29 (3·10, 11·48)	6·54 (1·91, 11·16)	11·64 (5·11, 18·18)	9·11 (–3·24, 21·46)
Tot. MUFA	Adjusted[a] W	0·40 (–5·61, 6·42)	–1·98 (–7·81, 3·84)	–9·00 (–14·60, –3·41)	–8·67 (–15·48, –1·85)	–8·84 (–19·22, 1·55)
	Adjusted[a] M	–1·46 (–7·23, 4·31)	5·65 (1·29, 10·00)	2·24 (–2·27, 6·75)	7·57 (1·07, 14·08)	5·96 (–4·37, 16·39)
			(e) Meatscore			
	Crude W	–1·59 (–2·71, –0·48)	–1·96 (–2·89, –1·03)	–2·03 (–2·89, –1·18)	–1·80 (–2·80, –0·79)	–1·30 (–2·87, 0·27)
14 : 0	Crude M	–0·34 (–1·29, 0·61)	–0·47 (–1·26, 0·32)	–1·04 (–1·87, –0·20)	–1·30 (–2·16, –0·44)	–1·30 (–2·42, 0·53)
	Adjusted[a] W	–1·19 (–2·25, –0·13)	–1·85 (–2·89, –0·82)	–2·25 (–3·12, –1·38)	–1·64 (–2·69, –0·58)	–1·37 (–2·93, 0·20)
	Adjusted[a] M	–0·62 (–1·58, 0·33)	–0·65 (–1·52, 0·22)	–0·90 (–1·72, –0·08)	–1·28 (–2·19, 0·36)	–1·15 (–2·54, 0·24)
	Crude W	–0·24 (–0·46, –0·03)	–0·34 (–0·51, –0·18)	–0·30 (–0·49, –0·11)	–0·26 (–0·53, 0·01)	–0·27 (–0·65, 0·10)
15 : 0	Crude M	–0·20 (–0·41, –0·00)	–0·23 (–0·42, –0·05)	–0·26 (–0·43, –0·09)	–0·20 (–0·41, 0·01)	–0·09 (–0·46, 0·28)
	Adjusted[a] W	–0·30 (–0·50, –0·10)	–0·30 (–0·50, –0·11)	–0·23 (–0·46, –0·02)	–0·12 (–0·37, 0·13)	–0·24 (–0·60, 0·12)
	Adjusted[a] M	–0·13 (–0·34, 0·09)	–0·17 (–0·37, 0·03)	–0·19 (–0·38, –0·03)	–0·19 (–0·41, 0·04)	–0·07 (–0·40, 0·27)
	Crude W	1·75 (–2·39, 5·89)	–1·49 (–4·99, 2·00)	–3·28 (–5·91, –0·65)	–5·58 (–9·55, –1·61)	–3·85 (–8·95, 1·24)
16 : 0	Crude M	0·37 (–2·75, 3·49)	–2·11 (–5·09, 0·86)	–0·95 (–3·88, 1·97)	–3·93 (–7·36, –0·51)	–1·89 (–5·93, 2·16)
	Adjusted[a] W	0·06 (–4·20, 4·31)	–1·10 (–4·64, 2·44)	–3·04 (–5·81, –0·26)	–4·79 (–8·92, –0·66)	–5·21 (–10·12, –0·31)
	Adjusted[a] M	–0·44 (–3·64, 2·76)	–2·54 (–5·48, 0·40)	–1·40 (–4·41, 1·62)	–4·37 (–7·85, –0·88)	–3·00 (–6·93, 0·93)
	Crude W	0·25 (–0·39, 0·89)	0·31 (–0·20, 0·82)	0·49 (–0·05, 1·03)	0·44 (–0·39, 1·26)	0·01 (–1·30, 1·33)
18 : 0	Crude M	0·40 (–0·21, 1·02)	0·36 (–0·11, 0·83)	0·24 (–0·28, 0·76)	0·64 (–0·01, 1·30)	0·64 (–0·01, 1·30)
	Adjusted[a] W	0·20 (–0·46, 0·86)	0·40 (–0·13,0·94)	0·45 (–0·12;1·02)	0·47 (–0·34, 1·29)	–0·13 (–1·38, 1·12)
	Adjusted[a] M	0·62 (0·03, 1·21)	0·49 (0·03, 0·95)	0·15 (–0·38,0·70)	0·71 (0·06,1·37)	1·13 (–0·06, 2·33)
	Crude W	–1·66 (–5·55, 2·22)	–7·18 (–11·87, –2·48)	–11·10 (–16·71, –5·48)	–8·69 (–16·90, –0·48)	–15·91 (–28·00, –3·82)
16 : 1n-7	Crude M	2·99 (–0·17, 6·14)	2·08 (–1·48, 5·66)	2·99 (–1·54, 7·52)	6·04 (–1·61, 13·70)	6·15 (–8·51, 20·80)
	Adjusted[a] W	–3·37 (–7·34, 0·59)	–7·62 (–12·36, –2·87)	–10·66 (–16·53, –4·79)	–10·81 (–18·88, –2·73)	–14·09 (–26·31, –1·87)
	Adjusted[a] M	1·15 (–1·99, 4·30)	0·00 (–3·49, 3·66)	–0·40 (–5·20, 4·41)	6·34 (–0·77, 13·44)	0·97 (–12·63, 14·57)
	Crude W	–0·00 (–10·53, 10·52)	–6·37 (–14·62, 1·87)	–4·82 (–13·10, 3·47)	–2·37 (–11·84, 7·09)	–7·77 (–21·75, 6·21)
18 : 1n-9	Crude M	9·30 (–2·56, 2·17)	7·91 (1·31, 14·52)	9·48 (2·22, 16·74)	15·48 (6·95, 24)	9·27 (–5·49, 2·03)
	Adjusted[a] W	4·47 (–5·15, 14·10)	–0·71 (–10·16, 8·72)	–7·28 (–14·86, 0·30)	–8·78 (–17·64, 0·06)	–4·36 (–18·36, 9·64)
	Adjusted[a] M	6·73 (–3·91, 17·26)	7·31 (0·32, 14·29)	8·93 (0·89, 16·97)	9·68 (1·05, 18·31)	7·74 (–6·53, 22)
	Crude W	0·26 (–0·60, 0·07)	–0·58 (–0·97, –0·20)	–0·91 (–1·38, –0·44)	–0·60 (–1·26, 0·07)	–0·79 (–1·86, 0·28)
16 : 1 n-7/16 : 0	Crude M	0·19 (–0·10, 0·47)	0·23 (–0·07, 0·53)	0·24 (–0·11, 0·59)	0·67 (0·02, 1·32)	0·70 (–0·40, 1·80)
	Adjusted[a] W	–0·25 (–0·63, 0·13)	–0·69 (–1·10, –0·27)	–1·13 (–1·63, –0·63)	–0·97 (–1·59, –0·34)	–1·09 (–2·18, 0·01)
	Adjusted[a] M	–0·06 (–0·34, 0·21)	0·15 (–0·15, 0·45)	0·08 (–0·30, 0·45)	0·49 (–0·13, 1·10)	0·21 (–0·72, 1·14)
	Crude W	0·74 (–4·32, 5·80)	–1·53 (–5·63, 2·56)	–4·84 (–8·31, –1·36)	–7·50 (–12·46, –2·54)	–9·95 (–16·67, –3·23)

Tot. SFA	Crude M	–0·69 (–4·24, 2·87)	–2·74 (–6·79, 1·31)	–1·99 (–5·83, 1·84)	–2·71 (–7·25, 1·83)	–5-·55(–12·43, 1·32)
	Adjusted[a] W	–0·07 (–5·22, 5·09)	–2·24 (–6·11, 1·63)	–3·93 (–7·58, –0·27)	–6·44 (–11·46, –1·43)	–8·24 (–15·21, –1·27)
	Adjusted[a] M	–1·38 (–5·30, 2·54)	–3·04 (–7·04, 0·96)	–2·22 (–6·02, 1·57)	–3·30 (–8·01, 1·42)	–6·87 (–13·67, –0·08)
	Crude W	–9·14 (–21·63, 3·34)	–10·32 (–22·14, 1·50)	–14·12 (–26·01, –2·22)	–22·07 (–36·09, –8·05)	–15·87 (–42, 10·27)
Tot. MUFA	Crude M	7·24 (–4·85, 19·32)	11·32 (2·06, 20·59)	11·93 (1·80, 22·05)	18·09 (3·32, 32·86)	17·69 (–8·48, 43–86)
	Adjusted[a] W	–4·90 (–18·15, 8·35)	–8·82 (–22·31, 4·67)	–24·22 (–37·05, –11·40)	–20·39 (–35·88, –4·90)	–22·30 (–46·94, 2·33)
	Adjusted[a] M	2·53 (–9·91, 14·98)	7·07 (–2·27, 16·41)	3·68 (–6·27, 13·64)	13·71 (–0·46, 27·88)	17·33 (–5·46, 40·13)

To improve readability, percentile difference values were multiplied by 100. [a]Model adjusted for sex, physical activity, and smoking.

Table 8a: Percentile differences (PD) with 95% confidence intervals (CI) at the 10th, 25th, 50th, 75th and 90th percentile of serum fatty acids across levels of Sandwiches. Results represent quantile change in fatty acids for each points increase of Sandwiches in crude and adjusted model in 4,030 participants of a Swedish cohort of 60-year-olds.

Sandwiches

		Percentiles differences of fatty acids				
FA		**10 th**	**25 th**	**50 th**	**75 th**	**90 th**
20:4 n-6	Crude	–30·96 (–50·34;11·59)	–26·98 (–42·70, –11·25)	–29·27 (–45·29, –13·24)	–35 (–54·79, –15·20)	–41·47 (–72·94, –10·01)
	Adjusted[1]	–25·52 (–46·97, –4·06)	–26·34 (–42·37, –10·31)	–30·23 (–46·83, –13·63)	–33·78 (–54·35, –13·21)	–44·34 (–74·45, ·14·22)
18:3 n-3	Crude	6·67 (2·95, 10·38)	5·74 (3·00, 8·48)	6·78 (4·05–9·51)	8·62 (5·08, 12·16)	9·60 (4·56, 14·64)
	Adjusted[1]	5·71 (2·10, 9·32)	5·23 (2·35, 8·12)	6·48 (3·59, 9·37)	9·93 (6·61, 13·25)	10·26 (5·43, 15·08)

[1] Model adjusted for sex, physical activity and smoking. To show the exact changes in percentiles of fatty acids the beta coefficient values have been multiplied by 100.

Table 8b: Percentile differences (PD) with 95% confidence intervals (CI) at the 10th, 25th, 50th, 75th and 90th percentile of serum fatty acids across levels of Fruits. Results represent quantile change in fatty acids for each points increase of Fruits in crude and adjusted model in 4,030 participants of a Swedish cohort of 60-year-olds.

Fruits

		Percentiles differences of fatty acids				
FA		**10 th**	**25 th**	**50 th**	**75 th**	**90 th**
EPA	Crude	8·01 (5·12, 10·91)	8·17 (4·74, 11·60)	7·32 (3·14, 11·50)	8·43 (2·64, 14·23)	12·10 (0·25, 23·96)
	Adjusted[1]	4·73 (1·38, 8·07)	6·77 (3·14, 10·39)	5·78 (1·28, 10·28)	9·02 (2·23, 15·80)	10·87 (–0·66, 22·40)
DHA	Crude	3·18(1·99, 4·37)	4·43 (3·22, 5·63)	4·58 (3·37, 5·79)	4·63 (3·18, 6·08)	5·82 (3·34, 8·29)
	Adjusted[1]	1·88 (0·61, 3·16)	3·43 (2·04, 4·82)	3·96 (2·71, 5·21)	4·42 (2·87, 5·98)	5·40 (2·86, 7·93)

[1] Model adjusted for sex, physical activity and smoking. To show the exact changes in percentiles of fatty acids the beta coefficient values have been multiplied by 100.

Table 8c: Percentile differences (PD) with 95% confidence intervals (CI) at the 10th, 25th, 50th, 75th and 90th percentile of serum fatty acids across levels of Vegetables. Results represent quantile change in fatty acids for each points increase of Vegetables in crude and adjusted model in 4,030 participants of a Swedish cohort of 60-year-olds.

		Vegetables				
		Percentiles differences of fatty acids				
FA		10 th	25 th	50 th	75 th	90 th
18:1 n-9	Crude	–25·51 (–39·26, –11·77)	–21·14 (–31·13, –11·15)	–23·85 (–34·31, –13·40)	–33·19 (–44·31, –22·08)	–46·32 (–64·17, –28·46)
	Adjusted[1]	–16·14 (–30·63, –1·64)	–22·56 (–32·59, –12·52)	–24·71 (–35·04, –14·37)	–22·66 (–34·96, –10·36)	–40·27 (–57·51, –23·02)
EPA	Crude	6·51 (2·86;10·16)	10·48 (6·74;14·23)	15·68 (11·43;19·93)	17·32 (10·03;24·60)	32·20 (19·69;44·70)
	Adjusted[1]	4·57 (1·01;8·12)	9·38 (5·48;13·27)	15·30 (10·88;19·73)	15·71 (8·56;22·86)	28·13 (15·57;40·69)
DHA	Crude	4·00 (2·71;5·29)	4·82 (3·39;6·25)	5·26 (3·97;6·56)	5·25 (3·58;6·92)	7·43 (4·70;10·17)
	Adjusted[1]	2·95 (1·76;4·14)	3·77 (2·40;5·15)	4·41 (3·00;5·83)	5·06 (3·27;6·87)	5·25 (2·40;8·09)
PUFA	Crude	85·07 (46·92;123·23)	56·29 (35·52, 80·06)	24·48 (8·75, 40·21)	34·67 (17·78, 51·55)	26·26 (4·72, 4·78)
	Adjusted[1]	64·34 (29·14, 99·53)	32·64 (10·17, 55·10)	21·26 (2·56, 39·97)	21·31(4·14, 38·50)	23·59 (0·75, 44·44)

[1] Model adjusted for sex, physical activity and smoking. To show the exact changes in percentiles of fatty acids the beta coefficient values have been multiplied by 100.

Table 8d: Percentile differences (PD) with 95% confidence intervals (CI) at the 10th, 25th, 50th, 75th and 90th percentile of serum fatty acids across levels of Potato dishes. Results represent quantile change in fatty acids for each points increase of Potato dishes in crude and adjusted model in 4,030 participants of a Swedish cohort of 60-year-olds.

		Potato dishes				
		Percentiles differences of FA				
FA		10 th	25 th	50 th	75 th	90 th
EPA	Crude	–8·91 (–12·76, –5·06)	–8·83 (–12·60, –5·06)	–8·46 (–13·03, –3·90)	–11·65 (–18·08, –5·21)	–16·20 (–29·61, –2·80)
	Adjusted[1]	–6·16 (–9·94, –2·38)	–8·56 (–12·76, –4·37)	–8·63 (–13·43, –3·82)	–13·43 (–20·84, –6·02)	–17·12 (–30·35, –3·88)
DHA	Crude	–3·30 (–4·70, –1·90)	–3·49 (–4·80, –2·20)	–3·44 (–4·75, –2·14)	–4·02 (–5·62, –2·41)	–6·49 (–9·05, –3·93)
	Adjusted[1]	–2·27 (–3·81, –0·74)	–3·11(–4·55, –1·67)	–3·17 (–4·58, –1·76)	–4·31 (–6·10, –2·51)	–6·81 (–9·42, –4·20)

[1] Model adjusted for sex, physical activity and smoking. To show the exact changes in percentiles of fatty acids the beta coefficient values have been multiplied by 100.

Table 8e: Percentile differences (PD) with 95% confidence intervals (CI) at the 10th, 25th, 50th, 75th and 90th percentile of serum fatty acids across levels of Dessert and Snack. Results represent quantile change in fatty acids for each points increase of Dessert and Snack in crude and adjusted model in 4,030 participants of a Swedish cohort of 60-year-olds.

		Dessert and Snack				
		Percentiles differences of fatty acids				
FA		**10 th**	**25 th**	**50 th**	**75 th**	**90 th**
16:0	Crude	–9·75 (–13·77, –5·74)	–11·89(–15·96, –8·42)	–12·78 (–16·40, –9·17)	–14·23 (–18·19, –10·28)	–11·38 (–17·48, –5·29)
	Adjusted[1]	–10·11 (–14·55, –5·66)	–12·65(–16·44, –8·87)	–12·85 (–16·27, –9·43)	–14·29 (–18·70, –9·88)	–9·63 (–15·01, –4·25)
16:1 n-7	Crude	–9·13 (–13·67, –4·59)	–12·42 (–17·96, –6·87)	–21·77 (–27·76, –15·77)	–31·04 (–40·43, –21·65)	–60·38 (–75·55, –45·22)
	Adjusted[1]	–6·79 (–11·58, –2·00)	–12·70 (–17·61,–7·80)	–21·12 (–26·87, –15·36)	–29·17 (–37·71, –20·62)	–47·36 (–63·20, –31·53)
18:2 n-6	Crude	118·51 (84·51, 152·52)	87·91 (63·07, 112·74)	76·27 (55·85, 96·68)	51·34 (29·78, 72·91)	31·52 (5·59, 57·45)
	Adjusted[1]	89·92 (53·83, 126·03)	83·10 (59·10, 107·10)	71·35 (51·17, 91·54)	61·97 (40·71, 82·24)	25·51 (2·84, 48·18)
Tot n-6	Crude	78·53 (46·33, 110·73)	68·52 (48·12, 88·92)	44·62 (30·13, 59·10)	36·97 (19·72, 54·22)	26·79 (4·68, 48·89)
	Adjusted[1]	80·96 (45·11, 116·80)	61·59 (41·09, 82·10)	56·03 (39·19, 73·88)	33·07 (15·47, 50·66)	33·60 (11·70, 55·50)
	Crude	78·53 (43·33, 110·73)	68·52 (48·12, 88·92)	44·62 (30·13, 59·10)	36·21 (20·88, 51·53)	29·57 (11·34, 47·79)
Tot PUFA	Adjusted[1]	76·59 (41·69, 11·50)	58·48 (38·44, 78·53)	44·84 (29·40, 60·28)	25·42 (20·41, 50·44)	32·12 (12·89, 51·35)

[1] Model adjusted for sex, physical activity and smoking. To show the exact changes in percentiles of fatty acids the beta coefficient values have been multiplied by 100.

Table 9a: Risk of Incident Cardio Vascular Disease (CVD) and Total Mortality According to Sex-specific Quartiles of Serum Cholesteryl Ester Eicosapentaenoic acid (EPA) and Docosahexaenoic Acid (DHA). Sex specific results based on the participants of the cohort of 60-year-old men and women.

		Quartiles of EPA				
		1	2	3	4	P_{trend}
Female	Median EPA, % of TFA	1.24	1.66	2.13	3.05	
CVD incidence	Events (person-years)	60 (6371)	42 (6653)	42 (6648)	39 (6797)	
	Crude HR (95% CI)	1.00 (reference)	0.67(0.45-0.99)	0.62(0.41-0.93)	0.60(0.40-0.90)	0.032
	Adjusted HR (95% CI)	1.00 (reference)	0.65(0.44-0.98)	0.64(0.43-0.97)	0.59(0.39-0.91)	0.043
All-cause mortality	Deaths (person-years)	61 (6708)	43 (6893)	54 (6841)	33 (6976)	
	Crude HR (95% CI)	1.00 (reference)	0.68 (0.46-1.01)	0.86 (0.60-1.25)	0.52 (0.34-0.79)	0.007
	Adjusted HR (95% CI)	1.00 (reference)	0.68 (0.46-1.01)	0.90 (0.62-1.31)	0.53 (0.34-0.82)	0.015
Male	Median EPA, % of TFA	1.16	1.66	2.14	3.08	
CVD incidence	Events (person-years)	77 (5,466)	81 (5,548)	74 (5,599)	72 (5,661)	
	Crude HR (95% CI)	1.00 (reference)	1.04(0.76-1.42)	0.94(0.68-1.29)	0.90(0.65-1.24)	0.41
	Adjusted HR (95% CI)	1.00 (reference)	1.09(0.79-1.49)	0.99(0.71-1.38)	0.99(0.70-1.40)	0.80
All-cause mortality	Deaths (person-years)	81 (5,879)	70 (5,971)	65 (5,990)	49 (6,077)	
	Crude HR (95% CI)	1.00 (reference)	0.85 (0.62-1.17)	0.78 (0.56-1.08)	0.59 (0.41-0.83)	0.002
	Adjusted HR (95% CI)	1.00 (reference)	0.85 (0.61-1.18)	0.82 (0.58-1.14)	0.62 (0.43-0.91)	0.014

		Quartiles of DHA				
		1	2	3	4	P_{trend}
Female	Median DHA, % of TFA	1.24	1.66	2.13	3.05	
CVD incidence	Events (person-years)	60 (6371)	42 (6653)	42 (6648)	39 (6797)	
	Crude HR (95% CI)	1.00 (reference)	0.67(0.45-0.99)	0.62(0.41-0.93)	0.60(0.40-0.90)	0.032
	Adjusted HR (95% CI)	1.00 (reference)	0.65(0.44-0.98)	0.64(0.43-0.97)	0.59(0.39-0.91)	0.043
All-cause mortality	Deaths (person-years)	61 (6708)	43 (6893)	54 (6841)	33 (6976)	
	Crude HR (95% CI)	1.00 (reference)	0.68 (0.46-1.01)	0.86 (0.60-1.25)	0.52 (0.34-0.79)	0.007
	Adjusted HR (95% CI)	1.00 (reference)	0.68 (0.46-1.01)	0.90 (0.62-1.31)	0.53 (0.34-0.82)	0.015
Male	Median DHA, % of TFA	1.16	1.66	2.14	3.08	
CVD incidence	Events (person-years)	77 (5,466)	81 (5,548)	74 (5,599)	72 (5,661)	
	Crude HR (95% CI)	1.00 (reference)	1.04(0.76-1.42)	0.94(0.68-1.29)	0.90(0.65-1.24)	0.41
	Adjusted HR (95% CI)	1.00 (reference)	1.09(0.79-1.49)	0.99(0.71-1.38)	0.99(0.70-1.40)	0.80
All-cause mortality	Deaths (person-years)	81 (5,879)	70 (5,971)	65 (5,990)	49 (6,077)	
	Crude HR (95% CI)	1.00 (reference)	0.85 (0.62-1.17)	0.78 (0.56-1.08)	0.59 (0.41-0.83)	0.002
	Adjusted HR (95% CI)	1.00 (reference)	0.85 (0.61-1.18)	0.82 (0.58-1.14)	0.62 (0.43-0.91)	0.014

CVD, cardiovascular disease; HR, hazard ratio; DHA, docosahexaenoic acid; TFA, total fatty acids

[1]Adjusted for smoking, physical activity, education, BMI, alcohol intake, diabetes, hypertension, hypercholesterolemia.

Table 9b: Risk of Incident Cardio Vascular Disease (CVD) and Total Mortality According to Sex-specific Quartiles of Serum Cholesteryl Ester linoleic acid (LA) and linolenic Acid (ALA). Sex specific results based on the participants of the cohort of 60-year-old men and women.

		Quartiles of LA				
		1	2	3	4	P_{trend}
Female	Median LA, % TFA	43.8	47.4	49.9	53.0	
CVD incidence	Events (person-years)	52 (6,583)	41 (6,661)	42 (6,694)	45 (6,531)	
	Crude HR (95% CI)	1.00 (reference)	0.78 (0.52-1.17)	0.79 (0.53-1.19)	0.87 (0.59-1.30)	0.50
	Adjusted HR (95% CI)	1.00 (reference)	0.86 (0.57-1.32)	0.94 (0.61-1.44)	1.08 (0.70-1.66)	0.74
All-cause mortality	Deaths (person-years)	55 (6,828)	39 (6,910)	50 (6,872)	47 (6,807)	
	Crude HR (95% CI)	1.00 (reference)	0.70 (0.46-1.05)	0.90 (0.62-1.32)	0.86 (0.58-1.27)	0.63
	Adjusted HR (95% CI)	1.00 (reference)	0.75 (0.49-1.15)	1.00 (0.67-1.50)	0.96 (0.63-1.47)	0.92
Male	Median LA, % TFA	43.8	47.6	50.1	53.0	
CVD incidence	Events (person-years)	79 (5,401)	84 (5,517)	64 (5,708)	77 (5,649)	
	Crude HR (95% CI)	1.00 (reference)	1.04 (0.76-1.41)	0.76 (0.55-1.06)	0.93 (0.68-1.27)	0.32
	Adjusted HR (95% CI)	1.00 (reference)	1.11 (0.81-1.51)	0.83 (0.59-1.17)	1.03 (0.73-1.44)	0.77
All-cause mortality	Deaths (person-years)	84 (5,851)	74 (5,946)	56 (6,065)	51 (6,056)	
	Crude HR (95% CI)	1.00 (reference)	0.86 (0.63-1.18)	0.63 (0.45-0.89)	0.58 (0.41-0.82)	<0.001
	Adjusted HR (95% CI)	1.00 (reference)	0.90 (0.65-1.23)	0.64 (0.45-0.90)	0.59 (0.41-0.85)	0.001

		Quartiles of ALA				
		1	2	3	4	P_{trend}
Female	Median ALA, % of TFA	0.69	0.82	0.94	1.11	
CVD incidence	Events (person-years)	33 (6637)	45 (6606)	46 (6626)	56 (6601)	
	Crude HR (95% CI)	1.00 (reference)	1.38 (0.88-2.16)	1.40 (0.90-2.20)	1.72 (1.12-2.64)	0.015
	Adjusted HR (95% CI)	1.00 (reference)	1.43 (0.91-2.24)	1.47 (0.93-2.31)	1.72 (1.12-2.66)	0.016
All-cause mortality	Deaths (person-years)	45 (6840)	46 (6824)	52 (6878)	48 (6876)	
	Crude HR (95% CI)	1.00 (reference)	1.03 (0.68-1.55)	1.16 (0.78-1.72)	1.07 (0.71-1.60)	0.67
	Adjusted HR (95% CI)	1.00 (reference)	1.02 (0.67-1.55)	1.15 (0.79-1.72)	1.02 (0.68-1.55)	0.82
Male	Median ALA, % of TFA	0.66	0.88	0.92	1.09	
CVD incidence	Events (person-years)	77 (5,569)	83 (5,558)	72 (5,586)	72 (5,561)	
	Crude HR (95% CI)	1.00 (reference)	1.08 (0.79-1.47)	0.93 (0.68-1.29)	0.94 (0.68-1.29)	0.52
	Adjusted HR (95% CI)	1.00 (reference)	1.16 (0.85-1.58)	0.97 (0.70-1.34)	1.02 (0.74-1.42)	0.86
All-cause mortality	Deaths (person-years)	62 (5,967)	58 (6,034)	70 (5,976)	75 (5,940)	
	Crude HR (95% CI)	1.00 (reference)	0.92 (0.64-1.31)	1.13 (0.80-1.59)	1.22 (0.87-1.71)	0.15
	Adjusted HR (95% CI)	1.00 (reference)	0.97 (0.67-1.39)	1.12 (0.79-1.58)	1.23 (0.88-1.73)	0.16

CVD, cardiovascular disease; HR, hazard ratio; ALA, α-linolenic acid, TFA, total fatty acids

[1]Adjusted for smoking, physical activity, education, BMI, alcohol intake, diabetes, hypertension, and hypercholesterolemia.

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