Intake of Fish and Fish Oil in Adolescence and Midlife and Risk of Coronary Heart Disease in Older Women

Álfheiður Haraldsdóttir

Thesis submitted for the degree of Master in Public Health Sciences

MPH committee:

Unnur Anna Valdimarsdóttir, Ph.D.
Laufey Steingrímsdóttir Ph.D.
Jóhanna Eyrún Torfadóttir M.Sc.

Faculty of Medicine
School of Health Sciences, University of Iceland
October 2010

Tengsl fisk- og lýsisneyslu á unglingsárum og á miðjum aldri við kransæðasjúkdóma í eldri konum

Álfheiður Haraldsdóttir

Lokaritgerð til meistaraprófs í lýðheilsuvísindum

Meistaraprófsnefnd:

Dr.Unnur Anna Valdimarsdóttir
Dr. Laufey Steingrímsdóttir
Jóhanna Eyrún Torfadóttir

Læknadeild
Heilbrigðisvísindasvið Háskóla Íslands
Október 2010

Abstract

The role of lifestyle and dietary patterns in the development of coronary heart disease (CHD) is relatively well established in the literature. Consumption of fish and fish oil have specifically been reported to be protective for the development of CHD while data are scarce on the potential protective role of these dietary items in different phases of life. The aim of this study was to examine the association between fish and fish oil consumption, both in adolescence and midlife and coronary heart disease in Icelandic women. We undertook a case-control study nested in a cohort of 3326 women (aged 69-95 years) who took part in the AGES-Reykjavik study. Fish and fish oil consumption were evaluated retrospectively by the subjects with a validated food frequency questionnaire (FFQ). Women underwent clinical examination at entry and were divided into cases and controls based on presence of coronary heart disease. Multivariate logistic regression was used to calculate adjusted odds ratios (AORs) and 95% confidence intervals (95% CI) of CHD when considering teenage and midlife exposure to fish and fish oil. Compared to women with no teenage intake of fish oil, women with consumption of 3-6 times per week had a lower risk of CHD (AOR 0.59; 95% CI 0.40 - 0.91) as were women with daily consumption (AOR 0.75; 95% CI 0.58 - 0.97). Similar findings were observed for midlife consumption of fish oil. No association was observed for teenage fish intake and CHD whereas medium consumption of fish during midlife (more than two portions up to four per week) presented increased risk of CHD (AOR 1.65, 95% CI 1.08-2.52). Fish oil consumption, both in adolescence and midlife, may be protective for developing CHD later in life.

Ágrip

Neysla vissra fæðutegunda eru talin geta dregið úr líkum á kransæðajsúkdómum og tilheyra fiskur og lýsi þeim flokki. Skortur er á rannsóknum á tengslum fæðu í æsku við kransæðasjúkdóma síðar á lífsleiðinni. Tilgangur rannsóknarinnar var að kanna tengsl á milli neyslu lýsis og fisks, bæði á unglingsárum og miðjum aldri, við kransæðasjúkdóma kvenna. Um er að ræða aftursýna tilfella-viðmiða rannsókn í ferilrannsókn. Þátttakendur voru voru 3326 konur á aldrinum 69-96 ára, sem tóku þátt í Öldrunarransókn Hjartaverndar sem fór fram á árunum 2002-2006. Konurnar svöruðu spurningalista um fæði á unglingsárum og á miðjum aldri og var skipt niður í viðmið og tilfelli eftir því hvort þær greindust með kransæðajsúkdóm í byrjun rannsóknar. Lógístísk aðhvarfsgreining var notuð til að reikna hlutfallslíkur. Helstu niðurstöður voru að konur sem neyttu lýsis 3-6 sinnum í viku á unglingsárum voru 41% (0.59, 95% öryggisbil 0.40-0.91) minna líklegar til að fá kransæðasjúkdóma miðað við þær sem aldrei neyttu lýsis. Konur sem neyttu lýsis daglega á unglingsaldri voru 25% minna líklegar miðað við sama viðmiðunarhóp (0.75, 95% öryggisbil 0.58 - 0.97). Svipaðar niðurstöður fundust fyrir neyslu á miðjum aldri. Ekki fundust marktæk tengsl á milli neyslu fisks á unglingsárum og kransæðsjúkdóma. Marktæk áhætta fyrir kransæðasjúkdómum fannst hins vegar fyrir neyslu á meira en tveimur upp að fjórum skömmtum af fiski á viku, á miðjum aldri (1.65, 95% CI 1.08 - 2.52), borið sama við neyslu á tveimur skömmtum eða á viku eða minna. Neysla lýsis á unglingsárum og á miðjum aldri getur verið verndandi gegn kransæðasjúkdómum.

Acknowledgements

I would like to think all of my supervisors, Jóhanna, Unnur and Laufey, for their great help with this project. You are all my role models in the world of science and I have learned so much from you this winter. I hope to get a chance to work with you again in the future. Extra special thanks for everything I am forgetting to mention…

Jóhanna, thank you for your endless good advice, precious time, patience and statistics lessons and for your way of making my never feel stupid when I was having one clueless moments after another, regarding statistics and more.

Unnur, thank you for your time and patience, your good moral and for always believing in my result, no matter what doubts I was having. Thank you for not suggesting having me locked up after tons of obsessive-compulsive emails concerning covariates, data missing and life in general.

Laufey, thank you for your enthusiasm, encouragement, all the good advice, for references and for always having time for me in your busy schedule, especially during the first sunny weekend this year. Your knowledge and experience have been a great influence for me and I have learned so much.

I would like to thank the AGES-Reykjavik Study executive committee and the Icelandic Heart Association for their contribution to this study and giving me access to their data. Special thanks to Guðný Eiríksdóttir and Þórunn Grétarsdóttir for their help with variables and more, and to Vilmundur Guðnason for his support and good advice.

I would like to thank all my dear friends in Stapi. When I got accepted for the Public Health program in 2007, I remember hoping that I would get to know someone to sit next to in class and have polite superficial conversations with during coffee breaks. I never expected to make friends for life, which I know I have. Thanks to ALL of you for your help and support, good advices and always being there for me. Without you, this winter would have been very different. Thank you. Extra special thanks to Agnes, Elsa and Halldóra for your endless help with everything!

This thesis would never been submitted without the love, support and endless help from my beloved husband Jóhann, my parents and my mother in law. Thank you.

Table of contents

Table of figures

Background

Despite a decline in mortality rates for coronary heart disease (CHD) over the last decades, it is still a major global health concern. CHD alone was considered responsible for 7.2 million, or 12.2% of total deaths in the world in 2004.[1] CHD is the most common cause of death in Iceland for both men and women[2] and is estimated to have caused 16% of all deaths in OECD countries in 2006.[3] CHD is one of the leading cause of disease burden in the developing countries as well.[4]

The most common cause of CHD is atherosclerosis which leads to myocardial ischemia by two primary mechanisms. It can either cause anoxia of the heart due to narrowed lumen and decreased flow of blood, or through a rupture of an atheromatous plaque. The ruptured plaque can form a thrombus, which may dislodge and travel further down the artery and cause an occlusion. The sudden anoxia can lead to myocardial infarction (MI) which may result in severe myocardial dysfunction or even death. The long term consequences of non fatal MI, which include heart failure, arrhythmias etc. are associated with considerable morbidity. There is no single dominant risk factor for atherosclerosis, but among the best established ones are hypertension, diabetes, high serum cholesterol, smoking and family history of CHD.[5] Early life exposures (diet, overweight etc.) are also believed to influence development of CHD later in life. For example, studies of fatty streaks in children and young adults show a correlation between the number of CHD risk factors and severity of asymptomatic coronary and aortic atherosclerosis.[6-8]

Most of the gained knowledge concerning CHD comes from studies on males only. There are indications to suggest that women might be different regarding CHD in certain aspects.[9] Women are believed to experience atypical symptoms more often than men and have different physiology, structure and function of the vascular and myocardial systems. Also, women are

more likely to receive delayed diagnosis, less-aggressive treatment and higher rates of in-hospital mortality.[9-11] Women are also believed to be more sensitive to some risk factor than men, for example smoking and diabetes.[11-13] Effects of fish and fish oil consumption on women in relation to CHD is not clear since most of the studies in this field have been conducted on men only or results not reported separately for gender.[14 15]

Diet and CHD

Diet, both nutrient composition and food choice, is considered among major strategies for preventing CHD. Diet is believed to influence development of CHD by many different mechanisms, including changes in blood lipid levels, blood pressure, thrombotic tendency, cardiac rhythm, endothelial function, systematic inflammation, insulin sensitivity, oxidative stress and homocysteine level.[16]

Studies on the beneficial or harmful effects of specific foods or nutrients in relation to CHD are not always consistent. However, fish, whole-grain products, fruit and vegetables, nuts, garlic and moderate amounts of red wine, are the foods that have shown most consistency in being beneficial, as have antioxidant nutrients, folic acid, dietary fiber and long chain polyunsaturated fatty acids (LC-PUFA). Foods rich in saturated fatty acids, trans-unsaturated fatty acids, dietary cholesterol and coffee have been linked with increased risk.[17]

Hu and Willet systematically reviewed evidence from epidemiologic and clinical investigations regarding various dietary factors and their effect on CHD. Database search through May 2002 retrieved 147 original investigations. Their results indicated that 3 dietary strategies were effective in preventing CHD. 1) Emphasize consumption of diet rich in unsaturated fatty acids (especially polyunsaturated fatty acids) rather than food high in saturated

- and *trans* fatty acids. 2) Increase consumption of omega-3 fatty acids from fish oil or plant sources and 3) consume high amount of fruits, vegetables, nuts and whole grain and moderate amounts of refined grains. These strategies, along with regular physical activity, avoidance of smoking, and maintenance of healthy body weight yielded the best results.[16]

Mente and colleges conducted a systematic review of prospective cohort studies and randomized controlled trials (RCT) supporting a causal link between dietary factors and coronary heart disease. The search yielded 146 prospective cohort studies and 43 randomized controlled trials published between 1950 -2007. Four criteria (strength, consistency, temporality and coherence) of the Bradford-Hill guidelines[18] were used to obtain causation score for each dietary exposure. For cohort studies, there is strong evidence (four criteria of the above fulfilled) that vegetables, nuts and Mediterranean diet can be protective against CHD. The same criteria support harmful effects of consumption of food rich in trans-fatty acids and foods with a high glycemic index. Moderate evidence (three criteria fulfilled) exists for consumption of fish, marine omega-3 fatty acids, folate, whole grains, dietary vitamins E and C, beta carotene, fruit, and fiber. However, for RCT, only the consumption of marine or total omega-3 fatty acids showed sufficient protective support according to the Bradford Hill criteria.[19]

A recent meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease (CVD) yielded that intake of saturated fat was not associated with an increased risk of CHD, stroke or CVD. The authors discuss that one of the reason for this findings may be that reduced intake saturated fat is often replaced by polyunsaturated fat that has been estimated to reduce CHD risk. However, more data are needed to elucidate whether CVD risks are likely to be influenced by the specific nutrients used to replace saturated fat.[20]

Few studies exist of early life diet on CHD events and mortality. Between 1937 and 1939, 4028 people in1234 families (4999 children, aged 0-19 years) in Britain took part in the Boyd Orr's Survey of family diet and health. Childhood intake of fruit, vegetables, fish, oily fish, total fat, saturated fat, carotene, vitamin C, and vitamin E were estimated and linked to death from all causes later in life. No association was found between diet in childhood and CHD and death due to CHD.[21]

Fish and fish oil consumption and CHD

Studies on fish consumption and its effect on CHD have shown inconsistent results but meta-analysis and critical reviews show overall a protective pattern against developing CHD.

Marckmann and Grönbæk[22] reviewed prospective cohort studies examining the relationship between fish intake or n-3 polyunsaturated fatty acids and CHD mortality. The studies were divided into categories of high, intermediate or insufficient quality after the strength and consistency of their findings. The evaluation was based on the quality of dietary assessment method, CHD death ascertainment, number of CHD death, statistical presentation, study duration and completeness of follow-up. Eleven studies, published between 1966 until January 1998 where identified. Four studies were judged to be of high quality. Two of them were performed in populations at low risk of coronary heart disease and found no protective effect of fish consumption. The other two studies were relatively small and included individuals at higher risk. They both found an inverse association between increased fish consumption and CHD, suggesting that 40-60 g of fish per day was an optimal dose to reduce the risk of CHD. Results of four studies of intermediate quality supported that increased fish consumption is inversely associated with CHD mortality in high risk populations only. Three studies were found to be of insufficient quality for conclusions to be drawn.

A meta analysis by He and colleagues on fish consumption and CHD mortality revealed similar results. Studies were included if they provided a relative risk (RR) estimate corresponding 95% confidence intervals and the frequency of fish intake. Eleven eligible studies were defined, including 222,364 individuals, with an average of 11.8 years of follow up after database search from 1966 to September 2003. Pooled RR showed that compared with those who

never consumed fish or ate fish less than once per month, individuals with higher intake of fish had lower CHD mortality.[23]

Despite a recent decline in fish consumption in Iceland[24 25] there is a strong tradition of fish and fish oil intake, especially in the last century.[26] Icelandic food based dietary guidelines recommend taking one teaspoon of fish oil per day and consuming fish at least twice a week, preferably more often.[27]

Protective mechanisms of fish and fish oil on CHD

The beneficial effect of fish and fish oil consumption are believed to be mainly from omega-3 LC-PUFA. Therefore, most studies do not separate these exposures from different sources and frequently estimate the amount of omega-3 LC-PUFA, based on the amount of fish and other food rich in these fatty acids that are consumed. Omega-3 fatty acids are a family of unsaturated fatty acids that have carbon-carbon double bounds starting at the third carbon molecule from the methyl end.[28] Omega-3 fatty acids include alfa-linolenic acids (ALA), and the LC-PUFAeicosaptenaenic acid (EPA) and doxosahecaenoic acid (DHA). ALA is nutritionally essential since the body is unable synthesize it. ALA is mostly found in vegetable oils and can be converted to both EPA and DHA, however, the conversion is both slow and ineffective in certain tissues. Therefore these fatty acids may be essential too, at least for the fetus and growing child.[29] EPA and DHA are mostly found in fat fish and seafood products.[30] Omega-3 fatty acids are believed to reduce the odds of developing a heart disease through a variety of actions, although the mechanism behind those actions is not known with confidence. They have been shown to prevent arrhythmias, they are prostaglandin and leukotriene precursors, have anti-

inflammatory properties, inhibit synthesis of cytokines and mitogens, stimulate endothelial-derived nitric oxide, are antithrombotic, have hypolipodemic properties, inhibit atherosclerosis and to be mildly hypotensive.[31]

Fish and fish oil consumption are believed to have different effects on different cardiovascular outcomes. This difference is likely to be related to varying dose and time-responses of the effects of omega-3 fatty acids on different cardiovascular risk factors. A typical dietary intake (< 750 mg/d of EPA and DHA) reduces the risk of sudden death and death within weeks to months due to CHD. At higher doses, maximum antiarrhythmic effects might have been achieved but other physiologic effects may modestly reduce the risk of other clinical outcomes that require longer durations of intake. Although not well established, favorable effects of fish might also to be due to selected protein, vitamin D and selenium found in fish, which each may provide additional benefits.[32]

Fish and fish oil consumption and CHD in women

Despite a fairly well established causality for preventive effects of fish and fish oil consumption and credible mechanism to support the relationship with CHD, current knowledge may not apply to women. The majority of published epidemiological studies on the subject have been done on males and based on possible gender differences in relation to CHD, the validity of those results may be limited. Further, data are scarce on the impact of both early- and midlife consumption on diseases later in life. In the review by Marckmann and Grönbæk, only three studies included females, one of high quality[33] that found protective effects of fish in their main conclusion, one of intermediate quality[34] with no such finding and one lacking credibility for drawing conclusion.[35] In the meta-analysis by He and colleges, five studies with women were included. Gender separated pooled analysis for four of those studies[34 36-38], showed overall protective effects of fish.

Other studies regarding women report differently. A prospective cohort study on Finnish women did not support a causal relationship between fish consumption and CHD. The cohort existed of 2445 women, free from cardiovascular disease, aged 30-79 years. Mean follow-up time was 21.5 years. Data on diet (from the year previous to the study) and risk factors was assessed at baseline. The endpoint was death from CHD. Multivariate adjustment for age, major risk factors for CHD and demographic factors showed that high consumption of fish was associated with decreased risk of CHD. After adjustment for other dietary items, this association was not significant.[39]

Similar results were found in a prospective cohort study of 3984 Danish women, aged 30 -70 years and free from cardiovascular disease. Follow-up was 39174 person years (approx. 10 years mean). Data on food consumption (no time interval reported), biological and behavioral

cardiovascular risk factors were collected with clinical examinations and questionnaires. Endpoints were fatal and non-fatal CHD. Adjustments were made for major risk factors, diet, alcohol, physical activity and education. Frequent fish consumption was not related to CHD morbidity or mortality after adjustments for risk factors.[40]

Folsom and Demissie did a prospective cohort study on intake of fish, and omega 3 LC-PUFA and mortality from several causes in a cohort of 41.836 postmenopausal women, aged 55-69 years. Baseline dietary intake was assessed by using a food frequency questionnaire (FFQ) and average daily intake of fish and omega-3 was calculated from the diet. Follow-up time was 14 years. Adjusted for age and energy intake only, eating 1.5-2.5 servings per week of fish was protective for CHD mortality. After adjustment for multiple risk factors, this association became insignificant. Estimated marine omega-3 fatty acid intake was not associated with CHD mortality.[15] Other studies has shown that fish and fish oil consumption can be protective in diabetic women[41] and women with known atherosclerosis.[42]

However, these results do not clarify whether fish and fish oil consumption is beneficial for CHD in women and further research is needed to conclude if a causal relationship exists, particularly as it relates to early exposure and CHD.

The aim of this study is to examine the relationship between fish and fish oil consumption in adolescence and midlife and CHD later in life among Icelandic women. The research question that will be addressed is whether high consumption of fish or fish oil in adolescence and/or midlife can reduce the risk of developing CHD.

Article

To be submitted to BMJ: British Medical Journal

Intake of Fish and Fish Oil in Adolescence and Midlife and Risk of Coronary Heart Disease in Older Women

Correspondence: Álfheiður Haraldsdóttir, Centre of Public Health Sciences, University of Iceland, Stapi v/Hringbraut, 101 Reykjavik, Iceland
alh1@hi.is

Running title: Fish and fish oil consumption and CHD in older women.

Abstract

Objectives: To examine the association between fish and fish oil consumption in adolescence and midlife and coronary heart disease later in life among Icelandic women.

Design: Case-control study nested in the Reykjavik-AGES cohort.

Setting: The AGES-Reykjavik Study.

Participants: 3326 Icelandic women aged 69-95 years, who took part in the AGES-Reykjavik study.

Methods: After clinical examination, the women were divided into CHD cases and controls at entry, based on presence of coronary heart disease (CHD) defined by abnormal ECG, answers to the Rose angina questionnaire and previous heart procedures. Fish and fish oil consumption were evaluated retrospectively by the subjects with a validated food frequency questionnaire.

Main outcome measure: Multivariate logistic regression was used to estimate adjusted odds ratios (AORs) and 95% confidence intervals (95% CI) of CHD according to fish or fish oil exposure status. Adjustments were made for age, education, smoking, family history of heart disease, physical activity, alcohol consumption, cholesterol levels, diabetes, hypertension and concurrent dietary factors.

Results: 406 (13.5%) of the women had developed CHD at entry. Compared to women with no teenage intake of fish oil, women who consumed fish oil 3-6 times per week had a decreased risk of CHD (AOR 0.59; CI 95% 0.40 - 0.91) as were women with daily consumption (AOR 0.75; 95% CI 0.58 – 0.97). Similar findings were observed for midlife consumption for fish oil. No beneficial association was observed for teenage fish consumption and CHD. No association was observed for teenage fish intake and CHD whereas medium consumption of fish during midlife (more than two portions up to four per week) presented increased risk of CHD (AOR 1.65 ; 95% CI 1.08-2.52)

Conclusions: Fish oil consumption, both in adolescence and midlife stages may reduce the risk of CHD in older women.

Introduction

The role of lifestyle and dietary patterns in the development of coronary heart disease (CHD) is relatively well established.[16 19] Consumption of fish and fish oil has specifically been associated with reduced risk of CHD.[14 22 23] Omega-3 long chain polyunsaturated fatty acids (LC-PUFA) from fish and fish oil may reduce the odds of developing CHD through a variety of mechanisms such as by preventing arrhythmias, being antithrombotic, mildly hypotensive as well as through hypolipodemic properties.[31]

Most of the knowledge on the impact of fish and fish oil consumption in relation to CHD comes from studies on males only. The few studies that have been done on women have generated conflicting results.[15 37 39 40] Further, data are scarce on the impact of fish and fish oil intake in early life on CHD in later life. However, early life exposures such as diet and overweight may have considerable impact on CHD risk in later life as fatty streaks in arteries of children and young adults have been reported to increase with severity of early life risk factors.[7 8 43]

Nested in a population known for its high intake of fish and fish liver oil, the aim of this study is to assess the impact of fish and fish liver oil consumption in teenage years and midlife on the risk of CHD in older Icelandic women.

Methods

Study design and population

This is a case-control study nested within the Reykjavik-AGES cohort of the Icelandic Heart Association. The data consist of 3,326 women aged 66-96 years derived from the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavik) study population. The AGES-Reykjavik study originated from the Reykjavik Study which started in 1967 with 30,795 men and women born between 1907 and1935 and living in Reykjavik at that time.[44] The Reykjavik Study has been described in details elsewhere.[45] The main aim of the AGES-Reykjavik is to examine risk factors, including genetic susceptibility and gene/environment interaction, in relation to disease and disability in old age.

The data collection for the AGES-Reykjavik study started in 2002. At that time, 11,549 members from the Reykjavik Study were still alive. A random sample of 5,764 men (42%) and women (58%) was recruited to participate. Examinations began in 2002 and finished in 2006 and consisted of three clinic visits within a 4-6 week time window. In the first visit, the participants, among other things, underwent clinical examination and filled out an extensive questionnaire including questions on food intake. The clinical visits have been described in details elsewhere.[44]

Assessment of fish and fish oil consumption

During the first visit participants answered questions on current diet, midlife diet (between the age of 40 to 50) and teenage diet (between the age of 14 to 19) using a food frequency questionnaire (FFQ) designed for this project. Two questions regarding fish consumption in the FFQ were used for this analysis. The first one was about frequency of fish meals and the second one about fish (herring, salmon) as topping on bread and in salad. Possible responses to the questions were; 1) never, 2) less than once a week, 3) 1-2 times a week, 4) 3-4 times a week, 5) 5-6 times a week 6) daily, and 7) more than once a day. The total fish consumption was estimated by computing the variables from these two questions and combining them into one variable. Numerical values were obtained by converting the weekly average estimates into daily estimates. Never became zero fish per day, less than once a week became 0.07 fish dose per day, 1-2 times per week became 0.21 fish dose per day, 3-4 times a week became 0.5 fish dose per day, 5-6 times per week became 0.79 fish dose per day, daily became 1 fish dose per day and more than once a day became 1.5 dose per day. The standard dose for a single fish meal was defined as 150 grams and fish on bread as 40 grams. These values were obtained from the National Nutrition Survey 2002.[46] The estimated proportion of fish on bread/salad of a total fish meal became 0.27, or 40/150. The converted numerical value of fish on bread was therefore multiplied with 0.27 and that value computed with the converted value of fish meal per day. The total outcome was multiplied with 7 to gain the total consumption per week. Values of total fish consumption were categorized into three groups, two portions and less per week, more than two portions up to four per week and four portions and more per week.

Fish liver oil (cod or saithe liver) supplements (liquid or capsules) were evaluated with one question with the same response alternatives as fish. Fish oil consumption was categorized

into four categories in table 4, 1) Never 2) less than 2 times per week, 3) 3-6 times per week and 4) daily. Fish oil consumption was also categorized as low (1-2 times a week and less) and high (3-4 times a week and more) for data analysis shown in tables 2 and 3.

Coronary heart disease – outcome assessment

The end point for this study was CHD according to finding on electrocardiogram (ECG), responses to Rose Angina questionnaire, and questions concerning previous heart procedures. Coronary events were defined according to standardized Minnesota Codes[47] and considered present if one or more of the codes 1:1:1 – 1:1:7, 1:2:1–1:2:8 and 9:2:0 were evident. The Rose questionnaire was introduced in 1962 as a standardized method of measuring angina and myocardial infarction. It defines angina as chest pain that limits exertion, is situated over the sternum or in the left chest and left arm, and disappears within 10 minutes by rest.[48] CHD was also confirmed if participants ever had a coronary bypass surgery, heart bypass or CABG or angioplasty of coronary arteries.

Covariate assessment

Information on possible confounders was retrieved from questionnaires filled out at entry into the Reykjavik Study and the AGES-Reykjavik.

From the Reykjavik Study information was obtained on diabetes, hypertension, total blood cholesterol and BMI when participants were in their midlife period. Diabetes (type 1 and 2) was either self reported or based on abnormally high glucose value after overnight fasting. Blood pressure was measured with a mercury sphygmomanometer, with a large cuff and the mean value of two blood pressure measurements from separate occasions was used for reference. Blood pressure measurements were divided into no hypertension (sys<120 and did<80), pre hypertension (sys >140 or 80 ≤ did<90) and hypertension (sys ≥140 or did >=90). Blood chemistry was measured with a Technicon Auto-Analyser after overnight fasting. Body Mass Index was calculated from weight and height measured at the same time point by standardized methods.

From AGES-Reykjavik we retrieved information on age at entry, level of education, smoking status, family history of heart disease, physical activity in teen and midlife and midlife alcohol consumption and dietary habits in youth, midlife and at present time. Education was categorized into elementary school, secondary school, college education (including junior college, business- and teacher's school) and university education. Smoking status was categorized into being current, previous smoker or never. Current and previous smokers were defined as having at least smoked 100 cigarettes or 20 cigars during lifetime. Family history of coronary disease was recorded if father, mother, siblings or children had coronary thrombosis. Variables of physical activity was pooled for midlife and early adulthood into never, rarely, occasionally, moderate and high. Responses for alcohol consumption in midlife were daily, 2-3

times a week, once a week, 2-3 times a month, less than once a month and never. 41 women reported not knowing the amount of alcohol consumed in midlife and were excluded in this analysis.

Possible dietary covariates were considered to be consumption of fruits, vegetables, milk products and amount of spread used on bread. For fruit and vegetables and milk there were seven responses possible, the same as for fish and fish oil consumption. Available response options for usual amount of spread used on bread were four, 1) usually did no use spread on bread 2) little 3) medium and 4) much. There were pictures showing the relevant amount of spread on bread for category 2, 3 and 4.

Statistical Analyses

325 women were excluded from the analysis due to missing data on either heart disease, fish and/or fish oil consumption, leaving 3001 women in the study.

We used Chi-square tests and their corresponding p-values to estimate different characteristics of women with and without CHD, fish and fish oil consumers in midlife and fish and fish oil consumers in adolescence. P-values ≤ 0.05 were considered statistically significant. Taking into account the influence of potential covariates, we used logistic regression to calculate adjusted odds ratios (AOR) with 95% confidence interval (CI), of CHD by differential fish and fish oil consumption, for midlife and teenage consumption separately. In the first model we adjusted for age (as a continuous variable) at entry to the AGES-Reykjavik. In the second model we adjusted for age (as in the first model), education (three categories, 1) primary, 2) secondary 3) college and university), smoking status, physical activity (three categories, never or rarely, occasionally, moderate or high), alcohol consumption (three categories, 1) never , 2) monthly 3) weekly), fish consumption 1) two portions and less per week 2) more than two portions up to four per week and 4) more than four portions per week, and fish oil consumption 1) never, 2) less than two times per week, 3) 3-6 times per week and 4) daily. In the third model we adjusted for the same variables as we did in the second model and added vegetable-, fruit- and milk consumption as well as the amount of spread used on bread. Midlife consumption for fruit and vegetables was divided into two categories (two times or less per week and three times and more per week). Midlife milk consumption was divided into two categories (less than daily and daily and more). Amount of spread was divided into two categories (never-little vs. medium-much). Teenage consumption for fruit and vegetables was divided into three categories (never, less than one time per week and more than one time per week). Teenager consumption for milk

consumption was also divided into three categories (less than daily, daily, and more than daily). The categories for fruit, vegetables and milk in adolescence are different from the midlife categories due to different consumption pattern and availability of foods. The fourth model was only calculated for midlife since variables were not available for teenage years. Adjustments were made for same factors as in model 3 plus diabetes, hypertension, total blood cholesterol, categorized into high (6 mmol/l ≤) and low (6 mmol/l >) and BMI (categorized into <30 and ≥ 30).

The two variables for fish oil consumption in midlife and adolescence were pooled in one variable with four categories to estimate the effect of longitudinal fish consumption on CHD outcomes. The first category included low consumption (two times or less per week) both in adolescence and midlife. The second one included low consumption in adolescence and high in midlife (3-4 times per week and more). The third one had high consumption in adolescence and low in midlife. The fourth had high consumption for both periods. Analysis for this variable was adjusted for same factors as for model 2 described above.

PASW version 18, Release Version 18.00 was used for all statistical analyses (SPSS, Inc., 2009, Chicago, IL, www.spss .com)

The study was approved by the Icelandic National Bioethics Committee (VSN: 00-063).[44]

Results

Out of 3001 women included in this analysis, 408 (13.5%) had either current or previously known CHD at entry into the AGES-Reykjavik study, while 2593 women had no history of CHD.

Major characteristics of women with and without CHD are presented in table 1. Secondary education was most common both for women with and without CHD, but higher proportion of the latter had gone to College/University. A higher proportion of women with CHD were previous smokers but no differences were observed with respect to alcohol consumption. Higher proportion of women with CHD had a family history of heart disease, high serum cholesterol and hypertension. Little difference between the groups was found concerning fish consumption in midlife. Majority of both groups were in the middle category of fish consumption (more than two portions up to four per week). Women without CHD had slightly higher daily fish oil intake. Women with known CHD ate less fruit and vegetables in midlife. Same pattern was found for teenage diet, with the exception regarding consumption of vegetables, which was similar between the groups.

Table 2 shows background characteristics of fish and fish oil consumers in midlife. The group of women with the highest fish intake (more than 4 portions per week) had the highest proportion of College/University education and moderate-high level of exercise. Women with the highest intake of fish were also most likely to have never smoked and they had higher prevalence of hypertension compared to women with lower intake. And finally women in the highest fish intake category had higher consumption of fruits, vegetables and milk and daily intake of fish oils.

Women with high fish oil intake in midlife were more likely to have higher level of education, non-smoking status, and high-moderate levels of exercise in midlife and early adulthood, and higher intake of fish, fruit, vegetables and milk.

Table 3 shows background characteristics of fish and fish oil consumers during teenage years. Women in the middle category of fish consumption were less likely to be current smokers compared to those who consumed less and more. They also had the highest proportion of fruit and vegetable consumption. Women with lowest intake of fish (less than two portions per week) had higher intake of fruit and vegetable consumption compared to those with high intake of fish. High consumers of fish as teenagers also reported highest daily intake of fish oil.

Looking at characteristics of fish oil consumers in adolescence we see that high consumption of fish oil was related to higher incidence of moderate-high exercise in midlife and early adulthood. Women who reported low consumption of fish oil were more likely to report never having had fruit and vegetables as teenagers. 43 % of women with high consumption of fish oil consumed high amounts of fish as teenagers, while 37 % of those with low consumption of fish oil had high fish intake.

Table 4a presents multivariate analysis on the association between CHD and fish and fish oil consumption in adolescence. Three logistic regression models were used to calculate odds ratio for CHD using fish oil and fish consumption as a predictor. No significant association was observed regarding fish consumption. All three models showed significant protective association between CHD and fish oil intake in adolescence. Using women with no intake of fish oil as a reference group, those with consumption of 3-6 times per week had an AOR of 0.57 (95% CI 0.38 – 0.85) to develop CHD in model 1, 0.56 (95% CI 0.36-0.87) in model 2 and 0.59 (95% CI 0.40 – 0.91) in model 3. Women with daily consumption had an AOR of 0.75 (95% CI 0.59 –

0.95) for developing CHD in model 1, 0.77 (95% CI 0.59 -0.99) in model 2 and 0.75 (CI 95% 0.58 - 0.97) in model 3.

Table 4b presents multivariate analysis for fish and fish oil consumption in midlife. The three first models have the same adjustment factors as the first three models in table 4a. The fourth model also includes well established risk factors for CHD (measured in midlife). Diet variables used for adjustment refer to midlife diet. Midlife's fish oil consumption was associated with decreased risk of CHD. For age adjusted analysis only, model 1 shows that women who consumed fish oil 3-6 times per week were less likely to develop a CHD (OR 0.54, 95% CI 0.37-0.79), compared to women who never consumed fish oil. Women with daily consumption were also less likely to have CHD (OR 0.70 95% CI 0.55 - 0.89). Model 2 and 3 showed similar trend for women who consumed fish oil 3-6 times per week and daily. Model 4 shows that women who consumed fish oil 3-6 times per week (OR 0.57, 95% CI 0.37 – 0.88) and daily (OR 0.76, 95% CI 0.59 -0.99) were less likely to develop CHD compared with those who never consumed fish oil.

Statistically significant increased risk for CHD was found for women in the middle category of fish consumption in all of the models (same models as described above) except when adjusting only for age at entry, using lowest fish intake category as a reference group. Model 2 showed AOR 1.54 (95% CI, 1.02-2.32), model 3 AOR 1.59 (95% CI, 1.05- 2.41) and model 4 AOR 1.65 (95% CI, 1.08-2.52).

Multivariate analysis for pooled fish oil consumption in midlife and as teenagers revealed that women who consumed high (3 times and more) amounts of fish oil in both adolescence and midlife were less likely to develop CHD compared to women who consumed low (2 times and less) amounts in both periods (AOR 0.65 95% CI 0.49 – 0.88). No significant association was found for women who had low consumption as teenagers and high in midlife or vice versa (data

not shown in table). Adjustments were made for age, education smoking status, and alcohol consumption in mid life, physical activity in midlife and early adulthood, and family history of heart disease. 37% of the participants had high intake of fish oil in both adolescence and midlife.

Discussion

In this study nested within a population with high consumption of both fish and fish oil, we found early and midlife consumption of fish oil to be protective against the risk of coronary heart disease in older women. We did not observe similar protective effects for fish consumption. On the contrary, women who consumed more than 2 up to 4 portions of fish in midlife fish had higher risk estimates than those with less frequent fish intake.

The protective effects of fish oil, consumed either in adolescence or midlife, on CHD risk, are intriguing. In adolescence, women who consumed fish oil 3-6 times per week were 41% less likely to develop CHD and women with daily consumption were 25% less likely, than those who never consumed fish oil. This association was independent of age at entry, education, smoking status, physical activity in adolescence and midlife, family history of heart disease and several dietary factors in adolescence. For midlife, women who consumed fish oil 3-6 times per week were 43% less likely to develop a CHD and daily consumption yielded in 24% risk reduction, compared with those who never consumed fish oil. This association was independent of age at entry, education, smoking status, physical activity in adolescence and midlife, family history of heart disease and several dietary factors in adolescence as well as major established risk factors of CHD and intake of several dietary factors in midlife. Our findings further imply that the main protection of fish oil consumption is achieved by taking cod liver oil during both adolescence and midlife.

Our findings on the protective effect of taking fish oil both in early life and midlife could be explained by several factors. Physiologic effects of omega-3 LC-PUFA are believed to differ according to dose- and time. Dietary intake of < 1g/d seems to have anti-arrhythmic benefits and risk reduction of cardiac death within weeks to months. Conversely, triglyceride lowering effects

might require months or years before clinical outcomes are evident.[49] The protective outcome for both periods could therefore be due to both long term and short term consumption. This may suggest that long term consumption of fish liver oil is important to protect women from developing CHD via mechanism that requires long time consumption to show effect, perhaps such as calcification of arteries or lipid profile. 37% of the study participants took fish liver oil more than three times per week at both periods. To our knowledge, such long term use of cod liver oil has not been studied previously, as few populations have used cod liver oil to this extent and for this long period of time as Icelanders have.

Furthermore, our findings show that consuming fish oil 3-6 times per week is more protective than daily consumption, for both periods. There is evidence that compared with little or no intake, modest consumption (250 mg/d) of marine omega-3 fatty acids lowers risk of cardiac mortality, whereas higher intake may not substantially lower risk any further, suggesting a threshold of effect.[50] In support of this hypothesis, an Icelandic study on the relationship between high consumption of marine fatty acids in early pregnancy and hypertensive disorders in pregnancy showed that consumption of high doses of n-3 LC-PUFA in early pregnancy, or other nutrients found in liquid cod-liver oil, may increase the risk of developing hypertensive disorders in pregnancy.[51]

We did not observe any protection of fish consumption – at either life stage – on the risk for CHD. In fact, consuming more than two portions up to four per week in midlife was associated with higher risk of CHD compared with consuming fish two portions or less per week. Possibly, this rather unexpected result may be explained by similar fish intake in the two groups of women, both those with CHD and those without CHD in both periods (see table 1). This suggests that some residual confounding remains despite the multiple adjustments. It should be emphasized that not all known risk factors for CHD were available for these women, notably not

the HDL/LDL ratio which is believed to be a more valid risk factor for CHD than total serum cholesterol. Neither were all dietary variables available from the FFQ, which only covered selected, major foods. Thus, the intake of melted stick margarine, high in *trans* fatty acids and most commonly used as condiment with fish meals in Iceland during the 1970ies and 1980ies, could not be adjusted for. According to the TRANSFAIR study, Icelanders had by far the highest *trans* fatty acid intake of all 14 participating countries[52] and Icelandic margarine was the highest in trans fatty acids of all margarine products measured in this European study.[53] *Trans* fatty acids can increase the risk of CHD in more ways than just through increased total cholesterol,[54] which was adjusted for in study, and thus they may be a plausible explanation for the increased risk with high fish consumption. In our study, the use of margarine or other fat, except for bread spread, was not included in the FFQ. We also do not have information on how the fish was cooked and women with CHD and without CHD could also differ in that way. Furthermore, information on the type of fish consumed as a main meal is lacking in our study which may be another possible explanation for not finding a protective effect of fish. Haddock, which is by far the most common fish type in the diet of Icelanders, is quite lean and contains consequently lower amounts of omega-3 LC-PUFA.[25] Fish consumed in Iceland may therefore not contribute much of omega-3 LC-PUFA in spite of its abundance in the diet. According to the national nutrition survey, cod liver oil contributes the greatest proportion of omega-3 fatty acids in the Icelandic diet, or 41% of total intake, which is much higher than in most western countries.[46 55] Other possible explanation for residual confounding could be that we only adjust for smoking status, not the amount of cigarettes currently or previously smoked.

Results from earlier studies on women and fish and omega-3 fatty acids have been inconsistent. Hu and colleges[37] reported that most beneficial effects found in fish intake on fatal and non-fatal CHD was consuming fish 2-4 times a week, compared to eating fish less than once

a month. Possibly the putative beneficial effect cannot be found in our study population since we lack a reference group of participants who seldom or never consume fish. The high and widespread fish intake of Icelandic women during these periods might therefore not be optimal to test the hypothesis. In our study the lowest fish intake category used for reference is defined as those who had fish two times or less per week. This is the amount that has been recommended to prevent CHD, and therefore a beneficial threshold level might already have been reached by the reference group. However, Jarvinen[39] and Folsom and Demissie[15] did not report any beneficial association between fish consumption and consumption of omega-3 fatty acids from diet, and fatal and non-fatal CHD, despite having a reference group with very low consumption. Osler et al showed insignificant increased risk for women who consumed fish two times or more per week, compared with women who consumed fish once a week. They found this relation to be unclear and blame some sort of residual confounding for the finding.[40] Their reference group of eating fish once a week might not have been optimal either. The most advantageous dose of fish for protective effect of CHD in women is therefore not clear.

All of the studies above on fish consumption and CHD in women adjust for multiple covariates in their statistical analysis, although none of studies use exactly the same variables as we do. Adjusting for major established risk factors may be subject to debate as fish and fish oil consumption are believed to have effect on those factors.[14 56 57] making them possibly effect modifiers rather than true confounders. The fact that our results are significant despite multiple adjustments, including BMI, diabetes, hypertension and total serum cholesterol may suggest a true causal relationship. The design of the studies is prospective which minimizes recall bias or dietary changes due to known disease, and the exposure precedes the outcome. However, during the often long follow-up, diet and risk factor can change and the results might be biased in that respect. Hu and colleges[37] used cumulative average of dietary intake from question list that were

submitted five times in 16 years of follow-up. The same method was used to estimate covariates. Information on covariates and questionnaires on food from different time periods used in our study might therefore give broader information on the causality.

There are few studies on early life dietary factors and CHD risk in later life. Research on children's diet in the Boyd Orr cohort from 1939 did not find any beneficial relationship between diet, including fish, and CHD later in life.[21] Other studies have shown that risk factors for CHD can develop early and affect formation of atherosclerosis. The amount of fatty streaks and fibrous plaques found in coronary arteries and the aortas of young people in autopsy did strongly correlate with increased body-mass index, systolic and diastolic blood pressure, serum concentrations of total cholesterol, triglycerides, low-density lipoprotein cholesterol, and decreased high-density lipoprotein cholesterol.[7] Another study showed that increased body max index in childhood and young adult life, as well as increased blood pressure and decreased HDL cholesterol levels in young adult life was associated with increased coronary artery calcification in autopsy.[8] The risk reduction found in our study for fish oil consumption in adolescence could therefore be due to less calcification in arteries, which is one of several mechanism that fish oil is believed to protect against CHD.[31]

Data on early life dietary exposures to fish and fish oil as well as detailed ascertainment of CHD outcomes later in life are limited and our study is therefore unique in that respect. Our study further benefits from prospective assessment of a multitude of important midlife covariates, e.g. blood pressure, cholesterol, diabetes rates and BMI, in the Reykjavik Study, as well as information retrieved in older age (when the same participants enter the AGES-Reykjavik). This includes comprehensive information on smoking habits during the life span, family history of CHD and education level. Adjusting our models for a multitude of potential confounding factors did not change our estimates significantly. Nevertheless, we cannot exclude

the possibility that some of our findings may be accounted for by unmeasured confounders. For instance, the fact that consistent fish oil consumption in both life stages carries a strong protective effect may be suspected to be due to healthy general lifestyle. However, adjusting for level of physical activity and other measures potentially associated with health consciousness or a healthy lifestyle did not alter our results. Furthermore, the protective effects of fish oil intake leveled off beyond 3-4 times per week which may suggest that occasional users – but not only persistent users – gain from their consumption. Possibly higher intakes may even be associated with less benefit, as studies on hypertension in pregnant women have suggested. .

Further we suggest that the increased risk for CHD associated with midlife consumption of fish may be attributed, among other; to confounding of *trans* fatty acids intake combined with the high fish intake of the reference group, which may already have reached the threshold level for possible protective effect of fish in our analysis.

Regarding the information on dietary habits in AGES-Reykjavik, our study is likely to suffer somewhat from recall bias since the women were asked to recall their diet many decades earlier. However a recent validation study of the FFQ used in the AGES-Reykjavik study shows that the question on fish oil consumption during midlife ranks subjects acceptably according to intake. When comparing retrospective food frequency assessments to current assessment (only available for a small subsample) the highest correlation of all food items was obtained for fish oil consumption in midlife ($r = 0.58$, $p = 0.001$). The question on midlife fish consumption showed lower correlation, but was still within acceptable range ($r = 0.281$, $p = 0.004$).[58] The questions on fish and fish consumption in adolescence have not been validated and studies on validity of answers of elderly people asked to recall their diet more than 10 years back in time are limited.[59] [60] Nevertheless, in order to be detrimental for our results, recall bias needs to be different between CHD cases and non-cases. We cannot exclude the possibility that women with long

history of CHD may have responded differently from those without CHD. The Icelandic Heart Association has a long reputation of valid endpoint assessment of CHD.[44] Still, the Rose angina questionnaire, used partly for case detection in this study, has been reported to lack specificity when diagnosing CHD in women.[61 62] As a result, the control group may contain women with undiagnosed CHD.

Lastly, different types of local fish oils consumed in adolescence may have varied in nutrient composition; this is however unlikely to affect our results since the variation is probably not differential across outcome categories (cases and controls). Similarly, the production of capsule containing fish oil started in the early 70's; capsule consumption usually carries smaller amount of fish oil than intake of the liquid itself and we cannot disentangle whether the midlife consumption of the women in our study consisted of capsules or liquid – or the amount of liquid consumed (teaspoon vs. tablespoon).

With few existing studies on early life dietary factors and CHD, our study provides important evidence for the potential preventive role of fish oil consumption on the development of CHD in women. Our results suggest that moderate early- and midlife fish oil consumption may be protective against the development of CHD in women. If confirmed in future studies – preferably with prospective ascertainment of fish oil consumption – these findings may have significant public health implications. Our study does not confirm previous findings on beneficial effect of fish consumption on CHD risk; if our findings of increased risk are replicated in future studies, further research in needed to understand the potential mechanism. Our findings on protective association of fish oil consumption in adolescence and CHD later in life, suggests that some CHD risk factors might develop early and that preventive measures could be initiated already in early life.

Acknowledgements

We thank the AGES-Reykjavik Study executive committee and the Icelandic Heart Association for their contribution to this study and access to their data. We also think Guðmundur Guðmundsson at Lýsi hf for sharing his knowledge on Icelandic fish oil production in Iceland in the past.

Disclosure of interest

No conflicts of interest are declared

Contribution to Authorship

All authors participated in designing and planning the study. AH drafter the manuscript and all authors critically revised the manuscript for important intellectual content. AH and JET were responsible for analyzing the data. The final version to be published has been approved by all authors.

Details of ethics approval

The study was approved by the Icelandic National Bioethics Committee (VSN: 00-063).

References

1. Materns C, Boerma T, and Ma Fat D. The global burden of disease 2004 update. Geneva: World Health Organization, 2008.
2. Number of deaths in 2008 by causes of death, age and gender (ICD-10). Reykjavik: Directorate of Health Iceland, 2010.
3. OECD. *Health at a Glance 2009*: OECD Publishing, 2009.
4. Gaziano TA, Bitton A, Anand S, Abrahams-Gessel S, Murphy A. Growing epidemic of coronary heart disease in low- and middle-income countries. *Curr Probl Cardiol*;35(2):72-115.
5. Fauci AS. *Harrison's principles of internal medicine : editors, Antony S. Fauci [et al.]*. New York McGraw-Hill, 2008.
6. Berenson GS, Srinivasan SR, Hunter SM, Nicklas TA, Freedman DS, Shear CL, et al. Risk factors in early life as predictors of adult heart disease: the Bogalusa Heart Study. *Am J Med Sci* 1989;298(3):141-51.
7. Berenson GS, Srinivasan SR, Bao W, Newman WP, 3rd, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *N Engl J Med* 1998;338(23):1650-6.
8. Mahoney LT, Burns TL, Stanford W, Thompson BH, Witt JD, Rost CA, et al. Coronary risk factors measured in childhood and young adult life are associated with coronary artery calcification in young adults: the Muscatine Study. *J Am Coll Cardiol* 1996;27(2):277-84.
9. Pilote L, Dasgupta K, Guru V, Humphries KH, McGrath J, Norris C, et al. A comprehensive view of sex-specific issues related to cardiovascular disease. *CMAJ* 2007;176(6):S1-44.
10. Jacobs AK. Women, ischemic heart disease, revascularization, and the gender gap: what are we missing? *J Am Coll Cardiol* 2006;47(3 Suppl):S63-5.
11. Jacobs AK. Coronary intervention in 2009: are women no different than men? *Circ Cardiovasc Interv* 2009;2(1):69-78.
12. Gierach GL, Johnson BD, Bairey Merz CN, Kelsey SF, Bittner V, Olson MB, et al. Hypertension, menopause, and coronary artery disease risk in the Women's Ischemia Syndrome Evaluation (WISE) Study. *J Am Coll Cardiol* 2006;47(3 Suppl):S50-8.
13. Jonsdottir LS, Sigfusson N, Gudnason V, Sigvaldason H, Thorgeirsson G. Do lipids, blood pressure, diabetes, and smoking confer equal risk of myocardial infarction in women as in men? The Reykjavik Study. *J Cardiovasc Risk* 2002;9(2):67-76.
14. Kris-Etherton PM, Harris WS, Appel LJ, American Heart Association. Nutrition C. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 2002;106(21):2747-57.
15. Folsom AR, Demissie Z. Fish intake, marine omega-3 fatty acids, and mortality in a cohort of postmenopausal women. *Am J Epidemiol* 2004;160(10):1005-10.
16. Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. *JAMA* 2002;288(20):2569-78.
17. Gibney JM, Margetts, B.M, Kearney, J.M. and Arab, L., editor. *Public Health Nutrition*. Oxford: Blackwell Publishing, 2008.
18. Rothman KJ. *Epidemiology. An Introduction*. New York: Oxford University Press, 2002.
19. Mente A, de Koning L, Shannon HS, Anand SS. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. *Arch Intern Med* 2009;169(7):659-69.
20. Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. *Am J Clin Nutr*;91(3):535-46.
21. Ness AR, Maynard M, Frankel S, Smith GD, Frobisher C, Leary SD, et al. Diet in childhood and adult cardiovascular and all cause mortality: the Boyd Orr cohort. *Heart* 2005;91(7):894-8.

22. Marckmann P, Gronbaek M. Fish consumption and coronary heart disease mortality. A systematic review of prospective cohort studies. *Eur J Clin Nutr* 1999;53(8):585-90.
23. He K, Song Y, Daviglus ML, Liu K, Van Horn L, Dyer AR, et al. Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. *Circulation* 2004;109(22):2705-11.
24. Steingrímsdóttir L, Thorgeirsdóttir H, Aegisdottir S. *Icelandic National Nutrition Survey 1990. I Main findings. Icelandic* Reykjavik: Nutrition Council, 1991.
25. Steingrímsdóttir L, Thorgeirsdóttir H, Aegisdottir S. *Icelandic National Nutrition Survey 1990. II Food and Culture. Icelandic* Reykjavík: Nutrition Council, 1992.
26. Sigurjónsson J. Diet and helth in Iceland.In Icelandic [Mataræði og heilsufar á Íslandi] Reykjavik Manneldisráð Íslands, 1943.
27. The Public Health Institute of Iceland. Food based dietary guidelines. [Cited May 2010] Available from lydheilsustod.is. Reykjavík: The Public Health Institute of Iceland 2006.
28. Harper CR, Jacobson TA. The fats of life: the role of omega-3 fatty acids in the prevention of coronary heart disease. *Arch Intern Med* 2001;161(18):2185-92.
29. Burdge GC, Calder PC. Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev* 2005;45(5):581-97.
30. Sandström B, Aro A, Becker W, Lyhne N, Pedersen JI, Þórsdótttir I. *Norrænar ráleggingar um næringarefni*. Reykjavík Rannsóknastofa í næringarfræði, Háskólaútgáfan, 1999.
31. Connor WE. Importance of n-3 fatty acids in health and disease. *Am J Clin Nutr* 2000;71(1 Suppl):171S-5S.
32. Mozaffarian D. Fish and n-3 fatty acids for the prevention of fatal coronary heart disease and sudden cardiac death. *Am J Clin Nutr* 2008;87(6):1991S-6S.
33. Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N Engl J Med* 1985;312(19):1205-9.
34. Mann JI, Appleby PN, Key TJ, Thorogood M. Dietary determinants of ischaemic heart disease in health conscious individuals. *Heart* 1997;78(5):450-5.
35. Norell SE, Ahlbom A, Feychting M, Pedersen NL. Fish consumption and mortality from coronary heart disease. *Br Med J (Clin Res Ed)* 1986;293(6544):426.
36. Fraser GE, Sabate J, Beeson WL, Strahan TM. A possible protective effect of nut consumption on risk of coronary heart disease. The Adventist Health Study. *Arch Intern Med* 1992;152(7):1416-24.
37. Hu FB, Bronner L, Willett WC, Stampfer MJ, Rexrode KM, Albert CM, et al. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA* 2002;287(14):1815-21.
38. Mozaffarian D, Lemaitre RN, Kuller LH, Burke GL, Tracy RP, Siscovick DS, et al. Cardiac benefits of fish consumption may depend on the type of fish meal consumed: the Cardiovascular Health Study. *Circulation* 2003;107(10):1372-7.
39. Jarvinen R, Knekt P, Rissanen H, Reunanen A. Intake of fish and long-chain n-3 fatty acids and the risk of coronary heart mortality in men and women. *Br J Nutr* 2006;95(4):824-9.
40. Osler M, Andreasen AH, Hoidrup S. No inverse association between fish consumption and risk of death from all-causes, and incidence of coronary heart disease in middle-aged, Danish adults. *J Clin Epidemiol* 2003;56(3):274-9.
41. Hu FB, Cho E, Rexrode KM, Albert CM, Manson JE. Fish and long-chain omega-3 fatty acid intake and risk of coronary heart disease and total mortality in diabetic women. *Circulation* 2003;107(14):1852-7.
42. Erkkila AT, Lichtenstein AH, Mozaffarian D, Herrington DM. Fish intake is associated with a reduced progression of coronary artery atherosclerosis in postmenopausal women with coronary artery disease. *Am J Clin Nutr* 2004;80(3):626-32.

43. Berenson GS, Srinivasan SR, Nicklas TA. Atherosclerosis: a nutritional disease of childhood. *Am J Cardiol* 1998;82(10B):22T-29T.
44. Harris TB, Launer LJ, Eiriksdottir G, Kjartansson O, Jonsson PV, Sigurdsson G, et al. Age, Gene/Environment Susceptibility-Reykjavik Study: multidisciplinary applied phenomics. *Am J Epidemiol* 2007;165(9):1076-87.
45. Jonsdottir LS, Sigfusson N, Sigvaldason H, Thorgeirsson G. Incidence and prevalence of recognised and unrecognised myocardial infarction in women. The Reykjavik Study. *Eur Heart J* 1998;19(7):1011-8.
46. Steingrímsdóttir L, Thorgeirsdóttir H, Ólafsdóttr AS. *Diet of Icelanders : dietary survey of The Icelandic Nutrition Council 2002 : main findings. In Icelandic* Reykjavik: Manneldisráð Íslands 2002.
47. Prineas R, Crow, R, and Blackburn H. The Minnesota Code Classification system for Electrocardiographic Findings. [Cited May 2010] Available from http://www.sph.umn.edu/epi/ecg/mncode.pdf: University of Minnesota, School of Public Health, 1982.
48. Rose GA. The Diagnosis of Ischaemic Heart Pain and Intermittent Claudication in Field Surveys. *Bull Wld Hlth Org.* 1962;27:645-58.
49. Mozaffarian D. Fish, mercury, selenium and cardiovascular risk: current evidence and unanswered questions. *Int J Environ Res Public Health* 2009;6(6):1894-916.
50. Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health: evaluating the risks and the benefits. *JAMA* 2006;296(15):1885-99.
51. Olafsdottir AS, Skuladottir GV, Thorsdottir I, Hauksson A, Thorgeirsdottir H, Steingrimsdottir L. Relationship between high consumption of marine fatty acids in early pregnancy and hypertensive disorders in pregnancy. *BJOG* 2006;113(3):301-9.
52. van de Vijver LP, Kardinaal AF, Couet C, Aro A, Kafatos A, Steingrimsdottir L, et al. Association between trans fatty acid intake and cardiovascular risk factors in Europe: the TRANSFAIR study. *Eur J Clin Nutr* 2000;54(2):126-35.
53. Aro. TransFatty Acids in Dietary Fats and Oils from 14 European Countries : The TRANSFAIR Study * 1. *Journal of Food Composition and Analysis* 1998;11(2):137.
54. Wilson TA, McIntyre M, Nicolosi RJ. Trans fatty acids and cardiovascular risk. *J Nutr Health Aging* 2001;5(3):184-7.
55. Olafsdottir AS, Thorsdottir I, Wagner KH, Elmadfa I. Polyunsaturated fatty acids in the diet and breast milk of lactating icelandic women with traditional fish and cod liver oil consumption. *Ann Nutr Metab* 2006;50(3):270-6.
56. Thorsdottir I, Tomasson H, Gunnarsdottir I, Gisladottir E, Kiely M, Parra MD, et al. Randomized trial of weight-loss-diets for young adults varying in fish and fish oil content. *Int J Obes (Lond)* 2007;31(10):1560-6.
57. Ramel A, Martinez JA, Kiely M, Bandarra NM, Thorsdottir I. Moderate consumption of fatty fish reduces diastolic blood pressure in overweight and obese European young adults during energy restriction. *Nutrition*;26(2):168-74.
58. Eysteinsdottir T, Gunnarsdottir, I, Thorsdottir, I and Steingrimsdottir, L. Validity of retrospective diet history: Assesing the recall of midlife diet using a Food Frequency Questionnaire in old age. Unpublished manuscript, 2010.
59. Rohan TE, Potter JD. Retrospective assessment of dietary intake. *Am J Epidemiol* 1984;120(6):876-87.
60. van Staveren WA, West CE, Hoffmans MD, Bos P, Kardinaal AF, van Poppel GA, et al. Comparison of contemporaneous and retrospective estimates of food consumption made by a dietary history method. *Am J Epidemiol* 1986;123(5):884-93.

61. Harris RB, Weissfeld LA. Gender differences in the reliability of reporting symptoms of angina pectoris. *J Clin Epidemiol* 1991;44(10):1071-8.
62. Nicholson A, White IR, Macfarlane P, Brunner E, Marmot M. Rose questionnaire angina in younger men and women: gender differences in the relationship to cardiovascular risk factors and other reported symptoms. *J Clin Epidemiol* 1999;52(4):337-46.

Table 1. Characteristics of women with and without CHD*‡			p-value¤
N = 3001	**With CHD**	**Without CHD**	
	n = 408 (%)	**n = 2593 (%)**	
Mean age[1]	77.57 years ± 5.42	76.25 years ± 5.69	0.000
Education[2]			
Primary school	159 (39)	724 (28)	0.000
Secondary	174 (43)	1241 (48)	
College-University	72 (18)	621 (24)	
Smoking status[3]			
Never	192 (47)	1394 (54)	0.04
Past smoker	160 (40)	869 (34)	
Current smoker	30 (13)	330 (12)	
Family history of heart disease[4]			
No	184 (45)	1612 (62)	0.00
Yes	223 (55)	973 (38)	
Physical activity-adolescence and midlife[5]			
Never-Rarely	197 (51)	1153 (48)	0.27
Occasionally	83 (22)	515 (21)	
Moderate-High	103 (27)	743 (31)	
Alcohol consumption[6]			
Never	178 (44)	1024 (40)	0.16
Monthly and less	196 (50)	1339 (53)	
Weekly	23 (6)	188 (7)	
Diabetes in midlife[7]			
Diabetes (type 1 and 2)	13 (3)	36 (1)	0.01
No diabetes	395 (97)	2557 (99)	
Hypertension midlife[8]			
No hypertension	85 (21)	703 (27)	0.01
Pre hypertension	191 (47)	1190 (46)	
Hypertension present	132 (32)	700 (27)	
Total Blood cholesterol in midlife[9]			
Low	117 (29)	1028(40)	0.000
High	291 (71)	1563 (60)	
BMI in midlife[10]			
<30	367 (91)	2324 (90)	0.47
>30	35 (9)	254 (10)	
Food consumption - Midlife[11]			
Fish			
≤ 2 portions p/w	35 (9)	309 (12)	0.14
2 > - 4 portions p/w	262 (64)	1591 (61)	
4 > portions p/w	111 (27)	693 (27)	
Fish oil			
Never	167 (41)	823 (32)	0.001
≤ 2 times p/w	44 (11)	289 (11)	
3-6 times p/w	35 (8)	335 (13)	
Daily	162 (40)	1146 (44)	
Vegetables			
≤ 2 times p/w	272 (67)	1579 (61)	0.02
≥ 3 times p/w	134 (33)	1003 (39)	
Fruit			
≤ 2 times p/w	282 (69)	1645 (63)	0.02
≥ 3 times p/w	124 (31)	939 (37)	
Milk			
Less than daily	183 (45)	1114 (43)	0.454
Daily and more	223 (55)	1496 (57)	
Spread-quantity			
Never or little	93 (23)	551 (21)	0.502
Medium or much	315 (77)	2033 (79)	

Food consumption - Adolescence[12]			
Fish			
≤ 2 portions p/w	196 (48)	1283 (49)	0.49
2 > - 4 portions p/w	40 (10)	289 (11)	
4 > portions p/w	172 (42)	1021 (40)	
Fish oil			
Never	202 (49)	1080 (42)	0.01
≤ 2 times p/w	46 (11)	287 (11)	
3-6 times p/w	31 (8)	302 (12)	
Daily	129 (32)	924 (35)	
Fruits			
Never	165 (41)	884 (34)	0.02
< 1 time p/w	159 (39)	1186 (46)	
≥ 1 time p/w	81 (20)	510 (20)	
Vegetables			
Never	96 (24)	507 (20)	0.02
< 1 time p/w	165 (40)	1128 (43)	
≥ 1 time p/w	146 (36)	950 (37)	
Spread			
Never or little	71 (17)	388 (15)	0.21
Medium or much	335 (83)	2191 (85)	
Milk			
Less than daily	95 (23)	640 (25)	0.62
Daily	260 (64)	1652 (64)	
More than daily	53 (13)	298 (11)	

*CHD is defined by ECG, Rose angina questionnaire and heart procedures.

‡ Due to missing cases, the number of women included in the table varies from 2794 to 3001.

¤ P-values are based on Chi-square test, except for age, where independent sample t-test was used.

[1]Status at entry in AGES-Reykjavik. ± values are standard deviations

[2] Status at entry in AGES-Reykjavik

[3] Status at entry in AGES-Reykjavik. Smoking defined as having smoked at least 100 cigarettes in a lifetime.

[4]Status at entry in AGES-Reykjavik. Family history confirmed if parents, siblings and children have had coronary thrombosis.

[5]Status at entry in AGES-Reykjavik.

[6]Reported status at entry in AGES-Reykjavik.

[7] Either self reported or abnormal glucose value after overnight fasting at entry to Reykjavik Study.

[8] Blood pressure at entry to Reykjavik Study. Divided into no hypertension (sys<120 and did<80), pre hypertension (sys >140 or 80 ≤ did<90) and hypertension (sys ≥140 or did >=90).

[9] Cholesterol measurements on entry to Reykjavik Study. Low is defined as less than 6 mmol/l and high 6 mmol/l and more

[10] Status at entry to Reykjavik Study. Body Mass Index was calculated from weight and height measured at the same time point by standardized methods.

[11]Data from FFQ on midlife diet from AGES-Reykjavik

[12]Data from FFQ on teenage diet from AGES-Reykjavik

Table 2. Characteristics of fish and fish oil consumers in midlife‡

N=3001	Fish consumption				Fish oil consumption•		
	≤ 2 portions p/w (%) n = 344	>2-4 portions p/w (%) n = 1853	> 4 portionsp/w (%) n = 804	p-value	Low (%) n = 1323	High (%) n = 1678	p-value
Education							
Primary	111 (32)	542 (29)	230 (29)	0.47	448 (34)	435 (26)	0.001
Secondary	175(51)	873 (47)	367 (46)		616 (47)	799 (48)	
College /University	58 (17)	434 (24)	201 (25)		252 (19)	441 (26)	
Smoking status							
Never	129 (38)	1006 (54)	451 (56)	0.00	667 (50)	919 (55)	0.047
Past	145 (42)	627 (34)	257 (32)		483 (37)	546 (32)	
Current smoker	70 (20)	220(12)	96 (12)		173 (13)	213 (13)	
Family history of heart disease							
No	213 (63)	1080 (58)	503 (63)	0.07	797 (61)	999 (60)	0.660
Yes	127 (37)	769 (42)	300 (37)		521 (39)	675 (40)	
Physical activity (midlife and adolescence)							
Never-rarely	164 (51)	873 (51)	313 (42)	0.000	680 (55)	670 (43)	0.000
Occasionally	80 (25)	364 (21)	154 (21)		248 (20)	350 (22)	
Moderate-High	76 (24)	493 (28)	277 (37)		304 (25)	542 (35)	
Alcohol consumption							
Never	108 (32)	737 (40)	357 (45)	0.001	560 (43)	642 (39)	0.066
Monthly and less	195 (58)	962 (53)	378 (48)		650 (50)	885 (54)	
Weekly	34 (10)	126 (7)	51 (7)		88 (7)	123 (7)	
Diabetes							
No	339 (99)	1822 (98)	791 (98)	0.57	1295 (98)	1657 (99)	0.063
Yes	5 (1)	31 (2)	13 (2)		28 (2)	21 (1)	
Hypertension							
Never	89 (26)	496 (27)	203 (25)	0.475	336 (25)	452 (27)	0.527
Pre-hypertension	168 (49)	852 (46)	361 (45)		609 (46)	772 (46)	
Hypertension	87 (25)	505 (27)	240 (30)		378 (29)	454 (27)	
Cholesterol							
Low	144 (42)	712 (38)	289 (36)	0.154	511 (38)	634 (38)	0.635
High	200 (58)	1139 (62)	515 (64)		811 (62)	1043 (62)	
Body max index kg/m^2							
Low	302 (88)	1669 (91)	720 (90)	0.257	1154 (88)	1537 (92)	0.000
High	41 (12)	168 (9)	80 (10)		157 (12)	132 (8)	
Fruit consumption							
≤ 2 times p/w	227 (66)	1239 (67)	461 (58)	0.02	956 (72)	971 (56)	0.000
≥ 3 times p/w	116 (34)	608 (33)	339 (42)		363 (28)	700 (42)	
Vegetable consumption							
≤ 2 times p/w	233 (68)	1188 (64)	430 (54)	0.00	920 (70)	931 (56)	0.000
≥ 3 times p/w	110 (32)	659(36)	368(46)		397 (30)	740 (44)	
Milk							
Less than once a day	177 (52)	809 (44)	311 (39)	0.000	609 (46)	688 (41)	0.005
Daily and more often	165 (48)	1039 (56)	490 (61)		708 (54)	986 (59)	
Spread							
No spread /little	76 (22)	386 (21)	182 (23)	0.564	285 (22)	359 (22)	0.937
Medium or much	266 (78)	1461 (79)	621 (77)		1035 (78)	1313 (79)	
Fish oil consumption							
Never	131 (38)	623 (34)	236 (29)	0.001			
2 ≤ times p/w	46 (13)	211 (12)	76 (10)				
3-6 times p/w	32 (9)	242 (13)	96 (12)				
Daily	135 (40)	777 (42)	396 (49)				
Fish consumption							
≤ 2 portions p/w					177 (13)	167 (10)	0.000
>2-4 portions p/w					834 (63)	1019(61)	
> 4 portions p/w					312 (24)	492 (29)	

‡ Due to missing cases, the number of women included in the table vary from 2794 to 3001. All variables defend in table 1

•Fish oil consumption is defined low (2 times and less p/w) and high (3 times and more p/w).

Table 3. Characteristics of fish and fish oil consumers in adolescence ‡

N=3001	Fish consumption				Fish oil consumption•		
	≤ 2 portions p/w (%)	>2-4 portions p/w (%)	> 4 portions p/w (%)	p-value	Low (%)	High (%)	p-value
	n = 1479	n = 329	n = 1193		n = 1615	n = 1386	
Education							
Primary	418 (28)	76 (23)	389 (33)	0.007	522 (32)	361 (26)	0.000
Secondary	722 (49)	163 (50)	530 (45)		754 (47)	661 (48)	
College/University	336 (23)	87 (27)	270 (22)		332 (21)	361 (26)	
Smoking status							
Never	784 (53)	147 (45)	655 (55)	0.006	822 (51)	764 (55)	0.019
Past	521 (35)	127 (39)	381 (32)		590 (37)	439 (32)	
Current	174 (12)	55 (16)	157 (13)		203 (12)	183 (13)	
Family history of heart disease							
No	875 (59)	204 (62)	717 (60)	0.639	970 (60)	824 (60)	0.789
Yes	598 (41)	124 (38)	474 (40)		640 (40)	556 (40)	
Physical activity (midlife and adolescence)							
Never - rarely	663 (48)	142 (47)	545 (49)	0.884	782 (52)	568 (44)	0.000
Occasionally	298 (22)	70 (23)	230 (20)		310 (21)	288 (22)	
Moderate-high	410 (30)	92 (30)	344 (31)		411 (27)	435 (34)	
Alcohol consumption							
Never	572 (39)	114 (35)	516 (44)	0.005	652 (41)	550 (40)	0.899
Monthly and less	776 (54)	174 (54)	585 (50)		825 (52)	710 (52)	
Weekly	106 (7)	34 (11)	71 (6)		111 (7)	100 (8)	
Fruit consumption							
Never	497 (34)	90 (28)	462 (39)	0.00	625 (39)	424 (31)	0.000
< 1 times p/w	652 (44)	132 (40)	561 (47)		705 (44)	640 (46)	
≥ 1 times p/w	320 (22)	106 (32)	165 (14)		277 (17)	314 (23)	
Vegetable consumption							
Never	286 (19)	57 (17)	260 (22)	0.000	382 (24)	221 (16)	0.000
< 1 times p/w	640 (44)	117 (36)	536 (45)		700 (43)	593 (43)	
≥ 1 times p/w	551 (37)	154 (47)	391 (33)		529 (33)	567 (41)	
Milk consumption							
Less than daily	397 (27)	73 (22)	265 (22)	0.048	477 (29)	258 (19)	0.000
Daily	909 (61)	213 (65)	790 (66)		964 (60)	948 (68)	
More than once a day	171 (12)	43 (13)	137 (12)		173 (11)	178 (13)	
Spread							
Never or little	226 (15)	46 (15)	187 (16)	0.763	251 (16)	208 (15)	0.658
Medium or much	1247 (85)	280 (85)	999 (84)		1353 (84)	1173 (85)	
Fish oil consumption							
Never	660 (45)	147 (45)	475 (40)	0.042			
≤ 2 times p/w	175 (12)	34 (10)	124 (10)				
3-6 times p/w	166 (11)	33 (10)	134 (11)				
Daily	478 (32)	115 (35)	460 (39)				
Fish consumption					835 (52)	644 (46)	0.005
≤2 portions p/w					181 (11)	148 (11)	
>2-4 portions p/w					599 (37)	594 (43)	
>4 portions p/w							

‡ Due to missing cases, the number of women included in the table vary from 2794 to 3001. All variables are defined in table 1

•Fish oil consumption is defined low (2 times and less p/w) and high (3 times and more p/w).

Table 4a. Odds ratio estimates (95% CI) for coronary heart disease by teenage fish and fish oil consumption

		Age adjusted OR		Model 2¤		Model 3¥
Fish	**N=3001**	**Cases 408**	**N=2735**	**Cases 370**	**N=2707**	**Cases 366**
	(%)		**(%)**		**(%)**	
≤ 2 portions p/w	49	1.0	49	1.0	49	1.0
>2 up to 4 portions p/w	11	0.87 (0.61 -1.26)	11	0.93 (0.63 -1.37)	11	0.90 (0.61 - 1.33)
> 4 portions p/w	40	1.10 (0.88 - 1.36)	40	1.04 (0.82 -1.32)	40	1.04 (0.82 - 1.33)
Fish oil						
Never	43	1.0	43	1.0	43	1.0
≤ 2 times p/w	11	0.88 (0.62 - 1.24)	11	0.92 (0.64 - 1.34)	11	0.94 (0.64 - 1.37)
3-6 times p/w	11	0.57 (0.38 - 0.85)	11	0.56 (0.36 - 0.87)	11	0.59 (0.40 - 0.91)
Daily	35	0.75 (0.59 - 0.95)	35	0.77 (0.59 - 0.99)	35	0.75 (0.58 - 0.97)

¤ Adjustment made for age, education, smoking status, physical activity, family history of heart disease, fish consumption (for fish oil) and fish oil consumption (for fish).

¥ Additional adjustments made for consumption of fruit, vegetable, and milk and amount of spread used in adolescence.

Table 4b. Odds ratio estimates (95% CI) for coronary heart disease by fish and fish oil consumption in midlife

		Age adjusted OR		Model 2 ¤		Model 3 ¥		Model 4*
Fish	**N=3001**	**Cases 408**	**N=2735**	**Cases 370**	**N=2706**	**Cases 366**	**N=2686**	**Cases 362**
	(%)		**(%)**		**(%)**		**(%)**	
≤ 2 portions p/w	11	1.0	11	1.0	11	1.0	11	1.0
> 2 up to 4 portions p/w	62	1.38 (0.95 - 2.00)	62	1.54 (1.02 - 2.32)	62	1.59 (1.05 - 2.41)	62	1.65 (1.08 - 2.52)
> 4 times p/w	27	1.26 (0.84 - 1.89)	27	1.40 (0.90 - 2.18)	26	1.49 (0.94 - 2.36)	26	1.56 (0.98 - 2.48)
Fish oil								
Never	33	1.0	33	1.0	33	1.0	33	1.0
≤ 2 times p/w	11	0.78 (0.54 - 1.11)	11	0.89 (0.61 - 1.30)	11	0.91 (0.62 - 1.32)	11	0.97 (0.66 - 1.43)
3-6 times p/w	12	0.54 (0.37 - 0.79)	12	0.56 (0.37 - 0.86)	12	0.56 (0.36 - 0.86)	12	0.57 (0.37 - 0.88)
Daily	44	0.70 (0.55 - 0.89)	44	0.75 (0.58 - 0.97)	44	0.76 (0.59 - 0.98)	44	0.76 (0.59 - 0.99)

¤ Adjustment made for age, education, smoking status, physical activity, family history of heart disease, fish consumption (for fish oil) and fish oil consumption (for fish).

¥ Additional adjustments made for consumption of fruit, vegetable, and milk and the amount of spread used in midlife.

*Additional adjustments made for cholesterol levels, diabetes, hypertension and body max index, measured in midlife.