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Health effects of lean fish consumption in overweight and obese young adults following an energy-restricted diet for eight weeks

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TABLE OF CONTENT

ABSTRACT

In the cross-European study SEAFOODplus YOUNG it was recently shown that consumption of lean fish increases weight loss in men and has other positive health effects. The aims of this thesis were to investigate whether lean fish consumption increases weight loss and improves cardiovascular risk factors in a dose-dependent manner during an eight-week energy restriction in young overweight or mildly obese healthy adults.

A total of 126 Icelandic individuals were grouped into three isocaloric diets, energy-restricted by 30%, which consisted of different amounts of cod; diet group 1 (control group) receiving no seafood, diet group 2 receiving 150 g cod three times a week and diet group 3 receiving 150 g cod five times a week. All subjects were 20-40 years of age and had a BMI of 27.5-32.5 kg/m^2. Participants followed a detailed individual-based meal plan and no changes were made in their physical activity level. Anthropometric measurements and cardiovascular risk factors were assessed at baseline and endpoint.

One hundred subjects completed the trial. During the eight-week trial, body weight decreased on average by 5.0 kg and significant reductions were also seen in other anthropometric measurements. According to multivariate analysis weight loss was 1.7 kg greater among subjects consuming the highest amount of cod compared to the control group. Trend analysis supported a dose-response relationship between cod consumption and weight loss, although these effects were mainly seen in women. Systolic and diastolic blood pressure, serum triglycerides and insulin concentration also decreased during the intervention and the prevalence of the metabolic syndrome dropped from 29% to 21%. Changes of other measured cardiovascular risk factors were similar between the diet groups and mainly thought to be attributed to weight loss and not cod consumption.

The present study indicates weight loss effects of lean fish consumption in a dose-dependent manner in young overweight or mildly obese adults, especially women, although further studies are needed on this mechanism.

ÁGRIP

Samevrópska rannsóknin SEAFOODplus YOUNG sýndi nýverið fram á að neysla magurs fisks eykur þyngdartap hjá karlmönnum auk þess að hafa ýmis önnur jákvæð heilsufarsleg áhrif. Markmið þessarar ritgerðar var að kanna hvort skammtaáhrif séu af neyslu magurs fisks, sem hluti af orkuskertu átta vikna mataræði, á þyngdartap og áhættuþætti hjarta- og æðasjúkdóma meðal ungra heilbrigðra fullorðinna í ofþyngd eða vægri offitu.

Í heildina var 126 íslenskum einstaklingum skipt niður í þrjá rannsóknarhópa sem innihéldu 30% orkuskerðingu en sama orkumagn. Rannsóknarhóparnir samanstóðu af mismiklu magni þorsks; rannsóknarhópur 1 (viðmiðunarhópur) innihélt enga fiskneyslu, rannsóknarhópur 2 innihélt 150 g þorsk þrisvar í viku og rannsóknarhópur 3 innihélt 150 g þorsk fimm sinnum í viku. Allir þátttakendur voru á aldrinum 20-40 ára og með líkamsþyngdarstuðul á bilinu 27.5-32.5 kg/m^2. Þátttakendur fylgdu ítarlegum einstaklings-miðuðum matseðli en engar breytingar voru gerðar á hreyfingu þeirra. Líkamsmál og áhættuþættir hjarta- og æðasjúkdóma voru metin í upphafi og lok rannsóknar.

Hundrað þátttakendur kláruðu íhlutunina. Á þessum átta vikum höfðu þátttakendur að meðaltali lést um 5.0 kg auk þess sem marktæk minnkun varð á öðrum líkamsmálum. Samkvæmt fjölþátta greiningu léttust þeir þátttakendur sem neyttu mesta magn þorsks um 1.7 kg meira en viðmiðunarhópurinn. Skammtaáhrif reyndust vera til staðar, þ.e. aukin fiskneysla leiddi til meira þyngdartaps, þó svo þessara áhrifa hafi aðallega gætt meðal kvenna. Slag- og hlébils blóðþrýstingur, styrkur þríglýseríða og insúlínstyrkur lækkuðu einnig meðan á rannsókninni stóð og tíðni efnaskiptavillu lækkaði úr 29% í 21%. Breytingar á öðrum blóðgildum voru svipaðar milli rannsóknarhópa og voru aðallega taldar vera vegna þyngdartaps en ekki þorksneyslu.

Núverandi rannsókn gefur til kynna skammtaáhrif af neyslu magurs fisks á þyngdartap meðal ungra fullorðinna í ofþyngd eða vægri offitu, sérstaklega kvenna, en þörf er á frekari rannsóknum á þessu ferli.

ABBREVIATIONS

%E = percent of total energy intake

BMI = body mass index (kg/m^2)

BP = blood pressure

CHD = coronary heart disease

CVD = cardiovascular disease

DHA = docosahexaenoic acid, 22:6(n-3)

EPA = eicosapentaenoic acid, 20,5(n-3)

FA = fatty acid

FFQ = food frequency questionnaire

HDL = high-density lipoprotein

LC n-3 PUFAs = long chain omega-3 polyunsaturated fatty acids

LDL = low-density lipoprotein

MUFA = monounsaturated fatty acid

NNR = Nordic Nutrition Recommendations

PUFA = polyunsaturated fatty acid

RI = recommended intake

SFA = saturated fatty acid

VLDL = very low-density lipoprotein

WHO = World Health Organization

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1. INTRODUCTION

The beneficial health effects of fish and seafood consumption, e.g. on cardiovascular risk factors, have mainly been attributed to long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs) whereas less is known of the health effects of other constituents in fish such as fish protein (He and Daviglus, 2005; Ortega, 2006). Epidemiological studies have shown that fish consumption within a healthy eating pattern is associated with lower body weight (Shubair et al., 2005; Schulze et al., 2006). However, dietary intervention studies which included fish in a weight loss diet are limited (Mori et al., 1999; Thorsdottir et al., 2007).

The present study continues the investigation started by the recently conducted randomized dietary intervention study SEAFOODplus YOUNG on the effects of lean fish consumption on weight loss (Thorsdottir et al., 2007) and cardiovascular risk factors (Gunnarsdottir et al., 2008) in humans. The SEAFOODplus YOUNG study showed promising results where 150 g lean or fatty fish consumption three times a week for eight weeks was associated with significantly lower body weight in men compared to isocaloric diet without seafood.

A controlled dietary intervention trial was conducted in young overweight and mildly obese healthy adults (20-40 years of age, BMI 27.5-32.5 kg/m^2) for eight consecutive weeks. Subjects were grouped into three diet groups; a control group (diet group 1) receiving no seafood, diet group 2 receiving 150 g cod three times a week and diet group 3 receiving 150 g cod five times a week. Two of the intervention groups were the Icelandic part of the SEAFOODplus YOUNG study, conducted during the winter 2004-2005. Diet group 3 was investigated in the wintertime 2006-2007. The protocol and inclusion and exclusion criteria for the third diet group were exactly the same as in the SEAFOODplus YOUNG study (Gisladottir, 2007), therefore the three diet groups are fully comparable.

The main aims of the present study were to investigate whether lean fish consumption improves weight loss and cardiovascular risk factors in a dose-dependent manner in overweight and obese healthy adults during an eight-week period of energy restriction. An article has been written on the main results of the investigation and is currently in review in an international scientific journal (see appendix I).

2. LITERATURE REVIEW

2.1 Overweight and obesity

2.1.1 Definition of obesity and assessment

According to The World Health Organization (WHO) overweight and obesity are defined as "abnormal or excessive fat accumulation that may impair health" (WHO, 2006). Body mass index (BMI), defined as body weight in kilograms divided by the square of height in meters (kg/m^2), is commonly used in categorizing adult populations and individuals as either underweight, normal weight, overweight or obese. Furthermore, WHO classifies obesity into three classes according to its risk of co-morbidities; from moderate risk to very severe risk (WHO, 2008a). These classifications can be seen in table 2.1.

Table 2.1: *The international classification of adult underweight, overweight and obesity according to body mass index (WHO, 2008a.)*

Classification	**BMI (kg/m^2)**
Underweight	< 18.5
Normal weight	18.5 - 24.9
Overweight	25.0 - 29.9
Obesity	≥ 30
Obesity class I	30.0 - 34.9
Obesity class II	35.0 - 39.9
Obesity class III	≥ 40

Despite its frequent use in classifying people's physical health, BMI has its limitations since it does not always reflect body composition and its accuracy can be greatly distorted by muscle mass, physical fitness, visceral adiposity, gender, age, ethnicity and bone structure. Hence, a person categorized as obese might not experience a higher risk of co-morbidities if the extra body weight is mainly caused by increased muscle mass due to high level of physical activity. Also, a normal weight person cannot be labelled as healthy because he/she

might lead an unhealthy lifestyle and/or have a poor muscle mass and therefore be in increased risk of impaired health.

It is well known that obese people store more fat in the abdomen, and since excess visceral abdominal tissue is often accompanied by elevated triglycerides, reduced high-density lipoprotein (HDL) cholesterol, elevated blood pressure, elevated fasting plasma glucose and/or elevated concentrations of inflammatory cytokines, this increases the risk of developing numerous chronic diseases (WHO, 2006; Despres, 2007). Clinical and epidemiological research has found waist circumference to be the best anthropometric indicator of intra-abdominal fat mass (Guagnano et al., 2007). Therefore it is advisable to include waist circumference, as well as BMI, in the evaluation of the risk of illness caused by overweight and obesity (Janssen et al., 2002).

2.1.2 Prevalence of overweight and obesity

Obesity has become a healthcare problem today and its prevalence has increased greatly in recent decades in almost every country and all age groups. The major cause in the recent obesity epidemic is a changing environment that promotes excessive calorie intake and discourages physical activity, causing an energy imbalance. According to WHO's latest projections approximately 1.6 billion adults (age 15+) were overweight worldwide in 2005 and at least 400 million adults were obese. WHO further estimates that by 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese (WHO, 2006).

In 2006, WHO reported that the average BMI in Europe was nearly 26.5 and that obesity affected up to a third of the adult European population or about 130 million people (WHO, 2008b). In Iceland, 45% of men and 28% of women were overweight in 2002 (age 15-80) and 12% of both men and women were obese (Steingrimsdottir et al., 2003). Among 6 year old Icelandic children, 17% of boys and 22% of girls were overweight in 2002 and 2.4% of boys and 6.1% of girls were obese (Gunnarsdottir and Thorsdottir, 2003).

2.1.3 Obesity and health risk

The co-morbidities of obesity include various cardiovascular diseases, non-insulin-dependent (type II) diabetes and certain forms of cancers such as breast, colorectal, prostrate, endometrial, kidney and gallbladder cancer (WHO, 2008b). The health consequences of

obesity can range from increased risk of premature death to disabilities that reduce the overall quality of life. Nowadays, obesity is responsible for 2-8 % of health care costs and 10-13 % of deaths in Europe (WHO, 2008b).

2.1.3.1 Obesity and the metabolic syndrome

Overweight and obesity can lead to a condition called the metabolic syndrome which is characterized by abdominal obesity, insulin resistance, elevated blood pressure, inflammation, dyslipidemia and hyperglycemia. Individuals with the metabolic syndrome are at elevated risk for cardiovascular diseases and type II diabetes. According to the International Diabetes Federation (IDF, 2005) these individuals are three times as likely to have a heart attack or stroke compared to people without the metabolic syndrome. Additionally, they have a five-fold greater risk of developing type II diabetes. The worldwide obesity epidemic has led to a marked increase in the metabolic syndrome and it is estimated that 25% of the world's adult population has this syndrome (IDF, 2005).

In the present study the IDF definition of the metabolic syndrome is used (Alberti et al., 2006). Subjects are defined as having the metabolic syndrome if they have central obesity and any two of the four additional factors shown in table 2.2. Central obesity is assumed if either body mass index or waist circumference reaches cut-off points.

Table 2.2: *Definition of the metabolic syndrome according to International Diabetes Federation (Alberti et al., 2006).*

Central obesity:	Body mass index *and/or* Waist circumference	> 30 kg/m^2 ≥ 94 cm for men ≥ 80 cm for women
And any two of the following four factors:	HDL cholesterol Blood pressure Triglycerides Fasting plasma glucose	< 1.03 mmol/L in men < 1.29 mmol/L in women SBP ≥ 130 mmHg, or DBP ≥ 85 mmHg ≥ 1.7 mmol/L ≥ 5.6 mmol/L

2.1.4 Young adults and obesity

Normally, people tend to put on weight as they age, therefore the prevalence of obesity should be greater among middle-aged people (Thorgeirsdottir, 1999). However, obesity also has become increasingly prevalent among children and young adults (WHO, 2006), i.e. the parents of an upcoming generation, which is of special concern. Research show that children's diet depends largely on their parents' diet (Benton, 2004; Kristjansdottir et al., 2006; Thorsdottir et al., 2006). Therefore it is likely that overweight or obese parents raise overweight or obese children, and since obesity in childhood often continues into adolescence and adulthood, this increases the prevalence of obesity (Johannsson et al, 2006; WHO, 2008b).

However, one should always bear in mind that even though environmental and behavioural factors play the greatest role in the complex pathogenesis of obesity, multiple genetic factors also affect an individual's inherent risk for this condition (Ichihara and Yamada, 2008). Overweight and obese young adults are at higher risk of co-morbidities later in life if they continue to gain weight and develop severe obesity. It is therefore a matter of prevention to help young overweight and obese adults to lose weight, and simultaneously decrease cardiovascular risk.

2.2 Seafood consumption and relation to health

For the last two decades many studies have reported the beneficial effects of seafood consumption on the risk of coronary heart disease mortality (Kromhout, 1985; Daviglus et al., 1997; He et al., 2004; Whelton et al., 2004). Intake of fish and fish oil is also thought to decrease the risk of other cardiovascular diseases, e.g. hypertension, stroke and cardiac arrhythmias (Sidhu, 2003). Fish intake might also increase insulin sensitivity (Ramel et al., 2008) and decrease the risk of non-insulin-dependent (type II) diabetes (Kromann and Green, 1980; Nkondjock and Receveur, 2003). Furthermore, fish consumption has been linked to decreased risk of many other diseases such as colorectal cancer (Gonzalez and Riboli, 2006), depression (Sontrop and Campbell, 2006) and rheumatoid arthritis (Calder, 2006).

The beneficial health effects of fish and seafood consumption have mainly been attributed to long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs) since these fatty

acids are almost exclusively derived from seafood (He and Daviglus, 2005; Ortega, 2006). Less is known of the health effects of other constituents in fish such as fish protein, even though quantitatively protein is the major energy-giving nutrient in seafood. According to The Icelandic Food Composition Database (ISGEM, 2003), lean fish such as cod contains 18.1% protein but only 0.5% fat, whereas fatty fish such as wild salmon contains 20.4% protein and 9.7% fat. Moreover, a recent review by Hooper et al. (2006) concluded that n-3 PUFAs alone do not have a clear effect on total mortality, combined cardiovascular events or cancer. However, clear positive effects on these endpoints were seen when only the fish-based studies were taken into account, suggesting that there are other constituents in fish as well that contribute to the beneficial health effects of fish consumption.

Epidemiological studies have shown that fish consumption within a healthy eating pattern is associated with lower body weight (Shubair et al., 2005; Schulze et al., 2006) and might therefore be an important factor in decreasing the prevalence of obesity. However, dietary intervention studies which included fish in a weight loss diet are limited (Mori et al., 1999; Thorsdottir et al., 2007). The recently conducted randomized dietary intervention study SEAFOODplus YOUNG showed promising results where lean and fatty fish consumption three times a week for eight weeks was associated with significantly lower body weight in humans compared to isocaloric control diet without seafood. The present study investigates whether higher doses of lean fish result in greater effects on weight loss and cardiovascular health.

2.3 LC n-3 PUFAs and health

Of the bioactive components in seafood, LC n-3 PUFAs have been the most frequently studied. The fatty acids eicosapentaenoic acid (EPA, 20:5(n-3)) and docosahexaenoic acid (DHA 22:6(n-3)) have been of special interest since they are the most abundant LC n-3 PUFAs found in seafood.

Humans cannot synthesize from scratch the essential n-3 fatty acid alpha-linolenic acid (ALA, 18:3(n-3)) which is widely distributed in plant oils. They can, however, synthesize to some extent the longer-chain n-3 fatty acids EPA and subsequently DHA from ALA, although it requires substantial metabolic work. It is recommended that people get EPA and DHA through the diet as well to fulfil the requirements of these important fatty acids.

Fish and seafood are the main sources of these LC n-3 PUFAs but they are also found in certain marine algae, in certain plants (e.g. soybean, canola) and, importantly for newborns, in human milk (Costa, 2007). LC n-3 PUFAs have been shown to reduce the risk of cardiovascular diseases, diabetes and rheumatoid arthritis as well as these fatty acids play a role in early neurodevelopment and may have beneficial effects on weight management. These effects will be discussed briefly.

2.3.1 Cardiovascular diseases

LC n-3 PUFAs might improve cardiovascular health by various mechanisms. They have been shown to decrease blood triglyceride concentrations, lower blood pressure and increase HDL cholesterol levels (Calder, 2004; Balk et al., 2006). LC n-3 PUFAs also decrease the production of chemoattractants, growth factors, adhesion molecules, inflammatory eicosanoids and inflammatory cytokines, and reduce platelet aggregation. They increase nitric oxide production, thereby causing endothelial relaxation and arterial compliance. In addition, they decrease thrombosis and cardiac arrhythmias, and increase heart rate variability (Calder, 2004; Covington, 2004). A recent study by Thies et al. (2003) suggests that LC n-3 PUFAs from fish oil might also incorporate into already formed atherosclerotic plaques, making them less infiltrated with macrophages and thereby stabilizing the plaques.

To sum it up, LC n-3 PUFAs have anti-thrombotic, anti-arrhythmic and anti-inflammatory effects which protect against both the pathological processes leading to cardiovascular diseases (i.e. atherosclerosis) and the cardiovascular processes that ultimately cause death (e.g. myocardial infarction and stroke) (Calder, 2004). Available evidence strongly indicates that fish or LC n-3 PUFAs consumption reduces the risk of coronary heart disease (CHD) and overall cardiovascular mortality, especially in high-risk populations (Bucher et al., 2002; Konig et al., 2005; Psota et al., 2006; Wang et al., 2006).

2.3.2 Diabetes mellitus

Studies of patients with insulin resistance and type II diabetes indicate that consumption of LC n-3 PUFAs delays disease progression (Nettleton and Katz, 2005). Furthermore, hypertension and dyslipidemia are common comorbidities of type II diabetes and major risk factors for cardiovascular complications (Derosa et al., 2006; Gerich, 2007). Consumption of LC n-3 PUFAs has also been shown to reduce blood pressure and improve lipid profiles and

may therefore prevent the onset of cardiovascular complications of diabetes (Calder, 2004; Nettleton and Katz, 2005). However, it remains to be established whether LC n-3 PUFAs can prevent subjects with insulin resistance or impaired glucose tolerance from developing type II diabetes (Nettleton and Katz, 2005).

2.3.3 Inflammatory diseases

Several studies (Pattison et al., 2004; Calder, 2006) have found beneficial effects of high consumption of LC n-3 PUFAs in patients with rheumatoid arthritis, a chronic inflammatory disease, by reducing the production of inflammatory mediators (eicosanoids, cytokines and reactive oxygen species) and the expression of adhesion molecules. However, evidence of clinical efficacy of LC n-3 PUFAs is weak in other inflammatory diseases such as asthma and inflammatory bowel diseases (Calder, 2006). Nevertheless, several recent studies suggest that maternal fish consumption during pregnancy might decrease the risk for asthma and allergy in early childhood (Salam et al., 2005; Calvani et al., 2006; Romieu et al., 2007).

2.3.4 Prenatal brain development

LC n-3 PUFAs, especially DHA, are necessary for brain development during pregnancy and the first two years of infancy. Infants can convert shorter-chain n-3 PUFAs to DHA, but it is unknown whether such conversion is adequate for the developing brain in the absence of maternal intake of DHA (Mozaffarian and Rimm, 2006). Maternal and infant DHA consumption is believed to be positively associated with cognitive function in infancy such as behavioural attention, visual recognition memory and language comprehension (Colombo et al., 2004; Daniels et al., 2004).

Consumption of fish and fish oil in pregnancy has been positively associated with higher birth weight (Thorsdottir et al., 2004; Olafsdottir et al., 2005), which is associated with lower risk of hypertension later in life (Gunnarsdottir et al., 2002). However, a very high intake of fish oil was associated with decreased infant size at birth, possibly due to threefold consumption of the recommended dietary allowance of vitamin A (Thorsdottir et al., 2004).

2.3.5 Obesity

Studies in rodents have shown that seafood-derived LC n-3 PUFAs decrease the growth of the adipose cell and suppress the development of obesity, probably through stimulated beta-oxidation (Couet et al., 1997; Nakatani et al., 2003; Ukropec et al., 2003; Flachs et al., 2005). In a recent review by Madsen et al. (2005) it is suggested that LC n-3 PUFAs have effects on the differentiation and function of white fat cells by targeting a set of key regulatory transcription factors involved in both adipogenesis and lipid homeostasis in mature adipocytes. Garaulet et al. (2006) have recently demonstrated that both LC n-3 and n-6 PUFAs consumption is related to a reduced size of the adipose cell in overweight or obese humans.

A human study by Mori et al. (1999), testing daily fatty fish consumption (3.65 g LC n-3 PUFAs/day) included in an energy-restricted diet for 12 weeks, did not result in more weight loss than an energy-restricted diet without seafood, whereas mean weight loss at the end of the trial was 5.6 kg. The fish-included diet, however, had more positive effects on several health related variables such as blood pressure, glucose-insulin metabolism and blood lipid profile (Mori et al., 1999; Bao et al., 1998).

On the other hand, the SEAFOODplus YOUNG study indicated that the addition of seafood to a nutritionally balanced energy-restricted diet may increase weight loss, since the inclusion of either lean or fatty fish, or fish oil as part of an energy-restricted diet resulted in 1 kg significantly more weight loss after eight weeks than did an isocaloric diet without seafood (Thorsdottir et al., 2007). However, the weight loss effect cannot only be contributed to LC n-3 PUFAs since lean fish (cod), which contains only small amounts of these fatty acids (0.3 g/day), resulted in similar amount of weight loss as fatty fish (salmon, 3.0 g LC n-3 PUFAs/day) and fish oil (1.5 g LC n-3 PUFAs/day), or 5.4-5.5 kg. Therefore it is likely that other constituents in fish also play an important role in the weight loss effects of seafood.

2.4 Fish protein and health

The effects of fish protein on health have been investigated considerably less than the health effects of LC n-3 PUFAs. Human studies on fish protein are few but several animal studies have shown that fish protein might have beneficial effects on health, e.g. by lowering blood pressure (Gutierrez et al., 1994; Murakami et al., 1994; Yahia et al., 2003a; Boukortt et al., 2004), total cholesterol (Iritani et al., 1985; Zhang and Beynen, 1993; Hurley et al., 1995; Demonty et al., 2003; Yahia et al., 2003b; Wergedahl et al., 2004) and triglycerides (Bergeron and Jacques, 1989; Hurley et al., 1995; Demonty et al., 2003) and increasing insulin sensitivity (Lavigne et al., 2001; Tremblay et al., 2003). Lean fish consumption might therefore decrease the risk of developing the metabolic syndrome and thereby reducing the risk of cardiovascular diseases and type II diabetes.

Fish protein has high biological value, is easily digestible and is rich in essential amino acids (Costa, 2007). The effects that fish protein has on lipid metabolism could in part be attributed to its special amino acid composition. Fish protein has a low content of isoleucine, leucine, phenylalanine and tyrosine but a high content of arginine, alanine, methionine, cysteine and glycine, in comparison to casein (Yahia et al., 2005), which might affect clinical outcomes and will be explained later. Animal and human studies have also shown promising effects of fish protein on weight loss, where these effects might mainly lie in the high content of the amino acid taurine in fish (Fujihira et al., 1970; Zhang et al., 2004; Tsuboyama-Kasaoka et al., 2006). Ample fish consumption might therefore be an important component in the battle against obesity.

The bioactive effects of fish protein have not been investigated thoroughly in humans. The beneficial effects of LC n-3 PUFAs are, on the other hand, well known and nowadays there are various supplements available containing fish oil, especially in Iceland. There is some evidence that the health effects of fish protein might have been underestimated and possibly there is a basis for the development of functional food made of fish protein. The health effects of fish protein will now be discussed in details.

2.4.1 Cardiovascular diseases

Animal studies indicate that fish protein may influence cardiovascular risk factors by improving lipid metabolism and blood pressure. Fish protein might therefore have beneficial effects on cardiovascular risk independent of the intake of LC n-3 PUFAs, even though human studies are limited.

The SEAFOODplus YOUNG study showed positive health effects of cod consumption on blood lipids (Gunnarsdottir et al., 2008) and oxidative stress (Parra et al., 2007) in humans. According to the USDA National Nutrient Database, 100 g cod contains ~180 mg EPA and DHA (USDA, 2007). It was estimated that subjects in the cod diet group consumed 0.26 g/day of EPA and DHA (Gunnarsdottir et al., 2008) but that amount is insufficient to achieve doses as recommended by, e.g., the American Heart Association for patients with heart disease (1 g/day) (Breslow, 2006) or doses used in studies to improve various clinical outcomes (2-4 g/day) such as hypertriglyceridemia (McKenney and Sica, 2007) and hypertension (Geleijnse et al., 2002). Therefore, LC n-3 PUFAs are unlikely to explain positive health effects of lean fish consumption.

2.4.1.1 Blood pressure

Studies in rats have shown blood pressure lowering effects of fish protein, compared to casein (Gutierrez et al., 1994; Murakami et al., 1994; Yahia et al., 2003a; Boukortt et al., 2004). These effects can be explained by the high arginine content of fish since nitric oxide, the metabolic product of arginine by the enzyme nitric oxide synthase, plays a crucial role as a vasorelaxant and lowers blood pressure (Gutierrez et al., 1994; Yahia et al., 2003a).

In addition, Yahia et al. (2003a) found out that the fish protein diet modified the fatty acid composition of liver microsomal total lipids and liver phospholipids essentially by decreasing the proportion of arachidonic acid (20:4(n-6)), which is attributed principally to a diminution in Δ6(n-6) desaturation activity, the first step of arachidonate biosynthesis. This might have been caused by the low lysine to arginine ratio in fish protein as compared to casein since a positive correlation has been reported between lysine/arginine ratio and liver microsomal Δ6(n-6) desaturase activity in rats (Koba and Sugano, 1990). Despite the decreased proportion of arachidonic acid, which is the precursor of eicosanoids such as prostacyclin (PGI_2), the production of the vasodilating PGI_2 was increased. These conflicting results indicate that other factors influence eicosanoids biosynthesis in hypertensive rats

independent of the arachidonic acid level. Yahia et al. (2003a) suggested that these factors might be vasoconstrictor hormones, including angiotensin II, vasopressin and epinephrine that have been shown to stimulate PGI_2 synthesis in vivo to constitute a homeostatic mechanism (Askari and Ferrerib, 2001).

2.4.1.2 Cholesterol

Fish protein has been found to reduce plasma total cholesterol levels in animal studies, compared to casein (Iritani et al., 1985; Zhang and Beynen, 1993; Hurley et al., 1995; Demonty et al., 2003; Yahia et al., 2003b; Wergedahl et al., 2004). Zhang and Beynen (1993) found that the cholesterol-affecting properties of a cod meal can be enhanced by the incorporation of higher proportions of fish protein in the diet, indicating a dose-dependent relation. Bergeron and Jacques (1989) demonstrated that fish protein diet caused fewer atherosclerotic lesions in rabbits, compared to casein diet, due to the cholesterol lowering effect.

Wergedahl et al. (2004) found that Acyl-CoA cholesterol acyltransferase activity (ACAT) in rats was significantly decreased by fish protein diet compared to casein diet. ACAT catalyzes the intracellular esterification of cholesterol and formation of cholesterol esters, thereby participating in accumulating cholesterol esters in macrophages and vascular tissues. By this mechanism, increased ACAT activity is thought to play a role in the progression of atherosclerosis (Carr et al., 1992) so this finding indicates that fish protein has a cardioprotective role and is in involved in the regulation of plasma cholesterol (Wergedahl et al., 2004).

The cholesterol lowering effect of fish protein can also be explained by its amino acid composition. Giroux et al. (1999) demonstrated that enrichment of a 30% casein diet with lysine and methionine caused a marked increase in serum total and low density lipoprotein (LDL) cholesterol levels in rabbits, which were partially prevented by supplementation with arginine. These amino acids could act diretly in the liver by regulating both synthesis of LDL cholesterol, apolipoprotein B (apo B) and catabolism of LDL cholesterol (Kurowska and Carroll, 1992; Kurowska and Carroll, 1996). Also, the hypercholesterolemic effect of lysine and methionine may be mediated in part by an increase in liver phosphatidylcholine (PC) synthesis since an elevation of hepatic PC was counteracted partially by arginine (Giroux et al., 1999). Glycine supplementation in rabbits and rats has also shown to have hypocholesterolemic effects (Katan et al., 1982; Sugiyama et al., 1986). Based on these

mechanisms, Wergedahl et al. (2004) also suggested that the amino acid composition of fish protein might be responsible for the cholesterol lowering effect, where low ratios of methionine to glycine and lysine to arginine in fish protein compared with casein may be involved in lowering the plasma cholesterol concentration.

A recent study in pigs showed that pigs fed fish protein for three weeks had lower cholesterol concentrations in plasma, LDL cholesterol and HDL cholesterol, compared to a control group fed casein (Spielmann et al., 2008). Spielmann et al. hypothesised that the hypocholesterolaemic effects of fish protein were in part caused by a stimulation of bile acid synthesis, since mRNA concentrations of genes involved in bile acid synthesis and cholesterol uptake were higher in pigs fed fish protein than in pigs fed casein.

In contrast to Spielmann's study, studies have also indicated that fish protein might increase the level of HDL cholesterol in rabbits (Bergeron and Jacques, 1989; Bergeron et al., 1992) and obese rats (Wergedahl et al., 2004), compared to casein. Bergeron and Jacques (1989) found that fish protein induced a decrease of LDL cholesterol in rabbits and an increase of HDL cholesterol, compared to casein. Since the LDL/HDL ratio has been shown to be a good indicator of atherosclerosis risk, fish protein may reduce the risk of atherosclerosis in humans as well.

In humans, on the other hand, the effects of cod protein on cholesterol metabolism, compared to other animal proteins, appear to be different from those observed in rodents (Demonty et al., 2003). Lacaille et al. (2000) found that fish protein did not affect total plasma cholesterol concentrations in men and a study by Gascon et al. (1996) on premenopausal women showed the same results. A possible reason for these conflicting findings in animal and human studies might be that doses in animal studies are usually much higher than in human studies. Also, in animal studies, isolated fish protein is used, leaving out nearly all possible effects of other nutrients, whereas lean fish as a whole is more often used in human studies which can interfere with the results.

Nevertheless, the SEAFOODplus YOUNG study resulted in a reduction in total cholesterol in the lean fish group that was of a similar degree as in the fatty fish group. The reduction was though only of borderline significance from the control group receiving no seafood when adjusted for weight loss (Gunnarsdottir et al., 2008), indicating that weight loss alone might be partly responsible for the cholesterol lowering effect. Unfortunately, the cholesterol lowering effect of the cod diet was mainly due to lowering of HDL cholesterol rather than LDL cholesterol, compared to the other diet groups, which is common in an

energy-restricted diet containing ≤ 30 %E from fat (Nordmann et al., 2006; Petersen et al., 2006), as in the SEAFOODplus YOUNG study.

2.4.1.3 Triglycerides

Fish protein might decrease plasma triglyceride levels in rodents (Bergeron and Jacques, 1989; Hurley et al., 1995; Demonty et al., 2003; Shukla et al., 2006) and increase lipoprotein lipase (LPL) activity in adipose tissue (Bergeron et al., 1992; Demonty et al., 1998) in comparison with casein. However, one should bear in mind that the protein-isolates tested in animal or human models are most likely not completely free of lipids. Thus it cannot be fully ruled out that some of the effects might originate from LC n-3 PUFAs, which is possible in this case. Nevertheless, Demonty et al. (2003) showed for the first time that cod protein lowers the rate of triglyceride secretion into the blood in rats, compared to casein, probably because of a reduction in hepatic triglyceride synthesis. The authors also suggested that the lowering effect of cod protein on triglyceride secretion may be related in part to its amino acid composition, since cod protein contains more arginine and has a lower lysine to arginine ratio than casein. However, Bergeron and Jacques (1989) found that serum triglycerides were higher in rabbits feeding on fish diet compared to casein diet.

Human studies have shown that fish protein, compared with other animal proteins, might lower plasma VLDL triglycerides in premenopausal women (Gascon et al., 1996) but did not affect total cholesterolemia and LDL-apoprotein B in postmenopausal women (Jacques et al., 1992). The human trial SEAFOODplus YOUNG revealed interesting results on the effects of fish protein on serum triglyceride concentration. The cod diet in that study lowered serum triglycerides to a similar degree as the fish oil diet, a result that has not been seen previously in a human study (Gunnarsdottir et al., 2008).

Based on studies in rodents, it has also been proposed that cod protein may interact with dietary n-6 (Bergeron et al., 1991) and n-3 (Demonty et al., 1998) PUFAs, mainly through specific alterations in hepatic lipid concentrations, to modulate plasma triglyceride and cholesterol concentrations (Demonty et al., 2003). Therefore LC PUFAs and fish protein might interact while improving cardiovascular health.

2.4.1.4 Taurine and CVD

Taurine (2-aminoethanesulfonic acid) is a free amino acid, derived from the sulfur-containing amino acids methionine and cysteine. It is concentrated in animal tissues, especially fish and seafood (Tsuboyama-Kasaoka et al., 2006) and has been used as a marker of seafood intake in several studies (Gonzalez et al., 1990; Yamori et al., 2001; Kim et al., 2003). Taurine has a role in several essential processes such as antioxidation, osmoregulation, membrane stabilization, neurotransmission, glycolysis and the synthesis of bile salts (Brosnan and Brosnan, 2006). Biasetti and Dawson (2002) found out that taurine protects against oxidative damage caused specificly by reactive quinones and oxygen radicals produced by catecholamine oxidation, explaining its antioxidant activity.

Taurine might have beneficial effects on cardiovascular risk factors. Animal studies have shown that taurine may attenuate hypertension (Nara et al., 1978) and increase HDL cholesterol (Matsushima et al., 2003), as well as suppressing reactive hypercholesterolemia in stroke-prone spontaneously hypertensive rats fed a hypercholesterolemic diet (Murakami et al., 1996a; Murakami et al., 1996b). The authors concluded that these cholesterol lowering effects are caused by an increase in cholesterol catabolism to bile acid through the enhancement of cholesterol 7 alpha-hydroxylase activity (Murakami et al., 1996b).

A human study by Mizushima et al. (1996) indicated that oral taurine supplementation might suppress reactive hypercholesterolemia in young men on high-fat cholesterol diets. Zhang et al. (2004) also found a positive effect on blood lipid profile in a human study with taurine supplementation of 3 g/day for seven weeks in overweight or obese adolescents, which resulted in significantly decreased triglyceride levels compared to a placebo group.

Inverse association has been observed between taurine excretion and ischemic heart disease mortality in humans in the cross-sectional WHO-CARDIAC study (World Health Organization coordinated Cardiovascular Diseases and Alimentary Comparison Study) (Yamori et al., 2001). The cardioprotective effects of taurine may in part be explained by its beneficial effects on the lipid profile, as listed above, but also by its antioxidant and anti-apoptosis activity that reduces the cardiomyocyte injury, which was the result of a study by Oriyanhan et al. (2005) in rats. Also, the beneficial effects on cardiovascular health in the WHO-CARDIAC study might in part be related to fish consumption in general, but not only to the taurine content. The human study SEAFOODplus YOUNG confirms these antioxidant effects of lean fish consumption (Parra et al., 2007), although taurine concentration was not measured, where the ample cod intake, combined with an energy-restricted diet, increased

plasma antioxidant capacity (AOP) and decreased lipid peroxidation (measured as plasma malondialdehyde, MDA). In fact, the inclusion of cod in the calorie-restricted intervention was the only diet group in the study that markedly decreased oxidative stress, expressed as the MDA/AOP ratio (Parra et al., 2007).

2.4.1.5 Arginine and CVD

Fish protein contains relatively high amounts of the amino acid arginine (~1 g/100 g fish) (FAO, 1970). Arginine is a nonessential amino acid because it can be synthesized from the amino acid glutamate (Kendler, 2006) which is also abundant in fish protein (He and Daviglus, 2005). Arginine is the major component of nitric oxide, synthesised via nitric-oxide synthase in the L-arginine-nitric oxide pathway, and therefore it appears to have beneficial effects on vascular endothelial function (Moncada and Higgs, 1993). Nitric oxide is the primary compound responsible for vasodilation in arteries and inhibits platelet aggregation. It also modulates leukocyte-endothelium interactions by altering cell adhesion molecule expression, reduces monocyte adherence and inhibits the proliferation of smooth muscle cells (Brown and Hu, 2001).

Arginine is known to have a cholesterol lowering effect, as discussed above, and its supplementation in animal models might counteract an elevation of serum total and LDL cholesterol induced by the high amount of essential amino acids in casein (Giroux et al., 1999). A review by Brown and Hu (2001) revealed that arginine supplementation might have beneficial effect on endothelial function in patients with hypercholesterolemia or coronary artery disease, but not in subjects with diabetes or healthy subjects. In addition, He and Daviglus (2005) have pointed out that the synergistic effect of the L-arginine-nitric oxide pathway and the effects of LC n-3 PUFAs on eicosanoid balance provide additional cardioprotective effects.

2.4.2 Diabetes mellitus

Previous studies on high-fat fed rats strongly suggest that fish protein prevents the development of insulin resistance (Lavigne et al., 2000; Lavigne et al., 2001; Tremblay et al., 2003). Tremblay et al. (2003) concluded that the better insulin sensitivity resulting from fish protein diet, compared to casein, was caused by a lower body and liver weight, whereas the

rats fed on casein diet developed visceral obesity and peripheral insulin resistance. However, a study by Lavigne et al. (2001) demonstrated that consumption of cod protein fully prevented the development of skeletal muscle insulin resistance in diet-induced obesity, compared to both casein and soy protein, without reduction in body weight or adiposity. This mechanism was believed to occur, at least in part, through a direct action of amino acids from cod on insulin-stimulated glucose uptake in skeletal muscle cells. Glutamine is a likely candidate there since its concentration is selectively decreased in cod protein-fed animals, compared to casein or soy protein-fed animals, and it has been reported that glutamine exposure inhibits insulin-stimulated glucose transport in skeletal muscles and promotes insulin resistance by routing glucose through the hexosamine pathway (Marshall et al., 1991; Traxinger and Marshall, 1991).

A recent human study by Ouellet et al. (2007) indicated that dietary cod protein improved insulin sensitivity in insulin-resistant men and women. Thus, fish protein could contribute to the prevention of non-insulin-dependent (type II) diabetes in humans.

2.4.2.1 Taurine and diabetes mellitus

In recent years, the amino acid taurine has been investigated in relation to diabetes. Since fish and seafood are the main dietary sources of taurine these studies will be discussed briefly. Several animal studies indicate that taurine supplementation could improve insulin sensitivity (Anuradha and Balakrishnan, 1999; Nandhini and Anuradha, 2002) and that taurine administration could reduce the complications induced by diabetes mellitus in the retina, lens and peripheral nerve (Franconi et al., 2004) where taurine is thought to act as a relatively specific antioxidant, reducing oxidative stress and inflammatory responses. Taurine is thought to increase glucose metabolism in rats and increase insulin activity, probably through the binding to insulin receptors (Hansen, 2001).

Human studies demonstrate that both type I and type II diabetic patients have a reduced plasma taurine concentration compared to healthy controls (Franconi et al., 1995; De Luca et al., 2001). Elizarova and Nedosugova (1996) documented that a supplementation of 0.5 g taurine in humans, twice a day for 30 days, decreased the average daily blood glucose concentration which indicates a glucose-lowering effect of taurine. However, a human study by Brons et al. (2004) revealed that daily supplementation with 1.5 g taurine for 8 weeks had no effect on insulin secretion or sensitivity in 20 non-diabetic overweight subjects. Taurine is thought to have beneficial effects on the lipid profile in overweight or obese non-diabetic

humans (Zhang et al., 2004) but further studies are needed on the possibility of taurine supplementations in diabetic or pre-diabetic subjects.

2.4.3 Obesity

A study by Yahia et al. (2005) suggested positive effects of fish protein on weight loss in rats since rats fed fish protein diet did not gain as much body weight as rats fed casein protein diet despite similar food and energy intake. The authors reported that rats fed the fish protein diet had significantly lower total lipids in adipose tissue and therefore lower fat deposition, compared to rats fed the casein diet (Yahia et al., 2005). However, the reduced essential amino acids (isoleucine, leucine, phenylalanine, tyrosine, valine and histidine) in fish protein, compared with casein, might have been responsible for this low growth (Yahia et al., 2003a).

The human study SEAFOODplus YOUNG found that regular consumption of lean fish (3 x 150 g per week) increased weight loss in overweight and obese men during an eight-week energy restriction in comparison to an isocaloric control diet without seafood (6.5 ± 2.8 kg vs. 5.3 ± 3.0 kg) (Thorsdottir et al., 2007). However, a significant effect of lean fish consumption on weight loss was not seen in women. Testing the weight loss effects of lean fish eaten more frequently or in higher amounts than in the SEAFOODplus YOUNG study is important as lean fish might be an interesting possibility to increase weight loss options for overweight or obese individuals.

2.4.3.1 Taurine and weight loss

Particular amino acids in fish protein might have beneficial effects on body weight, especially the amino acid taurine. Several studies have shown that taurine might promote weight loss. An early study by Fujihira et al. (1970) showed that taurine decreased body weight in obese mice, mainly due to inhibition of excess fat deposition in the body since taurine is highly concentrated in the gastrointestinal wall, influencing lipid metabolism. Tsuboyama-Kasaoka et al. (2006) recently reported that dietary taurine supplementation prevented obesity in mice fed high-fat diet by increasing both resting energy expenditure and gene expression involved in energy metabolism in white adipose tissue. The researchers concluded that obesity causes depletion of the blood taurine concentration, which then promotes further obesity, and that dietary taurine supplementation might interrupt this vicious circle and prevent obesity.

A human study by Zhang et al. (2004) also found that seven-week supplementation with 3 g taurine per day resulted in weight loss, possibly due to its beneficial effects on lipid metabolism. However, Mizushima et al. (1996) documented that taurine supplementation had no effect on body weight in 22 healthy young men on a high-fat and high-cholesterol diet. Further studies are needed on the effect of this promising weight loss agent.

2.5 Other nutrients in fish and relation to health

Regular consumption of fish and seafood, both fatty and lean varieties, contributes a lot to the intake of various vitamins and minerals, especially iodine, selenium and vitamin D, which are found in few other food groups.

2.5.1 Iodine

The main function of iodine is the synthesis of the thyroid hormones thyroxine (T4) and the biologically active form triiodothyronine (T3). The target tissues of T3 and T4 are liver, kidneys, muscles and developing brain where the thyroid hormones promote synthesis of enzymes and other proteins to increase metabolic activity in tissues (Shils et al., 2006). Iodine deficiency results in goitre, an enlargement of the thyroid gland, or even creatinism in more severe deficiencies. Hypothyroidism is uncommon in the Nordic countries but is a severe health problem in many countries of the world which are located far from sea (NNR, 2004). Furthermore, iodine status can be affected by selenium or iron intake, where low selenium or iron intake may have a negative impact on thyroid function (NNR, 2004).

Fish, especially marine fish and shellfish, are the main sources of iodine since this trace mineral is mainly found in seawater. Fish can contain 30-300 μg iodine per 100 g fish (Reykdal et al., 2000). Analysis from The Icelandic Food Composition Database (ISGEM, 2003) show that the iodine content in cod is 170 μg/100 g whereas it is 36 μg/100 g in wild salmon. Therefore, lean fish is usually a much better source of iodine than fatty fish. Milk and milk products can be quite good sources of iodine (9.7-12.7 μg/100 g in whole milk in Iceland) if the cows are fed fishmeal-based fodder or graze on soil located near the seaside (Reykdal et al., 2000). Eggs (57.2 μg/100 g (Reykdal et al., 2000)) and iodised table salt (5-50 μg/g (NNR, 2004)) can also be important iodine sources (NNR, 2004).

The recommended intake of iodine is 150 μg/day for adults and adolescents (NNR, 2004). The mean iodine intake in the Icelandic Dietary Survey conducted in 2002 was 163 μg/day with adolescents having the lowest intake, or 104 μg/day among adolescent girls. Icelanders used to have one of the highest iodine intake in the world but it has decreased since 1990 due to a decrease in fish consumption (Steingrimsdottir et al., 2003).

2.5.2 Selenium

Selenium is essential for selenoprotein activity and the main biological functions of selenium are mediated by glutathione peroxidases and other selenoproteins. There have been identified five selenium-containing glutathione peroxidases which all act as antioxidants in different tissues, protecting cells from oxidant molecules such as hydrogen peroxide (Shils et al., 2006). These selenium-containing enzymes may potentially retard the development of cardiovascular diseases via the antioxidant activity that inhibits atherosclerosis, and via the production of eicosanoids (Savige, 2001). Severe selenium deficiency is rare in humans but has caused cardiomyopathy in Chinese children and young women in 1979, a condition called Keshan disease. Kashin-Beck disease is a preadolescent or adolescent osteoarthritis endemic in China that has been associated with poor selenium status (Shils et al., 2006). Low selenium status has also been linked to increased incidence of cancer in several epidemiologic studies (Ujiie and Kikuchi, 2002; Ozgen et al., 2007). Some human intervention studies suggest that selenium supplements may be effective in cancer prevention (Clark et al., 1996; Bardia et al., 2008) although benefits of selenium supplementation are still uncertain and not recommended, requiring further studies (Navarro-Alarcon and Cabrera-Vique, 2008).

Like iodine, selenium in diet depends in large part on the selenium concentration in soil and in animal fodder and therefore varies in different countries. The main selenium dietary sources in most countries are fish and other seafood, offal and eggs. Meat and milk are also good selenium sources in some countries. According to The Icelandic Food Composition Database (ISGEM, 2003) selenium concentration is similar in various fish species; 26 μg/100 g cod, 23 μg/100 g wild salmon, 24 μg/100 g herring and 31 μg/100 g farmed trout. In other selenium dietary sources, its concentration is 6-143 μg/100 g in various offal, 21 μg/100 g eggs, 6-19 μg/100 g in various meat and 1.6 μg/100 g whole milk (ISGEM, 2003).

The recommended intake (RI) of selenium is 40 μg/day for women and 50 μg/day for men (NNR, 2004). The selenium intake in Iceland is high since the average intake in all age groups is 50% above the RI, according to the Icelandic Dietary Survey in 2002

(Steingrimsdottir et al., 2003). Fish is one of the main selenium sources in Iceland and accounts for around 25% of the general selenium intake (Steingrimsdottir et al., 2003). However, bread accounts for 27% (Steingrimsdottir et al., 2003) since the selenium intake in Iceland has been influenced by high-selenium wheat imported from North America (NNR, 2004).

2.5.3 Vitamin D

The main role of vitamin D is to maintain serum calcium and phosphorus concentration in a range that supports cellular processes, neuromuscular function and bone ossification. Vitamin D accomplishes this goal through the action of 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D) on regulating calcium and phosphorus metabolism in the intestines and bones (Shils et al., 2006). Vitamin D is also thought to inhibit cell proliferation and enhance cell differentiation activity and might therefore have physiological actions for cardiovascular health, cancer prevention, regulation of immune function and in decreasing the risk of autoimmune diseases (Shils et al., 2006). Vitamin D deficiency slows the production of calcium-binding proteins so that the intestines absorb only 10-15 % of dietary calcium, leading to calcium loss of bones, which results in rickets in children and osteomalacia and osteoporosis in adults (Insel et al., 2004; Shils et al., 2006). A recent Icelandic study demonstrated that adequate intake of vitamin D is more important for the calcium pool in the body than increasing dietary calcium intake beyond the recommended intake (Steingrimsdottir et al., 2005).

Vitamin D_3, or cholecalciferol, can be synthesised from 7-dehydrocholesterol in the skin via ultraviolet sunlight. This fat-soluble vitamin may also be derived from the diet and converted to its active form 1,25-$(OH)_2$D. However, dietary sources are very few since oily fish, margarine and vitamin D enriched milk are the only sources of importance. Other minor sources are certain freshwater fish and egg yolk (NNR, 2004). According to The Icelandic Food Composition Database (ISGEM, 2003), wild salmon contains 7.5 µg vitamin D per 100 g, farmed trout 32.9 µg/100 g whereas cod contains none (0 µg/100 g) due to its very low fat content (0.5 g/100 g compared to 14.6 g/100 g wild salmon). Vitamin D content in margarine is 8 µg/100 g, 1.4 µg/100 g in eggs and 0.38 µg/100 g in fortified Icelandic milk. Fish oil in supplemental form is an excellent source of vitamin D, containing 5-18.4 µg/10-15 ml (Lysi hf, 2008).

The recommended intake (RI) of vitamin D has recently been increased and is now 10 µg/day for children and adults and 15 µg/day for > 60 year old people (Public Health Institute

of Iceland, 2006). According to the Icelandic Dietary Survey in 2002, the average vitamin D intake in Iceland is far below the RI, or 6.0 μg/day in 15-80 years old individuals (Steingrimsdottir et al., 2003) where adolescent girls have the lowest intake of 2.2 μg/day, which is only 22% of RI.

2.5.4 Other constituents in fish

2.5.4.1 Calcium

Fatty fish and fish products are quite good sources of calcium, especially when eaten with the bones intact (Savige, 2001; NNR, 2004) like sardines which contain 420 mg/100 g (ISGEM, 2003). The skeletal effects of calcium are well known (NNR, 2004) but calcium may also protect against cardiovascular diseases by lowering blood pressure (Appel et al., 1997). Several population-based epidemiological studies have also shown an inverse association between calcium intake and risk of obesity (Zemel et al., 2000; Jacqmain et al., 2003) with the hypothesis that calcium might form a complex with fatty acids in the gut, preventing their absorption and thereby reducing fat absorption (Heaney, 2006).

2.5.4.2 Coenzyme Q_{10}

Concentrations of Coenzyme Q_{10} (CoQ_{10}) in fish is quite high or 4-64 μg/g of fish (Weber et al., 1997). The optimal dietary intake of CoQ_{10} is unknown but the average consumption in the Danish diet is 3-5 mg/day (Weber et al., 1997). A fish portion of 150 g would therefore give on average 4.5 mg CoQ_{10}, yielding the average daily CoQ_{10} consumption.

Coenzyme Q_{10} is well defined as a crucial component of the oxidative phosphorylation process in mitochondria which converts the energy in carbohydrates and fatty acids into ATP to drive cellular mechanism (Crane, 2001). Recent studies have also revealed the antioxidant properties of CoQ_{10} (Crane, 2001; Littarru and Tiano, 2007). In its reduced form, called ubiquinol, it inhibits protein and DNA oxidation as well as the peroxidation of cell membrane lipids and lipoprotein lipids present in the blood circulation (Littarru and Tiano, 2007). CoQ_{10} protects LDL cholesterol against oxidation in vitro and may therefore play a protective role against atherosclerosis (Weber et al., 1997).

2.5.4.3 Other vitamins and minerals

Nutritional benefits of fish consumption might, in part, also be due to other nutrients in fish. Fish is quite rich in other minerals than iodine, selenium and calcium; namely zinc and iron. Fish also contains various other vitamins than vitamin D such as vitamin B_{12} (cobalamine), vitamin B_3 (nicotinamide) and vitamin B_6 (pyridoxine), and fatty fish is also a good source of vitamin A (Costa, 2007) and vitamin E (Sidhu, 2003).

2.6 Other effects of fish consumption on weight loss

2.6.1 Protein intake, fish consumption and satiety

Macronutrients have different effects on satiety, independent of their caloric value. Studies have demonstrated that dietary protein is the most satiating macronutrient, compared with isoenergetic ingestion of carbohydrate or fat, both in human subjects and animal models (Porrini et al., 1997; Westerterp-Plantenga et al., 1999; Bensaid, 2002; Leidy et al., 2007). An additional mechanism that might explain the positive influence of fish protein on body weight is therefore its possible effect on satiety. Previous studies indicate that fish protein might have stronger satiating effect when compared with beef and chicken protein (Uhe et al., 1992; Borzoei et al., 2006).

However, the results of the SEAFOODplus YOUNG study indicate that lean fish consumption does not increase satiety compared to isocaloric diets of the same macronutrient composition, including fatty fish, fish oil or control diet without seafood (Arnarson, 2007). Furthermore, cod consumption even increased hunger scores measured on visual analogue scales (VAS) compared to the other diet groups, to an insignificant degree though. The researchers suggested that LC n-3 PUFAs in the two other seafood groups had favourable effects on appetite by increasing satiety feelings (Arnarson, 2007; Parra et al., 2008). Another possible mechanism might be that the cod diet increased metabolic rate to a greater degree than the other diets (Arnarson, 2007) since taurine has been shown to increase resting metabolic expenditure in mice (Tsuboyama-Kasaoka et al., 2006).

From the above-mentioned, one can conclude that fatty fish consumption, rich in LC n-3 PUFAs, might increase satiety to a greater degree than other protein sources of animal

origin and therefore have positive effects on body weight, but further studies are needed to verify this hypothesis.

2.6.2 High-protein diets

Protein plays a key role in body weight regulation not only by increasing satiety but also by increasing thermogenesis and having favourable effect on body composition through maintenance or accretion of fat-free mass (Westerterp-Plantenga, 2003; Paddon-Jones et al., 2008). Nutritional guidelines nowadays recommend that 10-20 % of the calorie content of the diet comes from protein. However, in many popular diets, such as the Atkins Diet, The Zone and The South Beach Diet, 30-40 % of the calorie content comes from protein, at the expense of carbohydrates (Astrup, 2005). The high-protein content in the aforementioned diets may actually be the reason for their partial success in inducing weight loss (Astrup et al., 2004).

However, high-protein/low-carbohydrate diets may suppress food intake, which e.g. causes the weight loss effects, by producing ketosis. Ketosis results from the depletion of glycogen stores in the body, induced by a severe restriction of carbohydrates, causing acidosis that can have life-threatening consequences (Herrin, 2003). High-protein intake (two or three times the recommended intake) has also been associated with progression of renal diseases, increased calcium losses and possible harmful effects on bone mass, and the risk of adiposity in children and juvenile diabetes (Friedman, 2004; NNR, 2004). Tremblay et al. (2007) pointed out that high-protein intake can have detrimental effects on glucose homeostasis by promoting insulin resistance and increasing gluconeogenesis. Emphasising the quality rather than the quantity of protein has been shown to modulate insulin resistance and revealed that protein derived from fish might have the most desirable effects on insulin sensitivity (Tremblay et al., 2007).

Additional potential harmful effects of high-protein diets on the cardiovascular system and cancer formation have been suggested though further research is needed (Eisenstein et al., 2002). High-protein diets should thus not be recommended. In the present study, protein intake was therefore set at 20% of the total energy content which can quite easily be attained with ample fish consumption.

2.7 Possible harmful effects of fish intake

Concern has arisen over potential harm from mercury, dioxins and polychlorinated biphenyls (PCBs) present in some fish species. These contaminants are present in low levels in lakes, rivers, seas and oceans but are concentrated in fish by bioaccumulation and biomagnification (Sidhu, 2003).

2.7.1 Methylmercury

Among metals, methylmercury (MeHg) is the compound of most concern (Costa, 2007). Larger, longer-living predators (e.g. swordfish, shark and tuna) have higher tissue concentrations of MeHg (as high as 1.0 mg/kg (Costa, 2007)) compared to smaller or shorter-lived species (Mozaffarian and Rimm, 2006). MeHg in fish is bound to proteins in muscles so cooking and removal of skin does therefore not significantly reduce its concentration (Costa, 2007).

MeHg is a highly reactive metal and promotes the formation of free radicals. It may also inactivate the antioxidant properties of glutathione and induce lipid peroxidation (Costa, 2007). Health effects of very high mercury exposure are paresthesias, ataxia and sensory abnormalities in adults, and delayed cognitive and neuromuscular development in fetuses. However, the health risks with chronic low exposure of MeHg, such as with fish consumption over time, are not as well documented (Mozaffarian and Rimm, 2006). It has been suggested that MeHg may increase the risk of cardiovascular diseases by promoting atherosclerosis (Costa, 2007) but studies have shown inconsistent results with an overall relative risk factor of 1.12 (0.71-1.75) in a recent meta-analysis (Mozaffarian and Rimm, 2006). The cardioprotective effects of LC n-3 PUFAs greatly outweigh the possible harm by MeHg so the net effect of fish consumption is still beneficial. Fish oil capsules contain little to no mercury and are therefore considered safe in moderate amounts (Foran et al., 2003). Among other metals, fish may also contain arsenic, cadmium and lead, which are not thought to be as harmful as MeHg (Costa, 2007).

2.7.2 PCBs and dioxins

Animal studies and few studies in humans indicate that PCBs and dioxins are carcinogenic, possibly through their effects on specific transcription factor affecting gene expression (Mozaffarian and Rimm, 2006), causing liver tumours (Sidhu, 2003). PCBs and dioxins are lipophilic, so high levels may be found in the adipose tissue of fatty fish (Sidhu, 2003). Therefore, the PCBs content in fish can be reduced 12-40 % by trimming fat, removing skin and by cooking (Mozaffarian and Rimm, 2006).

Largest contributors to total dietary intake of PCBs and dioxins are meats, dairy products and vegetables (86%) while only 9% of these toxic substances comes from fish and shellfish (Schecter et al., 2001). PCBs and dioxins have been measured in farmed and wild salmon, revealing that a consumption of 180 g salmon/week would result in 4 excess cancer deaths per 100.000 individuals, while consumption of either farmed or wild salmon would result in 7125 fewer CHD deaths (Hites et al., 2004; Hamilton et al., 2005). Therefore, cardiovascular benefits outweigh cancer risks 10 to 1000 fold, depending on the level of contamination (Mozaffarian and Rimm, 2006). Additional adverse effects of PCBs and dioxins include immunotoxicity, and reproductive and developmental toxicities (Costa, 2007) and perhaps diabetes (Fujiyoshi et al., 2006).

2.7.3 Special risk groups

Pregnant and lactating women, women of childbearing age and young children are advised to limit consumption of fish species that may contain high levels of MeHg or PCBs. However, since LC n-3 PUFAs, especially DHA, are necessary for brain development during gestation, pregnant women are advised to consume fish at least twice a week, even more often. The Icelandic recommendations (Icelandic Directorate of Health, 2004) advise pregnant women not to eat shark, swordfish, sushi, gravlax, cold-smoked fish, pickled whale, cod liver (since lean fish accumulates its fat in the liver rather than adipose tissue), halibut, fulmar and fulmar eggs. They should not eat tuna and orange roughy more than once a week and canned tuna, guillemot eggs and beaked whale not more than twice a week. In addition, pregnant women should not eat raw fish of any kind due to *Listeria monocytogenes*.

Common Icelandic fish species such as haddock, cod, salmon, trout, halibut, catfish and monkfish are considered safe for everyone and should be consumed as often as possible (Icelandic Directorate of Health, 2004). However, all individuals with very high fish

consumption (≥ 5 servings/week) should limit their intake of species highest in MeHg (Mozaffarian and Rimm, 2006).

In conclusion, overall benefits of moderate fish consumption twice a week outweigh the health risk of contaminants in fish. Mozaffarian and Rimm (2006) also point out that avoidance of fish consumption due to confusion regarding risks and benefits may result in excess CHD deaths in adults and suboptimal neurodevelopment in children.

2.8 Fish consumption in Iceland

2.8.1 Public recommendations on fish intake

The Public Health Institute of Iceland (2006) advises people to consume fish at least twice a week or more often, or at least 300 g/week. Consumption of a variety of lean and fatty fish is recommended. In addition to fish as a main course, people are advised to consume fish-containing fillings and dried fish as often as possible. These recommendations are similar to recommendations in other Nordic countries (NNR, 2004).

Following these recommendations contributes to the fulfilment of various nutrients such as protein, selenium and iodine. In addition, fatty fish is also rich in vitamin D and LC n-3 PUFAs. The health effects of these nutrients are discussed in details above in the *Literature review* of this thesis.

2.8.2 Present and past fish consumption in Iceland

For decades, Iceland has been well-known for the great fishing industry in this small Northern island. High fish consumption has been the main trait of the Icelandic diet and there used to be a firmly established tradition for the intake of fish oil. Many believe that the high consumption of fish and fish oil in Iceland is indeed the reason for how healthy and long-lived Icelanders have been. In 1990, Icelanders consumed the greatest amount of fish among all European countries or 73 g/day on average (or 3-4 times per week, considering a normal portion of 150 g) (Steingrimsdottir, 1991). However, fish consumption has decreased

remarkedly since then and in 2002 it was only 40 g/day, reaching a similar amount common in most European countries (Steingrimsdottir et al., 2003).

According to the Icelandic dietary survey in 2002 (Steingrimsdottir et al., 2003), the majority of Icelanders consume fish 1-2 times/week, or 55% of men and 52% of women, but one fourth of young women consume fish less than once a week. In addition, only one third of the Icelandic population consumed cod liver oil or concentrated cod liver oil capsules daily in 2002. In Iceland, lean fish species are much more popular than fatty fish species, where haddock is the most commonly consumed species, either eaten fresh or bought frozen (Icelandic Ministry of Fisheries, 2007).

Several Icelandic studies indicate a negative relation between fish consumption and BMI, i.e. that individuals that are overweight or obese consume on average less fish than normal weight Icelanders do. Among 67 overweight and mildly obese 20-40 year old Icelanders, fish consumption was only 22 g/day, which is equivalent to one fish meal per week (Magnusdottir, 2008). In the SEAFOODplus YOUNG study, 66% of the participants from Iceland, Ireland and Spain did not meet the recommendation of at least two fish meals per week (Thorsdottir et al., 2008).

Fish consumption has declined the most among young Icelanders. Young girls consume the least of fish or only 15 g/day on average, which corresponds to one fish meal every ten days. Elderly men consume the most, or 65 g/day on average, corresponding to three fish meals per week. Nowadays, the diet of young and older Icelanders is completely different. Young people consume six times more pasta then the elderly, ten times more soda, twelve times more French potatoes and twenty times more pizza. Therefore, pizza seems to be the new national dish of young Icelanders, who consume on average three times less of fish than the elderly (Steingrimsdottir et al., 2003). These relatively new dietary habits in Iceland are of special concern since the average fish consumption is likely to lower even further in the coming years, given the same continuation of dietary habits.

2.9 Recommendations on dietary intake

The food-based dietary guidelines published by the Public Health Institute of Iceland (2006) are in line with the Nordic recommendations (NNR, 2004) and their purpose is to inhibit vitamins and minerals deficiencies and promote a balance between energy-giving nutrients (figure 2.1). The guidelines are as follows (Public Health Institute of Iceland, 2006):

- 500 g of fruits, vegetables and fruit juices should be consumed daily, thereof at least 200 g vegetables and 200 g fruits, in addition to potatoes. Children younger than 10 years of age need smaller portions.
- Fish should be consumed at least twice a week (≥ 300 g/week), preferably more often.
- Consumption of whole grain cereals and other fiber-rich products should be increased. Fiber consumption should be at least 25 g/day (according to 2400 kcal diet).
- Two glasses, plates or cans of milk or dairy products should be consumed daily. Low-fat products and products that contain only small amount of added sugar are recommended. Cheese can substitute a part of the dairy products whereas 25 g of cheese corresponds to one portion.
- Consumption of oil and other unsaturated fatty acids should be increased at the expense of saturated and trans fatty acid. Fat and fatty foods should be consumed in moderation.
- Salt intake should be minimised; women should not consume more than 6 g of salt daily and men not more than 7 g.
- Fish oil supplements containing vitamin D should be consumed daily where one teaspoon (5 ml) of cod liver oil contains the RI of vitamin D for children and adults 60 years of age and younger.
- Water should be the most common drink whereas soft drinks and other drinks should be used in moderation.
- Sugar, cakes, sweets, ice cream and alcohol should be used in moderation.
- Regular meals during the day are recommended, 4-6 meals/day.

Macronutrient recommendations

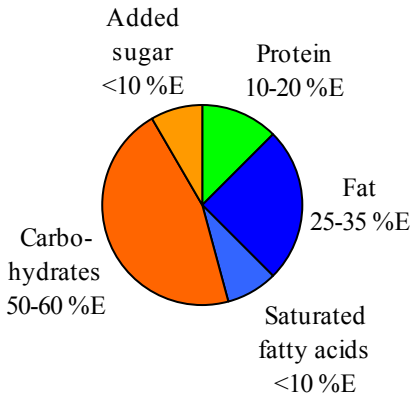

Figure 2.1: Recommended distribution of energy-giving nutrients; fat, protein and carbohydrates (Public Health Institute of Iceland, 2006).

3. SUBJECTS AND METHODS

3.1 Recruitment of subjects

Subjects in two of the three diet groups in the present study were recruited in the randomized controlled trial SEAFOODplus YOUNG which was conducted during the winter 2004-2005. That intervention was performed on 324 volunteers in three European countries; Iceland (n=140), Ireland (n=120) and Spain (n=64). The aim of the SEAFOODplus YOUNG intervention was to study the effects of different kinds of seafood on weight loss and other health variables in young European overweight and mildly obese adults on weight loss diets for eight consecutive weeks. The effects of four isocaloric diet groups were investigated; 1) a control group receiving no seafood but included sunflower oil supplements daily, 2) a lean fish group receiving 150 g cod 3x per week, 3) a fatty fish group receiving 150 g salmon 3x per week and 4) a fish oil group receiving no seafood but included fish oil supplements daily.

The results of the SEAFOODplus YOUNG intervention have been studied thoroughly in several master theses at the Unit for Nutrition Research in Reykjavik, Iceland (Gudnadottir, 2006; Sigurgeirsdottir, 2006; Arnarson, 2007; Arsaelsdottir, 2007; Gisladottir, 2007).

In the present study, subjects consisted of the Icelandic participants in diet groups 1 (n=35) and 2 (n=35) in the SEAFOODplus YOUNG intervention and then a third diet group (n=56) was added, receiving 150 g cod 5x per week. Subjects in the third group were recruited during the winter 2006-2007 by the author of this thesis. The protocol and inclusion and exclusion criteria for the third group were exactly the same is in the SEAFOODplus YOUNG study (Gisladottir, 2007) and therefore the tree diet groups are fully comparable. The aims of this thesis were to investigate whether various amounts of lean fish consumption, as part of an energy-restricted diet, had different effects on weight loss as well as other parameters of cardiovascular risk factors, i.e. if there were dose-response relations between lean fish consumption and weight loss or other cardiovascular parameters.

All participants were volunteers recruited through advertisements (see appendix II) in large workplaces in Reykjavik, Iceland, most of them coming from The University of Iceland and several banks in Reykjavik. A total of 126 Icelandic individuals participated (49 men and 77 women). The present study was approved by the National Bioethical Committee in

Iceland. The study followed the Helsinki guidelines and all subjects participating gave their written informed consent (see appendix III-V).

3.2 Inclusion and exclusion criteria

All potential subjects were screened for inclusion and exclusion criteria, first by phonescreening and then in person.

The inclusion criteria were:

- Body mass index (BMI) 27.5-32.5 kg/m^2
- Age 20-40 years
- Waist circumference of ≥ 94 cm and ≥ 80 cm for men and women, respectively

The exclusion criteria were:

- Weight loss (≥ 3 kg) within three months before the start of the intervention
- Use of supplements containing n-3 fatty acids, calcium or vitamin D during the last three months
- Drug treatment of diabetes mellitus, hypertension or hyperlipidemia
- Insulin-dependent (type I) diabetes
- Allergy for fish
- Women's pregnancy or lactation

3.3 Protocol

The subjects had three visits to the clinic during the eight-week trial; at baseline, midpoint and endpoint (figure 3.1). Additionally, they were contacted on two other occasions by phone and/or e-mail, in week 2 and in week 6. The subjects were also encouraged to contact the clinic of their own initiative if they had any questions regarding the research during the eight-week intervention period.

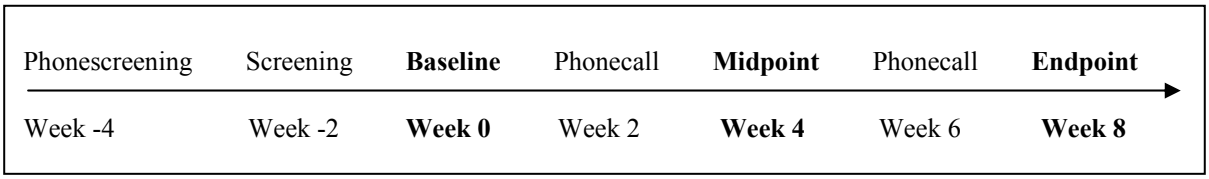

Figure 3.1: *The intervention setup.*

At baseline, midpoint and endpoint visits participants met a dietician, anthropometrical measurements were performed and compliance to seafood intake was assessed by a validated food frequency questionnaire (FFQ, see appendix VI) (Birgisdottir et al., 2008; Thorsdottir et al., 2008). The tasks that were performed during these visits can be seen in table 3.1. Total dietary intake was assessed by two-day weighed food records before baseline (investigating habitual diet) and during the two last weeks of the intervention trial (testing compliance to meal plans). At baseline, information on physical activity pattern during the last year, smoking habits and alcohol consumption were collected using a questionnaire (see appendix VII) (Martinez-Gonzalez et al., 2001). Subjects were instructed not to change their physical activity level during the eight-week intervention period and to keep their alcohol consumption to a minimum (max one drink of wine/beer per week).

Table 3.1: *Measurements and other tasks during the three visits to the clinic.*

Baseline (week 0)	**Midpoint (week 4)**	**Endpoint (week 8)**
Height	-	-
Weight	Weight	Weight
Waist circumference	Waist circumference	Waist circumference
Bioimpedance	Bioimpedance	Bioimpedance
Blood pressure	-	Blood pressure
Blood sample	-	Blood sample
FFQ; food frequency questionnaire	FFQ; food frequency questionnaire	FFQ; food frequency questionnaire
Lifestyle questionnaire (LSQ)	-	Lifestyle questionnaire (LSQ)
Interview with dietician	Interview with dietician	Interview with dietician
-	-	VAS; visual analogue scale (at home in week 6-7)
Two-day food registration (at home before baseline)	-	Two-day food registration (at home in week 6-7)

3.4 Intervention diets

3.4.1 Calculations of energy restriction

Basal metabolic rate (BMR) was calculated using Harris-Benedict equations (Cankayali et al., 2004) and a correction factor due to overweight and obesity of the subjects (Salvino et al., 2004). The calculations can be seen in tables 3.2 and 3.3. To estimate total energy expenditure the physical activity level was set to 1.3 since a low physical activity level was reported by all subjects (NNR, 2004). Each subject was instructed to follow a specific diet, energy-restricted by 30% from estimated total energy expenditure (~600 kcal/day energy restriction), for eight consecutive weeks. Since an energy deficit of 500-1000 kcal/day is believed to cause a 0.45-0.91 kg weight loss per week (NHLBI, 1998), an energy restriction of the present degree was believed to cause a roughly 0.5-1 kg weight loss per week, or 4-8 kg weight loss after eight weeks.

Table 3.2: *Calculations of the body weight that is used in the Harris-Benedict equations to evaluate BMR (see table 3.3) (Salvino et al., 2004).*

Ideal body weight	50 + (0.75*(Height cm – 150))
Weight used in the Harris-Benedict equations	((Actual body weight kg – Ideal body weight kg) *0.25) + Ideal body weight kg

Table 3.3: *Calculations of BMR for overweight subjects[1] by applying Harris-Benedict equations (Cankayali et al., 2004).*

Men	66.473 + (13.7516*Weight kg) + (5.0033*Height cm) – (6.755*Age years)
Women	655.0955 + (9.5634*Weight kg) + (1.8496*Height cm) – (4.6756*Age years)

[1] Weight given in the equation is calculated from ideal body weight (see table 3.2).

3.4.2 Diet groups

Each subject was allocated into one of three isocaloric diet groups consisting of different amounts of lean fish (table 3.4). Cod provided subjects in group 2 with 0.26 g LC n-3 PUFAs/day and 11.4 g fish protein/day, and group 3 with 0.43 g LC n-3 PUFAs/day and 19.1 g fish protein/day.

Table 3.4: *Diet groups in the present intervention*

Group:	**Fish:**	**Recruitment:**
1. Control	No seafood, but received lean meat instead	SEAFODplus YOUNG, 2004-2005
2. Lean fish I	150 g cod, 3x per week	SEAFODplus YOUNG, 2004-2005
3. Lean fish II	150 g cod, 5x per week	Current intervention, 2006-2007

The macronutrient composition of the three diets was according to public recommendations (NNR, 2004; Public Health Institute of Iceland, 2006) and designed to be identical in total fat (30% of total energy), carbohydrate (50% of total energy), protein (20% of total energy) and dietary fibre (20-25 g/day). Each subject received a detailed individual-based diet plan based on a food exchange system (see appendix VIII and IX) and instructions were given to standardize sources of fat, fruit and vegetable consumption and meal frequency.

3.5 Measurements

3.5.1 Anthropometric measurements

Anthropometric measurements were performed on three occasions during the eight-week period; at baseline, midpoint and endpoint (table 3.1). Body weight was measured in light underwear on a calibrated scale (SECA 708, Germany). Subject's height was measured at baseline with a calibrated stadiometer and waist circumference was measured with a tape measure following standardized methods (Gisladottir, 2007). Body fat mass, fat-free mass and fat percentage were assessed by bioelectrical impedance analysis (BIA) (Bodystat 1500, Bodystat Ltd, UK).

3.5.2 Blood parameters

Blood sampling (after overnight fasting) was conducted at baseline and endpoint of the study. Total cholesterol concentration in serum, serum triglycerides and fasting plasma glucose

concentrations were analysed using an enzymatic colorimetric assay and an automated analyzer (Hitachi 911; Roche Diagnostics). HDL cholesterol was determined in serum using PEG-modified enzymes and dextrane sulphate. Levels of serum LDL cholesterol were calculated using the Friedewald formula. Insulin concentration in plasma was measured with electrochemiluminescence immunoassay (ECLIA) on Modular Analytics E170 system from Roche Diagnostics (Manheim, Germany). A strict routine for blood pressure measurements was adhered to, which was defined in the research protocol (Gisladottir, 2007).

3.6 Statistical analysis

The data were analyzed using the SPSS statistical package 11.0. Values are reported as mean ± standard deviation (s.d.). Distributions of the investigated variables were estimated using the Kolmogornov Smirnov test. Differences between baseline and endpoint were calculated using paired-sample T-test or Wilcoxon signed ranks test, for normally and not normally distributed variables, respectively. Unadjusted differences between genders were calculated using the independent-samples T-test or Mann-Whitney U-test whereas unadjusted differences between dietary groups were calculated using one-way ANOVA (with LSD post hoc test) or Kruskal-Wallis H-test. A possible difference in dropouts between groups was investigated using chi-squared test. For correlation analysis, Pearson correlation or Spearman correlation were used.

In order to assess the effects of cod consumption (group 1 = control, group 2 = cod three times a week, group 3 = cod five times a week) on anthropometrical changes and improvements of blood variables (Δy) during the intervention, linear ANCOVA (analysis of covariance) models were constructed including the following variables: y at baseline, gender and diet group. The quality of the models was checked using Levene's test of homogeneity and the residuals of the models were checked for normality using Kolmogornov Smirnov test. Linear trend analysis was performed to investigate a possible dose-response relationship between cod consumption and outcome variables. The significance level in all statistical analyses was set at $P < 0.05$.

4. RESULTS

4.1 Participation and dropouts

Altogether, 126 subjects, or 49 men (39% of participants) and 77 women (61% of participants) started in one of the three diet groups. One hundred subjects (42 men and 58 women), or 79% of the participants, completed the intervention (Figure 4.1). 86% of men and 75% of women completed the intervention. Dropouts were not significantly different between women and men or between diet groups.

The main reasons for dropout were that the subject was unable to follow the restricted diet or had a lack of time to maintain the schedule of clinical visits. In addition, several women became pregnant during the intervention time.

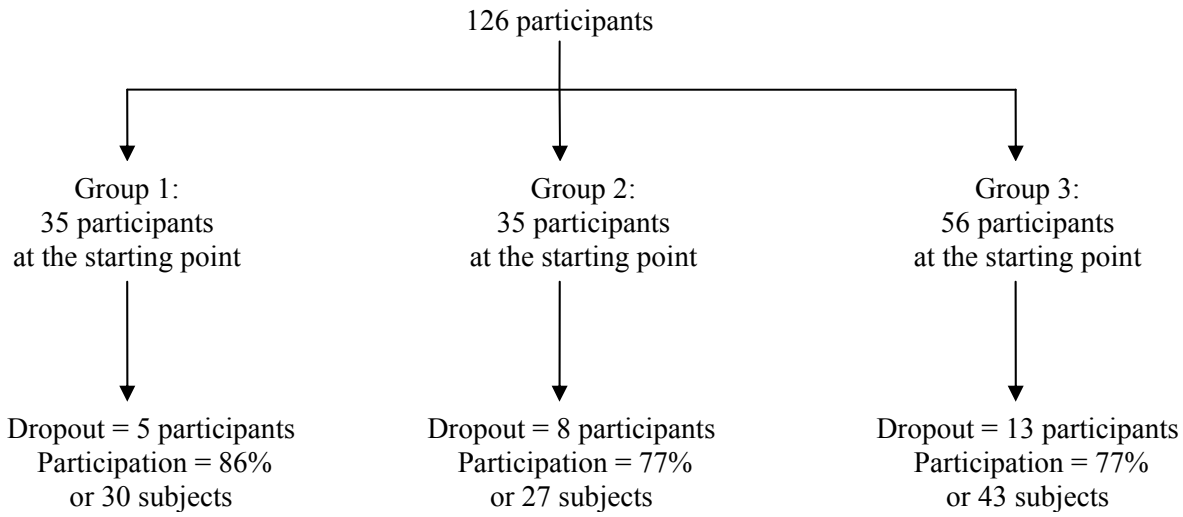

Figure 4.1: *Participation in each diet group and dropouts.*

4.2 Dietary intake

4.2.1 Energy-giving nutrients

The reported energy intake at baseline was higher than the calculated energy requirement (men: 2707 ± 860 vs. 2427 ± 143 kcal/day, P = 0.023; and women: 2081 ± 512 vs. 1935 ± 88 kcal/day, P = 0.016). The 30% energy restriction, calculated from energy requirements, was on average 634 ± 81 kcal/day (724 ± 43 vs. 576 ± 32 kcal/day for men and women, respectively, P < 0.001). Reported mean energy intake during the intervention in men was lower than the prescribed energy intake according to the meal plans (1465 ± 356 vs. 1703 ± 101 kcal/day, P < 0.001), but not in women (1322 ± 216 vs. 1359 ± 62 kcal/day, P = 0.221). There were no significant differences in total energy intake between intervention groups, neither at baseline nor endpoint.

Macronutrient intake at baseline and at the end of the trial were not different between the three intervention groups, with the exception of a lower protein intake in group 1 compared to groups 3 and 2 at endpoint, both in grams (66 ± 17 vs. 78 ± 14 g/day, P = 0.004 in LSD post hoc test, and 75 ± 15 g/day, P = 0.04) and as percentage of total energy intake (19 ± 2.2 vs. 23 ± 3.4 %E, P < 0.001, and 22 ± 3.4 %E, P = 0.02). Also, consumption of trans fatty acids at endpoint was higher in group 2 compared to group 3 (1.6 ± 1.1 vs. 1.1 ± 0.5 g/day, P = 0.009) and alcohol consumption at endpoint was higher in group 1 compared to group 3 (5.8 ± 12 vs. 0.3 ± 2.2 g/day, P = 0.002).

There was a significant difference in consumption of all energy-giving nutrients during the intervention, which usually resulted in consumption closer to the public recommendations in Iceland (table 4.1 and figure 3.1). Fat consumption decreased from 35 %E to 28 %E (P < 0.001), consumption of saturated fatty acids (SFA) decreased from 14 %E to 11 %E (P < 0.001) and consumption of added sugar decreased from 12 %E to 4.8 %E (P < 0.001). Also, carbohydrate consumption increased from 46 %E to 48 %E (P < 0.001) and fibre consumption increased from 18 g/day to 21 g/day (P < 0.001). However, consumption of PUFAs decreased from 4.6 %E to 4.0 %E (P = 0.009) and protein consumption increased from 17 %E to 22 %E (P < 0.001).

Table 4.1: *Comparison of energy-giving nutrient intake/day at baseline and endpoint. Values are unadjusted, shown as means ± s.d.*

	MEN			WOMEN			ALL SUBJECTS		
	Baseline	**Endpoint**	**P[1]-value**	**Baseline**	**Endpoint**	**P-value**	**Baseline**	**Endpoint**	**P-value**
Protein (g)	119 ± 39	79 ± 19	*< 0.001*	85 ± 20	70 ± 12	*< 0.001*	99 ± 33	74 ± 16	*< 0.001*
Protein (%E)	18 ± 3.4	22 ± 3.2	*< 0.001*	17 ± 3.7	21 ± 3.7	*< 0.001*	17 ± 3.6	22 ± 3.5	*< 0.001*
Fat (g)	106 ± 44	48 ± 17	*< 0.001*	80 ± 23	41 ± 12	*< 0.001*	91 ± 36	44 ± 15	*< 0.001*
Fat (%E)	35 ± 6.2	29 ± 6.6	*< 0.001*	35 ± 5.9	28 ± 5.9	*< 0.001*	35 ± 6.0	28 ± 6.2	*< 0.001*
SFA (g)	45 ± 22	19 ± 7.6	*< 0.001*	33 ± 11	17 ± 5.1	*< 0.001*	38 ± 17	17 ± 6.3	*< 0.001*
SFA (%E)	14 ± 3.7	11 ± 3.1	*< 0.001*	14 ± 2.9	11 ± 2.5	*< 0.001*	14 ± 3.2	11 ± 2.7	*< 0.001*
MUFA (g)	34 ± 14	16 ± 6.1	*< 0.001*	26 ± 8.2	13 ± 4.5	*< 0.001*	29 ± 12	14 ± 5.3	*< 0.001*
MUFA (%E)	11 ± 2.3	9.6 ± 3.0	*0.003*	11 ± 2.4	9.0 ± 2.6	*< 0.001*	11 ± 2.3	9.2 ± 2.8	*< 0.001*
PUFA (g)	13 ± 5.5	6.8 ± 3.4	*< 0.001*	11 ± 4.7	5.8 ± 2.5	*< 0.001*	12 ± 5.2	6.2 ± 2.9	*< 0.001*
PUFA (%E)	4.4 ± 1.6	4.1 ± 1.6	*0.424*	4.7 ± 1.7	3.9 ± 1.5	*0.007*	4.6 ± 1.7	4.0 ± 1.5	*0.009*
Trans FA (g)	3.9 ± 2.8	1.4 ± 0.9	*< 0.001*	2.9 ± 1.6	1.2 ± 0.7	*< 0.001*	3.3 ± 2.2	1.3 ± 0.8	*< 0.001*
Trans FA (%E)	1.3 ± 0.7	0.9 ± 0.6	*0.001*	1.2 ± 0.7	0.8 ± 0.4	*< 0.001*	1.3 ± 0.7	0.8 ± 0.5	*< 0.001*
Carbohydr-ates (g)	301 ± 98	173 ± 44	*< 0.001*	240 ± 71	162 ± 31	*< 0.001*	265 ± 88	166 ± 37	*< 0.001*
Carbohydr-ates (%E)	45 ± 6.6	48 ± 6.9	*0.005*	46 ± 6.3	49 ± 5.6	*0.009*	46 ± 6.4	48 ± 6.2	*< 0.001*
Fiber (g)	19 ± 7.1	21 ± 7.4	*0.074*	17 ± 5.2	21 ± 5.6	*< 0.001*	18 ± 6.0	21 ± 6.3	*< 0.001*
Added sugar (g)	85 ± 55	15 ± 9.8	*< 0.001*	65 ± 43	18 ± 11	*< 0.001*	73 ± 49	17 ± 10	*< 0.001*
Added sugar (%E)	12 ± 6.9	4.2 ± 2.4	*< 0.001*	12 ± 6.3	5.3 ± 2.9	*< 0.001*	12 ± 6.5	4.8 ± 2.8	*< 0.001*
Alcohol (g)	11 ± 20	2.5 ± 8.7	*0.001*	4.7 ± 10	2.4 ± 7.9	*0.009*	7.3 ± 15	2.4 ± 8.2	*< 0.001*
Alcohol (%E)	2.7 ± 4.6	1.0 ± 3.2	*0.007*	1.5 ± 3.2	1.1 ± 3.8	*0.196*	2.0 ± 3.8	1.1 ± 3.6	*0.020*
Cholesterol (mg)	370 ± 205	186 ± 97	*< 0.001*	236 ± 103	141 ± 42	*< 0.001*	290 ± 166	159 ± 73	*< 0.001*

[1] Paired-samples T-test / Wilcoxon signed ranks test

4.2.2 Vitamins and minerals

Micronutrient intake at baseline and endpoint can be seen in table 4.2. On average, the intake was higher than the recommended intake (RI) for most vitamins and minerals. However, the intake of some micronutrients did not reach recommendations. Vitamin D intake was far below the RI of 10 µg/day, or 2.7 ± 2.9 and 2.6 ± 1.6 µg/day at baseline and endpoint, respectively (insignificant decline). Vitamin E intake was also below RI except in women at endpoint. Thiamine, riboflavin and niacin intake was below RI at endpoint in men. Of special concern is the inadequate intake of folic acid (at baseline) and iron (at baseline and endpoint) in women of this fertile age group.

Table 4.2: *Comparison of vitamin and mineral intake/day at baseline and endpoint. Values are unadjusted, shown as means ± s.d.*

	MEN				WOMEN			
	RI	**Baseline**	**Endpoint**	**P-value**[1]	**RI**	**Baseline**	**Endpoint**	**P-value**
Vit A (µg)	900	912 ± 791	1308 ± 1184	*0.157*	700	898 ± 870	1371 ± 898	*0.004*
Vit D (µg)	10	2.4 ± 2.0	2.4 ± 1.3	*0.567*	10	2.9 ± 3.3	2.7 ± 1.7	*0.737*
Vit E (mg)	10	8.6 ± 4.6	6.1 ± 2.2	*0.008*	8	7.0 ± 2.6	11 ± 20	*0.234*
Thiamine (mg)	1.4	1.7 ± 0.8	1.3 ± 0.5	*0.004*	1.1	1.3 ± 0.5	1.6 ± 0.7	*0.046*
Riboflavin (mg)	1.7	2.2 ± 0.9	1.6 ± 0.5	*< 0.001*	1.3	1.8 ± 0.7	2.0 ± 0.8	*0.469*
Niacin (mg)	19	26 ± 12	18 ± 5.9	*< 0.001*	15	18 ± 7.1	21 ± 8.6	*0.057*
Vit B6 (mg)	1.6	2.2 ± 0.9	2.1 ± 0.7	*0.693*	1.2	1.7 ± 0.7	2.5 ± 1.1	*< 0.001*
Folic acid (µg)	300	345 ± 159	379 ± 131	*0.328*	400	313 ± 150	461 ± 225	*< 0.001*
Vit B12 (µg)	2.0	6.4 ± 3.7	5.0 ± 2.0	*0.078*	2.0	5.3 ± 3.1	5.1 ± 1.8	*0.417*
Vit C (mg)	75	90 ± 72	143 ± 88	*0.003*	75	95 ± 72	183 ± 139	*< 0.001*
Iodine (µg)	150	168 ± 136	203 ± 135	*0.243*	150	133 ± 94	231 ± 124	*< 0.001*
Selenium (µg)	50	86 ± 35	79 ± 29	*0.116*	40	64 ± 26	71 ± 26	*0.382*
Calcium (mg)	800	1245 ± 512	923 ± 289	*< 0.001*	800	1056 ± 339	901 ± 180	*< 0.001*
Phosphorus (mg)	600	1948 ± 620	1427 ± 360	*< 0.001*	600	1515 ± 343	1320 ± 199	*< 0.001*
Natrium (g)		4.2 ± 1.4	2.7 ± 0.7	*< 0.001*		3.1 ± 0.9	2.5 ± 0.6	*< 0.001*
Iron (mg)	9	15 ± 7.6	12 ± 5.2	*0.017*	15	13 ± 6.3	14 ± 5.7	*0.656*
Zinc (mg)	9	14 ± 5.4	10 ± 3.1	*< 0.001*	7	11 ± 3.7	12 ± 6.5	*0.633*

[1] Paired samples T-test / Wilcoxon signed ranks test

The intake of iodine decreased during the trial in the control group but increased significantly in group 3 receiving the highest amount of cod and also in women in diet group 2 (table 4.3). Also, the selenium intake decreased in the control group while it increased in diet group 3, significantly in women. The positive changes in iodine and selenium intake during the trial in diet groups 2 and 3 are most likely related to the increased lean fish consumption, since other dietary factors between groups were approximately the same.

Table 4.3: *Comparison of iodine and selenium intake/day from baseline to endpoint[1]. Values are unadjusted, shown as means ± s.d.*

	Iodine intake (µg)			**Selenium intake (µg)**		
	Baseline	**Endpoint**	**P-value[2]**	**Baseline**	**Endpoint**	**P-value**
Diet group 1						
Men	186 ± 119	79 ± 49	*0.007*	88 ± 29	54 ± 16	*0.008*
Women	131 ± 72	111 ± 66	*0.300*	65 ± 19	41 ± 7.8	*< 0.001*
Diet group 2						
Men	187 ± 158	199 ± 109	*0.674*	92 ± 39	81 ± 24	*0.105*
Women	131 ± 64	209 ± 105	*0.048*	73 ± 33	73 ± 18	*0.691*
Diet group 3						
Men	147 ± 139	300 ± 112	*0.005*	82 ± 39	98 ± 25	*0.319*
Women	135 ± 120	322 ± 93	*< 0.001*	58 ± 23	87 ± 23	*< 0.001*
All subjects						
Men	168 ± 136	203 ± 135	*0.243*	86 ± 35	79 ± 29	*0.128*
Women	133 ± 94	231 ± 124	*< 0.001*	64 ± 26	71 ± 26	*0.224*

[1] RI of iodine is 150 µg/day for men and women, and RI of selenium is 50 µg/day for men and 40 µg/day for women.
[2] Paired-samples T-test / Wilcoxon signed ranks test

4.3 Anthropometry

4.3.1 Baseline anthropometry

Anthropometric measurements at baseline are shown in table 4.4 (unadjusted values). The average age and BMI of men entering the trial was 31.0 ± 5.4 years and 30.4 ± 1.3 kg/m^2, respectively, and of women 29.9 ± 6.0 years and 30.0 ± 1.4 kg/m^2, respectively. Therefore, these values fitted well with the inclusion criteria for age (20-40 years) and BMI (27.5-32.5 kg/m^2). Men entering the trial weighed on average 100.0 ± 8.6 kg and women 85.2 ± 8.3 kg. Waist circumference at baseline was 102.3 ± 5.6 cm for men and 93.8 ± 6.3 cm for women, being well above the inclusion criteria of ≥ 94 cm for men and ≥ 80 cm for women. There were no significant differences in body weight, BMI and waist circumference between diet groups, neither among men nor women. Men in the control group (diet group 1) were significantly older (33.7 ± 5.0) than men in both cod groups (diet groups 2 and 3) (P = 0.042).

Body composition measurements at baseline of fat mass, fat-free mass and body fat percentage for men were 25.9 ± 4.6 kg, 74.1 ± 5.6 kg and 25.8 ± 3.1 %, respectively, and for women 33.1 ± 4.9 kg, 51.9 ± 4.5 kg and 38.8 ± 3.1 %, respectively. As can be seen in table 4.4, diet group 3 had significantly higher body fat percentage at baseline than diet groups 1 and 2, both among men (P < 0.001) and women (P = 0.012). Men in diet group 3 had significantly higher fat mass at baseline than men in diet groups 1 and 2 (P < 0.001), and women in diet group 2 had significantly higher fat-free mass than women in diet groups 1 and 3 (P = 0.023). There was a clear gender difference in body composition, as expected, where women had significantly more fat mass, less fat-free mass and hence, a higher body fat percentage than men (P < 0.001).

Table 4.4: *Baseline characteristics; anthropometric measurements and blood parameters. Values are unadjusted, shown as means ± s.d.*

	Group 1 - Control		Group 2 - Cod 3x /w		Group 3 - Cod 5x /w		All subjects		P-value[1]		post-hoc	
	Men (n=16)	Women (n=19)	Men (n=11)	Women (n=24)	Men (n=22)	Women (n=34)	Men (n=49)	Women (n=77)	Men	Women	Men	Women
Anthropometric measurements:												
Body weight (kg)	98.4 ± 8.4	82.7 ± 7.8	98.8 ± 7.4	86.8 ± 7.5	101.8 ± 9.2	85.4 ± 8.9	100.0 ± 8.6	85.2 ± 8.3	*0.441*	*0.266*		
Height (m)	1.81 ± 0.07	1.67 ± 0.06	1.81 ± 0.05	1.70 ± 0.06	1.82 ± 0.07	1.69 ± 0.07	1.81 ± 0.07	1.68 ± 0.07	*0.698*	*0.417*		
BMI (kg/m^2)	30.2 ± 1.6	29.7 ± 1.4	30.3 ± 1.0	30.2 ± 1.5	30.6 ± 1.2	30.0 ± 1.4	30.4 ± 1.3	30.0 ± 1.4	*0.544*	*0.500*		
Waist circumference (cm)	101.5 ± 5.9	91.7 ± 5.5	100.6 ± 4.6	93.2 ± 5.5	103.8 ± 5.8	95.5 ± 6.9	102.3 ± 5.6	93.8 ± 6.3	*0.255*	*0.094*		
Fat mass (kg)	23.9 ± 4.4	31.7 ± 5.2	23.7 ± 3.6	32.9 ± 4.2	28.6 ± 4.0	34.1 ± 5.2	25.9 ± 4.6	33.1 ± 4.9	*0.001*	*0.234*	3 > 1, 2	
Fat-free mass (kg)	74.6 ± 5.3	51.0 ± 3.4	75.1 ± 5.1	54.1 ± 4.3	73.2 ± 6.2	51.0 ± 4.8	74.1 ± 5.6	51.9 ± 4.5	*0.620*	*0.023*		2 > 3, 1
Body fat percentage (%)	24.1 ± 2.7	38.1 ± 3.1	24.0 ± 2.5	37.7 ± 2.6	28.1 ± 2.0	39.9 ± 3.0	25.8 ± 3.1	38.8 ± 3.1	*<0.001*	*0.012*	3 > 2, 1	3 > 2, 1
Blood parameters:												
Total cholesterol (mmol/L)	5.1 ± 0.9	5.1 ± 0.6	5.0 ± 0.8	4.9 ± 0.7	4.7 ± 1.0	4.6 ± 1.0	4.9 ± 0.9	4.8 ± 0.9	*0.502*	*0.121*		
HDL cholesterol (mmol/L)	1.2 ± 0.3	1.5 ± 0.2	1.0 ± 0.2	1.5 ± 0.3	0.9 ± 0.2	1.3 ± 0.4	1.0 ± 0.2	1.4 ± 0.3	*0.002*	*0.049*	1 > 3	3 < 1, 2
LDL cholesterol (mmol/L)	3.2 ± 0.9	3.1 ± 0.5	3.2 ± 0.9	3.0 ± 0.7	3.1 ± 0.9	2.8 ± 0.9	3.2 ± 0.9	3.0 ± 0.8	*0.938*	*0.305*		
Triglyceride (mmol/L)	1.5 ± 0.9	1.0 ± 0.4	1.5 ± 0.6	1.1 ± 0.4	1.5 ± 0.9	1.2 ± 0.6	1.5 ± 0.8	1.1 ± 0.5	*0.816*	*0.495*		
Glucose (mmol/L)	5.0 ± 0.3	4.6 ± 0.4	4.8 ± 0.4	4.6 ± 0.4	4.8 ± 0.5	4.5 ± 0.5	4.9 ± 0.4	4.6 ± 0.5	*0.383*	*0.374*		
Insulin (mU/L)	12.9 ± 8.2	9.8 ± 5.0	9.0 ± 2.5	10.5 ± 4.4	8.6 ± 6.3	6.1 ± 5.1	10.1 ± 6.6	8.4 ± 5.2	*0.075*	*<0.001*		3 < 2, 1
Systolic BP (mmHg)	133.4 ± 7.5	124.2 ± 7.6	133.9 ± 7.2	123.7 ± 9.1	139.3 ± 11.2	127.0 ± 10.7	136.2 ± 9.6	125.3 ± 9.5	*0.113*	*0.361*		
Diastolic BP (mmHg)	73.1 ± 8.0	73.0 ± 5.7	73.4 ± 5.9	74.0 ± 5.8	73.1 ± 8.5	71.3 ± 8.2	73.2 ± 7.7	72.6 ± 6.9	*0.996*	*0.329*		

[1] One-way ANOVA / Kruskal-Wallis H-test

4.3.2 Weight loss

Anthropometric measurements at the end of the trial are shown in appendix X, table A1. Unadjusted changes in body weight and other anthropometric measurements from baseline to endpoint are shown in table 4.5 and a clearer view of all subjects can be seen in figure 4.2.

Table 4.5: *Reductions in anthropometry at endpoint. Values are unadjusted, shown as means ± s.d.*

	Group 1 Control	**Group 2 Cod 3x /w**	**Group 3 Cod 5x /w**	**All subjects**	**P-value[1]**	**post-hoc[2]**
Body weight decrease (kg)						
Men	5.0 ± 3.5	5.6 ± 3.1	5.6 ± 3.5	5.4 ± 3.4	*0.873*	
Women	3.3 ± 2.2	4.2 ± 2.5	6.0 ± 2.3	4.6 ± 2.6	*0.002*	*3 > 1, 2*
All	4.1 ± 3.0	4.6 ± 2.7	5.8 ± 2.9	5.0 ± 2.9	*0.037*	*3 > 1*
				P=0.214 [3]		
BMI decrease (kg/m^2)						
Men	1.5 ± 1.1	1.7 ± 1.0	1.7 ± 1.0	1.7 ± 1.0	*0.852*	
Women	1.2 ± 0.8	1.5 ± 0.9	2.1 ± 0.9	1.6 ± 0.9	*0.003*	*3 > 1, 2*
All	1.3 ± 0.9	1.5 ± 0.9	1.9 ± 1.0	1.6 ± 1.0	*0.024*	*3 > 1*
				P=0.994		
Waist circumference decrease (cm)						
Men	4.2 ± 2.8	4.7 ± 2.7	6.1 ± 3.8	5.2 ± 3.3	*0.234*	
Women	2.5 ± 2.1	4.1 ± 2.8	7.3 ± 2.2	4.9 ± 3.1	*<0.001*	*3 > 1, 2*
All	3.3 ± 2.5	4.3 ± 2.8	6.8 ± 3.1	5.0 ± 3.2	*<0.001*	*3 > 1, 2*
				P=0.698		
Fat mass decrease (kg)						
Men	2.8 ± 3.2	3.2 ± 2.7	3.3 ± 2.3	3.1 ± 2.7	*0.853*	
Women	2.8 ± 2.6	3.1 ± 2.1	4.6 ± 3.1	3.6 ± 2.7	*0.090*	
All	2.8 ± 2.8	3.1 ± 2.3	4.0 ± 2.8	3.4 ± 2.7	*0.160*	
				P=0.418		
Fat-free mass decrease (kg)						
Men	2.2 ± 1.9	2.4 ± 2.2	2.4 ± 1.9	2.3 ± 1.9	*0.951*	
Women	0.5 ± 1.6	0.9 ± 1.2	1.3 ± 2.4	1.0 ± 1.9	*0.413*	
All	1.3 ± 1.9	1.4 ± 1.7	1.8 ± 2.2	1.6 ± 2.0	*0.485*	
				P=0.001		
Body fat percentage decrease (%)						
Men	1.7 ± 2.6	2.2 ± 2.4	1.9 ± 1.7	1.9 ± 2.1	*0.906*	
Women	1.9 ± 2.2	1.9 ± 1.6	2.8 ± 3.1	2.2 ± 2.5	*0.428*	
All	1.8 ± 2.4	2.0 ± 1.8	2.4 ± 2.6	2.1 ± 2.3	*0.577*	
				P=0.482		

[1] One-way ANOVA / Kruskal-Wallis H-test

[2] LSD post-hoc test revealing differences in diet groups 1, 2 and 3

[3] Independent samples T-test / Mann-Whitney U-test

After the eight-week intervention, body weight was significantly lower in all diet groups compared to baseline ($P < 0.001$) where the average reduction was 5.0 ± 2.9 kg, or 5.4 ± 3.4 kg for men and 4.6 ± 2.6 kg for women ($P = 0.214$). Unadjusted weight loss was greatest in diet group 3 receiving cod 5x /w, where subjects lost on average 5.8 ± 2.9 kg, being significantly greater compared to the control group, which lost on average 4.1 ± 3.0 kg ($P = 0.014$ in LSD post hoc test), but insignificantly greater than diet group 2 that received cod 3x /w and lost on average 4.6 ± 2.7 kg ($P = 0.097$).

When looking at the genders seperately it can be seen that women receiving cod 5x /w lost the most body weight during the trial, or 6.0 ± 2.3 kg, which was significantly greater than weight loss among women receiving less or no cod (diet groups 1 and 2, $P = 0.002$). Women in the control group lost the least body weight during the trial, or 3.3 ± 2.2 kg. Weight loss among men was more even when comparing their diet groups although men in the control group lost insignificantly less body weight (5.0 ± 3.5 kg) than men in the lean fish groups (5.6 ± 3.1 kg and 5.6 ± 3.5 kg in group 2 and 3, respectively).

On average, participants lost 5.5 ± 3.1 % of their baseline body weight. Relative weight loss in group 3 was significantly higher compared to group 1 (6.4 ± 3.1 % vs. 4.5 ± 3.0 %, $P = 0.026$). Women in diet group 3 lost the most relative body weight, or 7.1 ± 2.7 % of their baseline body weight, and women in diet group 1 the least, or 4.0 ± 2.5 % ($P = 0.002$). 59% of all subjects lost ≥ 5% of their baseline body weight (43%, 67% and 65% in diet groups 1, 2 and 3, respectively).

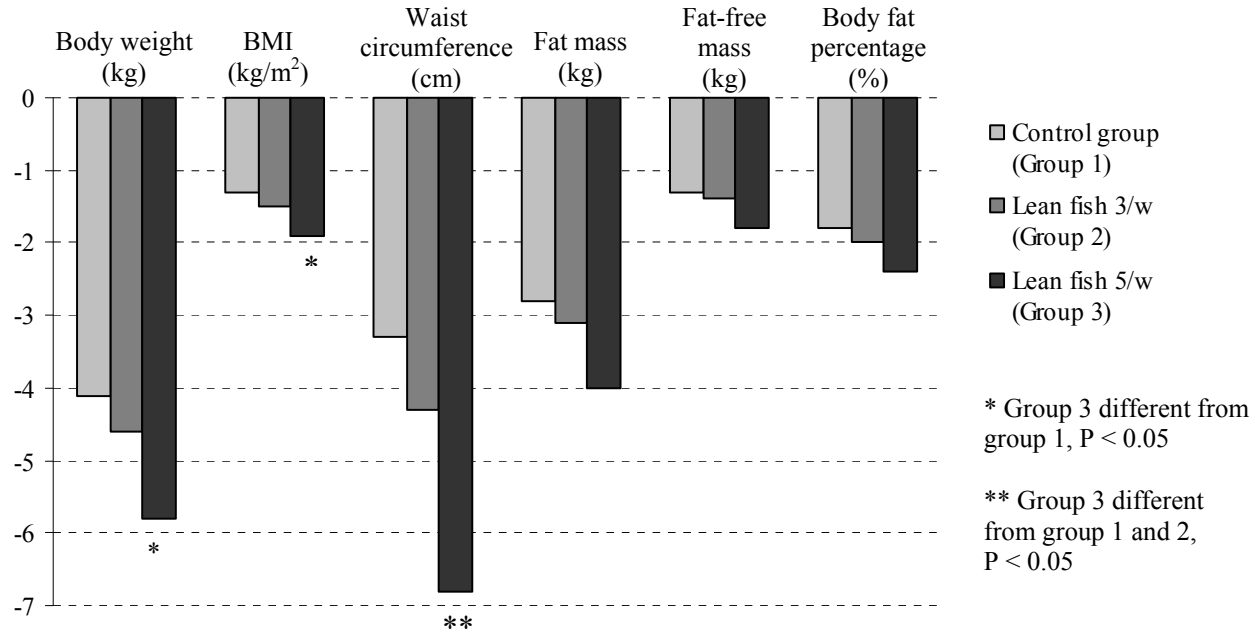

Figure 4.2: *Decreases in anthropometric measurements after the eight-week intervention (unadjusted data). One-way ANOVA / Kruskal-Wallis H-test*

Multivariate ANCOVA models for anthropometric measurements are shown in table 4.6 where adjustments are made for anthropometric measurements at baseline, gender and diet group. The ANCOVA model for weight loss showed 1.7 kg more weight loss (P = 0.015) after eight-week intervention in group 3 than in group 1. It also showed a 0.7 kg insignificantly greater weight loss in group 2 compared to group 1 (P = 0.396). Trend analysis showed a significant dose-response relationship (P = 0.011) for weight loss between diet groups. Addition of a gender*diet group interaction term to the multivariate analysis did not turn out significant (male*cod 3x /w: P = 0.839; male*cod 5x /w: P = 0.126).

Table 4.6: *Adjusted changes[1] in anthropometric measurements after eight-week intervention, compared with the control group receiving no seafood.*

	B-value Changes	**P- value**	**95% CI**	
			Lower	**Upper**
Body weight (kg)				
Lean fish 3/w	-0.666	*0.396*	-2.218	0.885
Lean fish 5/w	-1.729	*0.015*	-3.115	-0.344
BMI (kg/m2)				
Lean fish 3/w	-0.161	*0.527*	-0.663	0.341
Lean fish 5/w	-0.555	*0.015*	-1.001	-0.108
Waist circumference (cm)				
Lean fish 3/w	-0.994	*0.199*	-2.520	0.532
Lean fish 5/w	-3.419	*< 0.001*	-4.830	-2.007
Fat mass (kg)				
Lean fish 3/w	-0.191	*0.793*	-1.633	1.251
Lean fish 5/w	-0.873	*0.209*	-2.242	0.496
Fat-free mass (kg)				
Lean fish 3/w	-0.333	*0.533*	-1.388	0.722
Lean fish 5/w	-0.529	*0.254*	-1.444	0.387
Body fat percentage (%)				
Lean fish 3/w	-0.149	*0.815*	-1.409	1.111
Lean fish 5/w	-0.274	*0.664*	-1.522	0.974

[1] Variables included in the model: Anthropometric measurement at baseline, gender and diet group.

4.3.3 Reduction in BMI and waist circumference

BMI was on average 28.4 ± 1.6 kg/m^2 at the end of the eight-week trial and the average unadjusted reduction in BMI was 1.6 ± 1.0 kg/m^2 ($P < 0.001$). Therefore, the percentage of subjects classified as obese (BMI ≥ 30.0 kg/m^2) declined from 54% at baseline to 13% at endpoint, meaning a 76% decrease among subjects classified as obese at baseline. At baseline, 61% of men and 49% of women were classified as obese but at the end of the trial only 17% and 10% of men and women, respectively, had a BMI ≥ 30.0 kg/m^2. The average unadjusted reduction in waist circumference was 5.0 ± 3.2 cm, yielding an endpoint waist circumference of 96.7 ± 6.4 cm and 88.7 ± 5.9 cm for men and women, respectively, significantly lower than at baseline ($P < 0.001$).

Reduction in BMI was significantly greater in group 3 compared to the control group in the unadjusted bivariate analysis (1.9 ± 1.0 kg/m^2 vs. 1.3 ± 0.9 kg/m^2, $P = 0.008$ in LSD post hoc test; table 4.5), and remained significant in the multivariate model (0.6 kg/m^2 more loss, $P = 0.015$; table 4.6). The same was true for reduction in waist circumference (6.8 ± 3.1 cm vs. 3.3 ± 2.5 cm, $P < 0.001$; 3.4 cm more loss, $P < 0.001$). Also, women in diet group 3 lost significantly more waist circumference than women in diet group 2 (7.3 ± 2.2 cm vs. 4.1 ± 2.8 cm, $P < 0.001$) and their BMI also decreased significantly more (2.1 ± 0.9 kg/m^2 vs. 1.5 ± 0.9 kg/m^2, $P = 0.017$), although the same was not true for men when comparing groups 2 and 3. However, trend analysis showed a significant dose-response relationship between diet groups for BMI reduction and waist circumference reduction ($P = 0.007$ and $P < 0.001$, respectively).

4.3.4 Changes in body composition

On average, unadjusted fat mass decreased by 3.4 ± 2.7 kg ($P < 0.001$), fat-free mass by 1.6 ± 2.0 kg ($P < 0.001$) and body fat percentage decreased by 2.1 ± 2.3 % ($P < 0.001$) (table 4.5) during the eight-week diet intervention. The average body fat percentage at the end of the trial was 23.8 ± 3.7 % and 36.5 ± 3.5 % for men and women, respectively (appendix X, table A1). Body fat percentage at endpoint was significantly lower than at baseline, both in men and women and in all diet groups ($P < 0.001$). On average, 53% of weight loss was due to losses of fat mass while 47% occurred at the expense of fat-free mass. The composition of weight loss was more favourable in women where 67% of weight loss was due to losses of fat mass and 33% occurred at the expense of fat-free mass, compared to 35% fat mass loss and 65%

fat-free mass loss in men. Men lost significantly more fat-free mass than women (2.3 ± 1.9 kg vs. 1.0 ± 1.9 kg, P = 0.001) and women lost insignificantly more fat mass than men (3.6 ± 2.7 kg vs. 3.1 ± 2.7 kg, P = 0.418). There were no significant differences between diet groups in body composition although trend analysis resulted in borderline significance between cod consumption and reduction in fat mass (P = 0.057), indicating a possible dose-response relationship.

4.4 Blood parameters

4.4.1 Baseline blood parameters

Baseline values for blood parameters are shown in table 4.4. There were no significant differences in blood values between groups at baseline except for HDL cholesterol and insulin concentration. Men in group 1 had higher HDL cholesterol concentration than men in group 3 (P = 0.002) and women in group 3 had lower HDL cholesterol concentration than women in group 1 and 2 (P = 0.049). Women in group 3 also had lower insulin concentration than women in group 2 and 1 (P < 0.001). There were also a few gender differences in blood values at baseline. Women had higher HDL cholesterol concentration than men, as expected, lower glucose concentration, lower triglyceride concentration and lower systolic blood pressure.

4.4.2 Reduction in blood parameters

Endpoint values for blood parameters are shown in appendix X, table A1. Reductions in all blood parameters are shown in table 4.7 and figure 4.3. Comparison between baseline and endpoint blood values for all subjects and percentage of subjects within reference values are shown in table 4.8. Overall, the intervention resulted in significant reductions in systolic and diastolic blood pressure (P < 0.001 and P = 0.001, respectively), serum triglyceride (P = 0.012) and insulin concentrations (P = 0.022) (figure 4.3). Reduction in total cholesterol concentration was of borderline significance (P = 0.076). According to multivariate statistics, changes in blood lipids, insulin and glucose were not significantly affected by cod

consumption (table 4.9) and trend-analysis did not indicate a positive dose-response relationship for any of the blood parameters.

Table 4.7: *Reductions[1] in blood parameters at endpoint. Values are unadjusted, shown as means ± s.d.*

	Group 1 Control	Group 2 Cod 3x /w	Group 3 Cod 5x /w	All subjects	P-value [2]	post-hoc
Total cholesterol (mmol/L)						
Men	0.08 ± 0.84	0.40 ± 0.55	0.12 ± 0.68	0.16 ± 0.71	*0.564*	
Women	0.26 ± 0.54	0.02 ± 0.43	0.03 ± 0.77	0.09 ± 0.61	*0.436*	
All	0.18 ± 0.69	0.14 ± 0.49	0.07 ± 0.72	0.12 ± 0.65	*0.783*	
				p=0.600 [3]		
HDL cholesterol (mmol/L)						
Men	-0.05 ± 0.15	0.02 ± 0.17	-0.01 ± 0.13	-0.02 ± 0.14	*0.566*	
Women	0.10 ± 0.16	0.16 ± 0.21	-0.02 ± 0.27	0.07 ± 0.24	*0.042*	*2 > 3*
All	0.03 ± 0.17	0.12 ± 0.21	-0.01 ± 0.22	0.04 ± 0.21	*0.027*	*2 > 3*
				p= 0.022		
LDL cholesterol (mmol/L)						
Men	-0.01 ± 0.73	0.19 ± 0.34	0.15 ± 0.68	0.10 ± 0.64	*0.712*	
Women	0.20 ± 0.50	-0.12 ± 0.39	0.01 ± 0.51	0.02 ± 0.47	*0.161*	
All	0.10 ± 0.62	-0.03 ± 0.39	0.08 ± 0.59	0.05 ± 0.55	*0.673*	
				p= 0.453		
Triglyceride (mmol/L)						
Men	0.29 ± 0.70	0.41 ± 0.47	0.06 ± 0.91	0.20 ± 0.78	*0.496*	
Women	-0.04 ± 0.34	0.16 ± 0.28	0.07 ± 0.39	0.07 ± 0.34	*0.257*	
All	0.12 ± 0.56	0.23 ± 0.36	0.06 ± 0.68	0.13 ± 0.57	*0.477*	
				p= 0.295		
Glucose (mmol/L)						
Men	0.14 ± 0.41	0.30 ± 0.40	-0.03 ± 0.39	0.09 ± 0.41	*0.140*	
Women	-0.01 ± 0.30	0.01 ± 0.45	-0.08 ± 0.50	-0.03 ± 0.43	*0.780*	
All	0.06 ± 0.36	0.09 ± 0.45	-0.06 ± 0.45	0.02 ± 0.42	*0.298*	
				p= 0.142		
Insulin (mU/L)						
Men	4.8 ± 5.2	2.0 ± 4.0	0.1 ± 6.0	2.0 ± 5.7	*0.060*	
Women	0.4 ± 4.8	1.0 ± 4.9	0.5 ± 5.4	0.6 ± 5.0	*0.922*	
All	2.5 ± 5.4	1.3 ± 4.6	0.3 ± 5.6	1.2 ± 5.3	*0.230*	
				p= 0.211		
Systolic blood pressure (mmHg)						
Men	4.3 ± 6.1	4.9 ± 5.5	1.7 ± 9.9	3.1 ± 8.0	*0.523*	
Women	5.4 ± 5.5	3.3 ± 9.4	2.4 ± 11.9	3.5 ± 9.6	*0.636*	
All	4.9 ± 5.7	3.8 ± 8.3	2.0 ± 10.9	3.4 ± 8.9	*0.401*	
				p= 0.837		
Diastolic blood pressure (mmHg)						
Men	3.8 ± 7.5	2.6 ± 5.8	3.0 ± 9.3	3.2 ± 8.0	*0.937*	
Women	2.6 ± 6.4	-0.4 ± 5.2	3.3 ± 6.0	1.9 ± 6.0	*0.116*	
All	3.2 ± 6.8	0.5 ± 5.5	3.1 ± 7.6	2.4 ± 6.9	*0.232*	
				p= 0.367		

[1] Minus values indicate an increase from baseline to endpoint

[2] One-way ANOVA / Kruskal-Wallis H-test

[3] Independent samples T-test / Mann-Whitney U-test

At the end of the trial, the average diastolic blood pressure was 70.2 ± 6.0 mmHg and systolic pressure was 126.2 ± 10.4 mmHg, resulting in insignificant reduction from baseline values (72.8 ± 7.2 mmHg and 129.5 ± 10.9 mmHg). There was a significant negative effect of diet group 3 on systolic blood pressure reduction compared to group 1 (less reduction, B = 4.808, P = 0.009; table 4.9), and reductions in diastolic blood pressure were also significantly greater in the control group compared to group 2 (B = 2.960, P = 0.034).

The average total cholesterol concentration had declined insignificantly by 0.12 ± 0.65 mmol/L, resulting in a concentration of 4.7 ± 0.9 mmol/L at the end of the trial. HDL cholesterol concentration declined significantly more in group 2 compared to group 3 (P = 0.027; table 4.7). At the end of the trial, average HDL values were insignificantly higher in men, compared to baseline values, but significantly lower in women (P = 0.025; table 4.8). The intervention had little and insignificant effects on LDL cholesterol concentrations. The average concentration of LDL cholesterol was 3.0 ± 0.8 mmol/L, both at baseline and endpoint.

Triglyceride concentration declined insignificantly during the trial, from 1.5 ± 0.8 mmol/L to 1.3 ± 0.9 mmol/L in men (P = 0.096; table 4.8) and from 1.1 ± 0.5 mmol/L to 1.0 ± 0.5 mmol/L in women (P = 0.138). The same is true for glucose concentration whereas insulin concentration declined significantly in men (P = 0.024). Trend analysis indicated a negative dose-response relationship on insulin levels (P = 0.024), i.e. insulin concentration seemed to lower more in the control group than in the cod-diet groups.

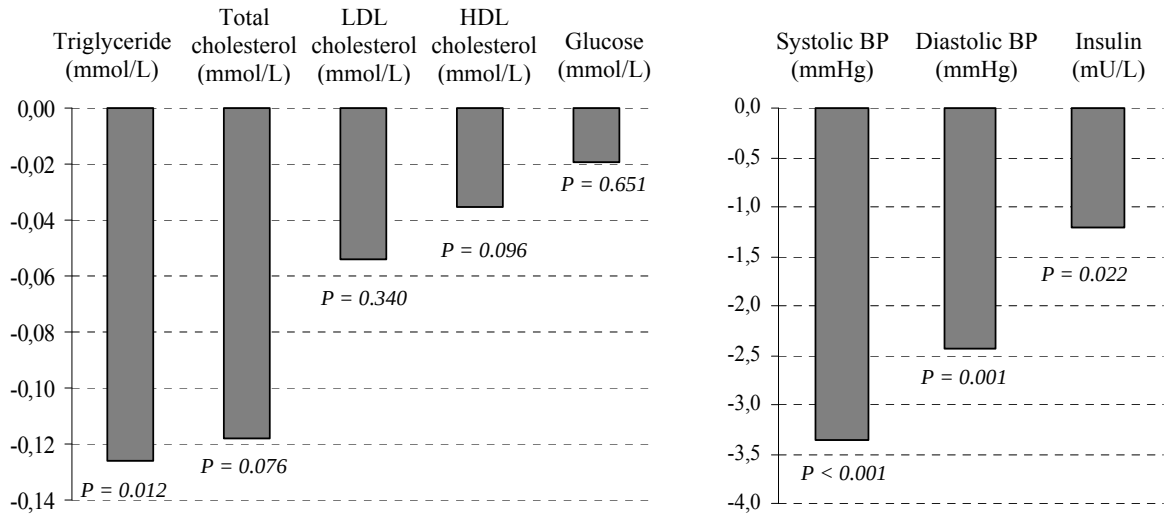

Figure 4.3: *Reductions in blood parameters after the eight-week intervention (unadjusted data). Paired samples t-test / Wilcoxon signed ranks test*

Table 4.8: *Blood parameters; comparison between baseline and endpoint values for all subjects and percentage of subjects within reference values. Values are unadjusted, shown as means ± s.d.*

	Reference values	MEN Baseline	MEN Endpoint	P-value[4]	WOMEN Baseline	WOMEN Endpoint	P-value
Total cholesterol (mmol/L)	< 5.2 [1]	4.9 ± 0.9	4.7 ± 0.9	*0.157*	4.8 ± 0.9	4.7 ± 0.8	*0.283*
% of subjects		61 %	79 %		68 %	79 %	
HDL cholesterol (mmol/L)	≥ 1.29 ♀ [2] ≥ 1.03 ♂ [2]	1.0 ± 0.2	1.1 ± 0.3	*0.454*	1.4 ± 0.3	1.3 ± 0.3	*0.025*
% of subjects		49 %	52 %		64 %	48 %	
LDL cholesterol (mmol/L)	< 2.6 [1]	3.2 ± 0.9	3.1 ± 0.8	*0.311*	3.0 ± 0.8	3.0 ± 0.8	*0.789*
% of subjects		31 %	29 %		29 %	30 %	
Triglyceride (mmol/L)	< 1.7 [2]	1.5 ± 0.8	1.3 ± 0.9	*0.096*	1.1 ± 0.5	1.0 ± 0.5	*0.138*
% of subjects		71 %	79 %		87 %	91 %	
Glucose (mmol/L)	≤ 5.6 [2]	4.9 ± 0.4	4.7 ± 0.5	*0.148*	4.6 ± 0.5	4.6 ± 0.4	*0.556*
% of subjects		96 %	93 %		100 %	98 %	
Insulin (mU/L)	≤ 24.9 [3]	10.1 ± 6.6	8.5 ± 5.3	*0.024*	8.4 ± 5.2	7.9 ± 4.4	*0.389*
% of subjects		98 %	100 %		97 %	100 %	
Systolic BP (mmHg)	< 130 [2]	136.2 ± 9.6	132.6 ± 9.4	*0.015*	125.3 ± 9.5	121.6 ± 8.5	*0.007*
% of subjects		31 %	36 %		73	81 %	
Diastolic BP (mmHg)	< 85 [2]	73.2 ± 7.7	70.0 ± 5.7	*0.014*	72.6 ± 6.9	70.3 ± 6.2	*0.020*
% of subjects		92 %	100 %		98 %	100 %	

[1] American Heart Association

[2] International Diabetes Federation

[3] American Diabetes Association

[4] Paired samples T-test / Wilcoxon signed ranks test

Table 4.9: *Adjusted changes[1] in blood parameters after eight-week intervention, compared with the control group receiving no seafood.*

Not adj. for weight loss	B-value Changes	P- value	95% CI Lower	95% CI Upper	Adj. for weight loss	B-value Changes	P- value	95% CI Lower	95% CI Upper
Total cholesterol (mmol/L)					***Total cholesterol (mmol/L)***				
Lean fish 3/w	-0.097	*0.553*	-0.422	0.228	*Lean fish 3/w*	-0.061	*0.699*	-0.374	0.252
Lean fish 5/w	-0.048	*0.747*	-0.340	0.245	*Lean fish 5/w*	0.053	*0.718*	-0.236	0.342
HDL cholesterol (mmol/L)					***HDL cholesterol (mmol/L)***				
Lean fish 3/w	-0.094	*0.057*	-0.191	0.003	*Lean fish 3/w*	-0.088	*0.075*	-0.184	0.009
Lean fish 5/w	-0.039	*0.407*	-0.131	0.054	*Lean fish 5/w*	-0.023	*0.630*	-0.117	0.071
LDL cholesterol (mmol/L)					***LDL cholesterol (mmol/L)***				
Lean fish 3/w	0.046	*0.746*	-0.238	0.331	*Lean fish 3/w*	0.054	*0.707*	-0.232	0.340
Lean fish 5/w	-0.025	*0.846*	-0.279	0.230	*Lean fish 5/w*	-0.004	*0.973*	-0.267	0.258
Triglyceride (mmol/L) - Male					***Triglyceride (mmol/L) - Male***				
Lean fish 3/w	-0.178	*0.588*	-0.839	0.483	*Lean fish 3/w*	-0.126	*0.670*	-0.722	0.469
Lean fish 5/w	0.203	*0.434*	-0.317	0.722	*Lean fish 5/w*	0.256	*0.274*	-0.212	0.725
Triglyceride (mmol/L) - Female					***Triglyceride (mmol/L) - Female***				
Lean fish 3/w	-0.174	*0.111*	-0.390	0.042	*Lean fish 3/w*	-0.142	*0.186*	-0.354	0.070
Lean fish 5/w	0.070	*0.503*	-0.278	0.138	*Lean fish 5/w*	0.024	*0.829*	-0.199	0.247
Glucose (mmol/L)					***Glucose (mmol/L)***				
Lean fish 3/w	-0.108	*0.259*	-0.297	0.081	*Lean fish 3/w*	0.080	*0.397*	-0.267	0.107
Lean fish 5/w	0.0003	*0.997*	-0.171	0.172	*Lean fish 5/w*	0.058	*0.510*	-0.116	0.232
Insulin (mU/L)					***Insulin (mU/L)***				
Lean fish 3/w	0.433	*0.689*	-1.710	2.575	*Lean fish 3/w*	0.637	*0.546*	-1.448	2.723
Lean fish 5/w	-0.142	*0.887*	-2.122	1.838	*Lean fish 5/w*	0.319	*0.746*	-1.635	2.273
Systolic BP (mmHg)					***Systolic BP (mmHg)***				
Lean fish 3/w	0.895	*0.656*	-3.083	4.874	*Lean fish 3/w*	1.091	*0.592*	-2.910	5.071
Lean fish 5/w	4.808	*0.009*	1.221	8.396	*Lean fish 5/w*	5.289	*0.005*	1.594	8.984
Diastolic BP (mmHg)					***Diastolic BP (mmHg)***				
Lean fish 3/w	2.960	*0.034*	0.233	5.688	*Lean fish 3/w*	3.177	*0.022*	0.472	5.882
Lean fish 5/w	-1.148	*0.352*	-3.586	1.290	*Lean fish 5/w*	-0.586	*0.641*	-3.070	1.898

[1] Variables included in the model: Blood parameter's concentration at baseline, gender, diet group. Weight loss is included in the model on the right side.

4.5 Other endpoint analysis

4.5.1 Prevalence of the metabolic syndrome

The prevalence of the metabolic syndrome declined during the diet intervention from 29% at baseline to 21% at endpoint, meaning a 27% decrease among participants diagnosed with the metabolic syndrome at baseline. The syndrome was more common among men; at baseline 49% of men fulfilled the definition of the metabolic syndrome and 16% of women, while at endpoint 36% of men had the syndrome (27% decrease) and 10% of women (34% decrease). At baseline, the metabolic syndrome happened to be most common in diet group 3 at baseline, where 43% of subjects fulfilled the definition, declining down to 33% at endpoint.

4.5.2 Correlations

Weight loss was positively correlated to reductions in all other anthropometric measurements ($r = 0.485$-0.978, $P < 0.001$), serum triglyceride reduction ($r = 0.344$, $P < 0.001$) and reduction in total cholesterol ($r = 0.224$, $P = 0.026$) (table 4.10). There were no significant correlations between weight loss and reduction in other blood parameters. Interestingly, there were no correlations between weight loss at the end of the trial and caloric intake in menus during the trial, energy intake at baseline or endpoint, energy reduction during the trial, or body weight at baseline. When looking at each gender, weight loss was positively correlated with total cholesterol reduction ($r = 0.324$, $P = 0.036$), HDL cholesterol reduction ($r = 0.354$, $P = 0.022$) and triglyceride reduction ($r = 0.374$, $P = 0.015$) in men, but only to triglyceride reduction in women ($r = 0.283$, $P = 0.033$).

As can be seen in appendix X, table A2, BMI at baseline correlated with other anthropometric measurements at baseline except body fat percentage, and also with HDL cholesterol concentration at baseline ($r = -0.186$, $P = 0.037$) and serum triglyceride at baseline ($r = 0.183$, $P = 0.040$). BMI correlations with fat intake and systolic blood pressure at baseline were of borderline significance ($r = 0.163$, $P = 0.069$ and $r = 0.165$, $P = 0.065$, respectively). BMI at baseline did not correlate with other blood parameters at baseline and neither with energy intake nor added sugar consumption at baseline. However, energy intake at baseline correlated with body weight at baseline ($r = 0.427$, $P < 0.001$).

Table 4.10: *Correlations[1] with weight loss at the end of the eight-week trial*

WEIGHT LOSS correlations:	**All subjects**		**Men**		**Women**		**Group 1**		**Group 2**		**Group 3**	
	r-value[1]	**P-value**	**r-value**	**P-value**	**r-value**	**P-value**	**r-value**	**P-value**	**r-value**	**P-value**	**r-value**	**P-value**
Body weight baseline	0.104	*0.303*	0.072	*0.652*	-0.008	*0.955*	0.489*	*0.006*	0.053	*0.795*	-0.173	*0.267*
Δ BMI	0.978*	*< 0.001*	0.992*	*< 0.001*	0.987*	*< 0.001*	0.985*	*< 0.001*	0.981*	*< 0.001*	0.971*	*< 0.001*
Δ Waist circumference	0.824*	*< 0.001*	0.880*	*< 0.001*	0.783*	*< 0.001*	0.854*	*< 0.001*	0.774*	*< 0.001*	0.849*	*< 0.001*
Δ Fat mass	0.756*	*< 0.001*	0.823*	*< 0.001*	0.756*	*< 0.001*	0.776*	*< 0.001*	0.788*	*< 0.001*	0.702*	*< 0.001*
Δ Fat-free mass	0.485*	*< 0.001*	0.629*	*< 0.001*	0.302*	*0.026*	0.424*	*0.020*	0.598*	*0.002*	0.451*	*0.004*
Δ Body fat percentage	0.548*	*< 0.001*	0.657*	*< 0.001*	0.517*	*< 0.001*	0.623*	*< 0.001*	0.643*	*0.001*	0.450*	*0.004*
Δ Total cholesterol	0.224*	*0.026*	0.324*	*0.036*	0.104	*0.440*	0.356	*0.058*	0.781*	*< 0.001*	-0.029	*0.852*
Δ HDL cholesterol	0.056	*0.581*	0.354*	*0.022*	-0.058	*0.674*	0.344	*0.073*	-0.002	*0.993*	0.034	*0.826*
Δ LDL cholesterol	0.057	*0.581*	0.127	*0.429*	-0.044	*0.750*	0.238	*0.224*	0.529*	*0.005*	-0.290	*0.066*
Δ Triglyceride	0.344*	*< 0.001*	0.374*	*0.015*	0.283*	*0.033*	0.179	*0.352*	0.329	*0.093*	0.525*	*< 0.001*
Δ Glucose	0.120	*0.236*	-0.012	*0.940*	0.210	*0.114*	-0.036	*0.852*	0.472*	*0.013*	0.104	*0.507*
Δ Insulin	0.000	*1.000*	-0.208	*0.186*	0.191	*0.150*	0.104	*0.584*	0.394*	*0.042*	-0.161	*0.301*
Δ Systolic BP	0.063	*0.536*	-0.048	*0.763*	0.159	*0.234*	0.028	*0.882*	0.343	*0.080*	0.027	*0.862*
Δ Diastolic BP	0.134	*0.183*	0.026	*0.870*	0.247	*0.062*	0.453*	*0.012*	0.303	*0.125*	-0.151	*0.335*
Calories in menus	0.127	*0.207*	0.070	*0.657*	-0.041	*0.758*	0.413*	*0.023*	0.162	*0.420*	-0.138	*0.378*
Energy restriction	0.129	*0.202*	0.032	*0.843*	-0.067	*0.616*	0.427*	*0.019*	0.167	*0.405*	-0.155	*0.321*
Calorie reduction from baseline to endpoint	0.030	*0.773*	0.198	*0.234*	-0.029	*0.832*	0.012	*0.952*	0.426*	*0.027*	-0.119	*0.466*

[1] Pearson correlation / Spearman correlation coefficient

* Significant r-value (P > 0.05)

5. DISCUSSION

5.1 Weight loss and other anthropometric changes

The present study is one of the first interventions in humans to test the effects of different amounts of lean fish consumption, in combination with energy restriction, on weight loss and risk factors for cardiovascular diseases. The most important finding of the study is that inclusion of lean fish to an energy-restricted diet for eight weeks resulted in significantly more weight loss than an isocaloric diet without seafood in young overweight or obese individuals. The average unadjusted weight loss was 5.8 kg in diet group 3, being significantly higher than the 4.1 kg weight loss in the control group. This finding was confirmed in the multivariate analysis, where there was on average 1.7 kg significantly more weight loss among subjects consuming 150 g cod five times a week compared to the control group receiving no seafood. The multivariate analysis also resulted in 0.7 kg insignificantly greater weight loss in diet group 2 compared to the control group, where subjects in diet group 2 lost on average 4.6 kg during the eight-week trial. The trend analysis clearly supports a dose-response relationship between cod consumption and weight loss.

The average weight loss was 5.0 kg during the eight-week intervention, which was in line with expected weight loss (NHLBI, 1998), indicating good compliance to energy restriction. The majority of participants (59%) in the present study lost ≥ 5% of their baseline body weight. Intentional weight loss of that modest degree is thought to reduce many of the health risk factors associated with obesity (Blackburn, 1995; Klein et al., 2004) since the distribution of fat loss is uneven and starts with greater relative reduction in visceral fat than subcutaneous fat (Ross et al., 2000). About one-third to a half more of body fat is lost from visceral adipose tissue (VAT) than from the abdominal subcutaneous adipose tissue compartment in response to weight loss interventions (Doucet et al., 2002). Results have demonstrated that for every kilogram of diet-induced weight loss, the corresponding reduction in VAT is 2-3 % (Ross, 1997). Thus a diet-induced weight loss of 5 kg, as the average weight loss in the present study, corresponds to a 10-15 % reduction in VAT.

Since weight loss maintenance is difficult to achieve, it is an important question whether an eight-week intervention is an appropriate tool to improve health or risk factors over a longer period. In a recent study by Magnusdottir (2008), subjects that were on an eight-

week weight loss diet were followed up for one year. After the eight-week trial those subjects had lost 5.7 kg on average and regained 3.0 kg 12 months later so the total weight loss was 2.7 kg in the long run. At the end of the eight-week trial, 68% of the subjects had lost ≥ 5% of their baseline body weight and 12 months later the proportion was 35%. Her study also indicated that the eight-week weight loss program is more effective to lose weight than nutritional education and monthly guidance during one year, as in the control group that had no prior weight loss program, which is in line with prior studies (Astrup and Rossner, 2000).

Dose-response relations were also observed between cod intake and reductions in waist circumference and body mass index. Lean fish consumption did not affect changes in body composition although trend analysis resulted in borderline significance between cod consumption and reduction in fat mass. On average, 53% of weight loss was due to losses of fat mass while 47% occurred at the expense of fat-free mass, where more favourable effects on body composition were seen in women than in men during the trial, which is in opposition to the fact that women generally lose less of their initial body fat than men even after a comparable weight loss (Doucet et al., 2002). It is not uncommon that as much as 50% or more of the weight loss achieved through dieting occurs at the expense of lean body mass if physical activity is not increased (McInnis et al., 2003). However, body fat percentage in the current study decreased significantly by 2.1% during the trial despite no changes in physical activity.

Currently it is not clear whether lean fish as a whole or particular proteins or amino acids are responsible for increased weight loss observed in our study, although taurine is a likely candidate (Fujihira et al., 1970; Zhang et al., 2004; Tsuboyama-Kasaoka et al., 2006), as discussed in the *Literature review* of this thesis. The weight loss effects of cod protein have only been studied to a limited degree, even in animal models (Yahia et al., 2005). It is not unlikely that fish protein, LC n-3 PUFAs, which are also available in small amounts from cod, and other nutrients in fish have additive beneficial effects on body weight control and other health variables.

Furthermore, frequent fish consumption can also help people to decrease meat consumption with a simultaneous reduction in saturated and total fat intake while maintaining an adequate intake of high quality protein and other nutrients (Bao et al., 1998). It is believed that a fish-based diet rather than meat-based diet reduces the risk of obesity and diabetes (Nkondjock and Receveur, 2003) and epidemiological studies indicate that ample fish

consumption within a nutritionally balanced eating pattern is associated with lower body weight (Shubair et al., 2005; Schulze et al., 2006).

5.1.1 Weight loss and gender

According to the multivariate analysis, cod consumption accounts for more weight loss in both genders than the diet without seafood, although the unadjusted data indicated that it had a more dose-dependent effect in women. On the other hand, the SEAFOODplus YOUNG study (Thorsdottir et al., 2007) resulted in significantly more weight loss only in male subjects receiving lean fish (150 g cod 3x/week) compared to the control group, but not in female subjects. The mechanism of increased weight loss due to cod consumption is not known, thus, this matter remains speculative. However, gender differences in the concentration of the obesity-related hormone leptin (Niskanen et al., 1997; Doucet et al., 2000) and metabolic pathways of alpha-linolenic acid (Burdge and Wootton, 2002; Burdge et al., 2002), with relevance for dietary interventions and body weight control (Thorsdottir et al., 2007) have been reported. In addition, the possible dose-dependent effect of LC n-3 PUFAs on weight loss in women cannot be ruled out.

Another interesting aspect of gender differences in the present study is how difficult it was to get men to participate in this weight loss study, whereas women were very interested and willing to participate. The advertisement, with the heading "Do you want to lose weight?" (see appendix II), seemed to be more appealing to women than men, which is not a surprise nowadays. The majority of women want to lose weight and many are dieting, even though they are within the medically defined healthy weight range, mainly due to psychological and socio-cultural factors (Germov and Williams, 2004). However, the prevalence of obesity is similar between the genders and men are even more frequently overweight than women (Kiefer et al., 2005).

5.2 Effects on blood parameters

During the intervention, systolic and diastolic blood pressure, serum triglycerides and insulin concentration decreased significantly. The prevalence of subjects diagnosed with the metabolic syndrome decreased by 27%, dropping from 29% to 21% during the intervention.

The overall decline in the prevalence of the metabolic syndrome was mainly due to fewer classifications of central obesity among subjects at endpoint compared to baseline. As a result of weight loss, the prevalence of obesity (BMI ≥ 30.0 kg/m^2) declined from 54% at baseline to 13% at endpoint. There was also a considerable reduction in waist circumference. In addition, blood pressure (both systolic and diastolic pressure) declined during the eight-week intervention and more subjects were within the reference value for serum triglyceride concentration at endpoint compared to baseline.

In the present study cod consumption did not affect the improvements of blood parameters during the intervention. However, in the SEAFOODplus YOUNG study significant effects of cod consumption (3 x 150 g/week) on total cholesterol and triglycerides were observed (Gunnarsdottir et al., 2008). Baseline values of total cholesterol and triglycerides were similar in the present study and the SEAFOODplus YOUNG study, thus, these findings might be explained by the larger sample size in the SEAFOODplus YOUNG study and higher statistical power. Several other animal and human studies have shown positive effects of fish protein or taurine supplementation on total cholesterol (Iritani et al., 1985; Zhang and Beynen, 1993; Hurley et al., 1995; Mizushima et al., 1996; Murakami et al., 1996a; Murakami et al., 1996b; Demonty et al., 2003; Yahia et al., 2003b; Wergedahl et al., 2004), HDL cholesterol (Bergeron et al., 1992; Matsushima et al., 2003; Wergedahl et al., 2004), or plasma triglycerides (Bergeron and Jacques, 1989; Hurley et al., 1995; Demonty et al., 2003; Zhang et al., 2004; Shukla et al., 2006).

In the present study the amount of cod consumed seemed to negatively affect the blood pressure reduction during the intervention, which is in contrast to previous animal researches (Gutierrez et al., 1994; Murakami et al., 1994; Yahia et al., 2003a; Boukortt et al., 2004;). However, these effects were not consistent, since, e.g., the negative effect on diastolic blood pressure seen in the diet group receiving cod three times a week was not observed in the diet group receiving cod five times a week. The effect might be explained by unequal distribution of relevant factors or covariates between the diet groups. Nevertheless, these findings deserve further investigation.

BMI at baseline was positively associated with triglyceride concentration and negatively associated with HDL cholesterol concentration. However, it did not correlate with other blood parameters, indicating that a BMI of 27.5-32.5 kg/m^2 does not necessarily result in higher blood pressure, higher total cholesterol or LDL cholesterol, or higher insulin or fasting glucose concentrations. The narrow BMI range in the present study might explain these results. Also, the limitations of BMI in reflecting body composition and medical

condition play a great role since its reflection of body conposition can be distorted by many other factors than body weight and height, such as muscle mass, fitness level and bone structure.

5.3 Dietary changes

Energy intake from baseline to endpoint decreased similarly in all three intervention groups, which is the main reason for weight loss in subjects. However, it does not explain the additional weight loss experienced in the cod-diet groups. The average macronutrient distribution at baseline was mainly consistent with the results from the Icelandic Dietary Survey conducted in 2002 (Steingrimsdottir et al., 2003). Saturated fatty acid intake and added sugar intake was too high (14 %E and 12 %E, respectively), carbohydrate intake too low (46 %E) and fat intake were in the upper limit of recommendations (35 %E). However, at the end of the trial the average macronutrient distribution was much closer to the Icelandic recommendations (Public Health Institute of Iceland, 2006) with 11 %E from saturated fatty acids, 4.8 %E from added sugar, 48 %E from carbohydrates and 28 %E from fat. In addition, fiber consumption at endpoint was closer to the recommendations, increasing from 18 g/day to 21 g/day.

Intake of most vitamins and minerals reached the recommendations (NNR, 2004) at baseline and endpoint although vitamin D intake was far below recommendations (2.7 and 2.6 µg/day, respectively) and also much lower than in the Icelandic Dietary Survey in 2002 (6.0 µg/day) (Steingrimsdottir et al., 2003). Steingrimsdottir et al. (2003) concluded that only those Icelanders who take fish oil supplements reach the RI of vitamin D so the main reason for this low vitamin D intake is the exclusion criteria of fish oil use three months before the trial. Vitamin D intake did not increase in the cod-diet groups since cod contains only 0.5 g fat /100 g and therefore almost no fat-soluble vitamins. Intake of iodine and selenium at baseline was mainly consistent with the results from the Icelandic Dietary Survey in 2002 (Steingrimsdottir et al., 2003) although daily iodine intake in men was considerably lower in the current study (168 µg vs. 205 µg). During the intervention, intake of iodine and selenium increased in subjects receiving lean fish five times a week, while it decreased in the control group.

Nutrient density is very important when consuming an energy-restricted diet where fish consumption is a critical source of various nutrients. Even though cod is an excellent source of high quality protein, iodine and selenium, varieties of lean and fatty fish should be recommended since fatty fish is a much better source of vitamin D and LC n-3 PUFAs and by far the best dietary source of these two nutrients. As indicated by the present study, lean fish consumption may promote body weight reduction in humans, which is an important public health message since the consumption of lean fish has been reported to be more common than consumption of fatty fish (Amiano et al., 2001; Philibert et al., 2006; Welch et al., 2006; Icelandic Ministry of Fisheries, 2007; van Gelder et al., 2007). Further studies are needed to verify the weight loss effects of lean fish consumption in general, as well as mechanisms behind that process.

An interesting future project would be to study the effects of similar research diets, including meal plans as in the present study, but without energy restriction. The aim of such study would be to investigate whether lean fish consumption causes weight loss in a dose-dependent manner as a part of a healthy diet based on estimated total energy expenditure without energy restriction. If lean fish consumption proves to have weight loss effects in such a study that would be in important message for the public who could use that knowledge permanently in daily life without transforming their life style.

5.4 Strength and limitation of the study

A limitation of the current study is that it was not fully randomized, because diet group 3, receiving cod five times a week, was investigated two years later than the two other diet groups. The same inclusion and exclusion criteria, recruitment area, research facilities, meal plans, procedures and laboratories were used for group 3. Additionally, there were no significant differences in body weight, BMI and waist circumference between the groups at baseline, and all subjects were of similar age although men in the control group were on average slightly older than men in both cod groups, which should not be of importance here since completers have been shown to be even older than dropouts in this age group (Sigurgeirsdottir, 2006). Habitual dietary intake of the groups was not significantly different, which altogether makes the three groups fully comparable.

A limitation of each dietary intervention trial is the uncertainty whether dietary intakes of subjects during the study period were as reported or prescribed. As there was an intense support of the study participants by the staff, frequent contact via phone and in person visits, and since compliance was tested during the intervention trial using a FFQ, validated for assessing frequency of seafood consumption (Birgisdottir et al., 2008), this risk was minimized. In addition, average weight loss during the trial was as expected, indicating good compliance to energy restriction.

The strength of the study is its design where participants were carefully supervised during the eight-week intervention and uniformity of baseline characteristics was monitored in the screening process. The fact that it is one of the first interventions in humans to test the effects of different amounts of lean fish consumption in combination with energy restriction on weight loss and risk factors for cardiovascular diseases, gives the study a meaningful value and novel results. The health effects of LC n-3 PUFAs have been well documented (Mori et al., 1999; Bucher et al., 2002; Calder, 2004; Daniels et al., 2004; Thorsdottir et al., 2004; Madsen et al., 2005; Nettleton and Katz, 2005; Balk et al., 2006; Calder, 2006) whereas a lack of knowledge on fish protein is prominent in the literature. The present study contributes to an increasing knowledge on the effects of fish protein consumption on weight loss and cardiovascular health.

6. CONCLUSION

Participants lost on average 5.0 kg during the eight-week trial that investigated the effects of different amounts of lean fish consumption combined with an energy-restricted diet. The present study indicates a dose-response relationship between cod consumption and weight loss during an eight-week period of energy restriction. Inclusion of 150 g lean fish five times per week resulted in 1.7 kg significantly greater weight loss in young overweight or obese individuals than an isocaloric diet without seafood. According to the results, the dose-dependent effects of cod consumption on weight loss were greater in women than in men.

Systolic and diastolic blood pressure, serum triglycerides and insulin concentration decreased during the eight-week intervention and the prevalence of the metabolic syndrome decreased by 27%. These changes are most likely attributed to weight loss and not cod consumption since no differences in blood parameters improvements were seen between diet groups.

From public health perspective, the weight loss effect of lean fish consumption is an important message since the consumption of lean fish has been reported to be more common than consumption of fatty fish. However, consumption of varieties of lean and fatty fish should be recommended, as part of a healthy eating pattern, due to different nutrient composition. Further studies are needed to verify the weight loss effects of lean fish consumption in general, as well as mechanisms behind that process. An interesting future project would be to study the effects of similar research diets, including meal plans as in the present study, but without energy restriction.

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APPENDIX

Overview of appendixes:

Appendix I: Article in review in an international scientific journal

Appendix II: Advertisement for recruitment

Appendix III: Introduction letter for participants

Appendix IV: Informed consent

Appendix V: Instructions for participants

Appendix VI: Food frequency questionnaire (FFQ)

Appendix VII: Life style questionnaire (LSQ)

Appendix VIII: Menu – an example of 1500 kcal in diet group 3

Appendix IX: Exchange list – an example for 1400-1600 kcal menu

Appendix X: Result tables – Endpoint characteristics and BMI correlations

Appendix I – Article in review

Title: Consumption of lean fish and weight loss in young overweight and obese adults on an energy reduced diet for eight-weeks

Running head: Lean fish consumption and weight loss

Keywords Weight loss diet, lean fish, fish protein, overweight, obesity

Wordcount abstract: 248

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Abstract

Background and Aims: In a cross-European study it was recently shown that consumption of lean fish increases weight loss in men and has other positive health effects. The aim was to investigate whether lean fish (cod) consumption increases weight loss and improves cardiovascular risk factors in a dose dependent manner during an eight-week energy restriction in young overweight and obese healthy adults.

Methods and Results: In this dietary intervention 126 subjects (20-40 years, BMI 27.5-32.5 kg/m^2) were grouped to energy-restricted diets (-30%) prescribing identical macronutrient composition but different amounts of cod: group 1 = control group, no seafood; group 2 = 150g cod 3x/week; and group 3 = 150g cod 5x/week. Anthropometric measurements and cardiovascular risk factors were assessed at baseline and endpoint. Body weight decreased after eight weeks (5.0±2.9kg, P<0.001), also waist circumference (5.0±3.2cm, P<0.001), BMI (1.65±0.95kg, P<0.001), systolic (3.4±8.9mmHg, P=0.001) and diastolic blood pressure (2.4±6.9mmHg, P<0.001), triglycerides (1.26±0.567mmol/L, P=0.030) and insulin (1.21±5.31mU/L, P=0.025). The prevalence of metabolic syndrome dropped from 29 to 21%. According to linear models weight loss was 1.7 kg greater among subjects consuming 150g 5x/week compared to the control group (P<0.015). The trend analysis supported a dose-response relationship between cod consumption and weight loss (P<0.001), but changes of other measured cardiovascular risk factors were similar between the groups.

Conclusion: A dose-response relationship between cod consumption and weight loss during an eight-week energy restriction is found and 5x150 g cod/week results in 1.7 kg greater weight loss in young overweight or obese adults than an isocaloric diet without seafood.

Introduction

Obesity is a cardiovascular risk factor [1] and has become increasingly prevalent among young adults and children [2]. Epidemiological studies have shown that fish consumption within a healthy eating pattern is associated with lower body weight [3,4], and there are also dietary intervention studies, which support the idea that fish consumption helps in body weight control [5,6]. Beneficial effects of fish consumption have often been attributed to long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) [7]. Little is known on the health effects of other constituents in fish, such as fish protein, which is quantitatively the most important fish component.

The recently conducted randomized dietary intervention study SEAFOODplus YOUNG [6] found that regular consumption of lean fish (3 x 150 g cod per week), which contains only small amounts of LC n-3 PUFA, increases weight loss in overweight and obese men during an eight-week energy restriction in comparison to an isocaloric control diet without seafood. Testing the weight loss effects of lean fish eaten more frequently or in higher amounts than in the SEAFOODplus YOUNG study is important as lean fish might be an interesting possibility to increase weight loss and therefore to decrease cardiovascular risk for overweight or obese individuals. The SEAFOODplus YOUNG study also showed positive health effects of cod consumption on blood lipids [8] and oxidative stress [9]. Although cod contains LC n-3 PUFA (~ 180 mg EPA and DHA/100 g) [10], the amount is insufficient to obtain doses as recommended by, e.g., the American Heart Association for patients with heart disease (1 g) [11] or doses used in studies to improve various clinical outcomes (2-4 g/d) [12,13]. Therefore, it is likely that other fish constituents than LC n-3 PUFA are responsible for the positive health effects of lean fish consumption.

Several animal studies have suggested positive effects of fish protein on weight loss and health: It has been shown that rats fed fish protein do not gain as much weight as rats fed casein protein diet despite similar food and energy intake [14]. Fish protein have also been reported to have beneficial effects on cardiovascular risk factors, independent of the intake of LC n-3 PUFA, e.g., blood pressure lowering effects [14] and beneficial changes in lipid metabolism by reducing cholesterol concentrations [15] compared to other animal protein. Fish protein also prevents the development of insulin resistance in high fat-fed rats [16].

The present study continues the investigation started by the SEAFOODplus YOUNG study on the effects of lean fish consumption on weight loss and cardiovascular risk factors in humans.

We conducted a controlled dietary intervention trial in young overweight and obese adults. The main goal of the present study was to investigate whether lean fish (cod) consumption at various doses improves weight loss and cardiovascular risk factors in a dose dependent manner during an eight-week period of energy restriction.

Methods

Study population

Participants were recruited through advertisements in Reykjavik, Iceland. All potential subjects were screened for inclusion and exclusion criteria. The inclusion criteria were body mass index (BMI) 27.5 to 32.5 kg/m^2, age 20 - 40 years, and waist circumference of ≥ 94 cm and ≥ 80 cm for men and women, respectively. Exclusion criteria were weight change due to a weight loss diet within three months before the start of the study, use of supplements containing n-3 fatty acids, calcium or vitamin D during the last three months, allergy for fish, drug treatment of diabetes mellitus, hypertension or hyperlipidemia and women's pregnancy or lactation. The experimental protocol and the process for obtaining informed consent were approved by the National Bioethical Committee in Iceland. The study followed the Helsinki guidelines (as revised in 2000) and all subjects participating gave their written consent. The study was performed during wintertime 2004-2005 for group 1 (n = 35) and 2 (n = 35) in a random design (as part of the Icelandic arm of the SEAFOODplus YOUNG study), and in 2006-2007 group 3 (n = 56) was investigated. Altogether, 126 subjects started in one of the three groups, and one hundred (79 %) subjects completed the intervention (Figure 1). Dropouts were not significantly different between women and men or between diet groups.

Protocol

The subjects had three visits to the clinic during the eight-week trial; at baseline, midpoint and endpoint, where they met a dietician, anthropometrical measurements were performed, and compliance to seafood intake was assessed by a validated food frequency questionnaire (FFQ) [17,18]. Dietary intake was assessed by two-day weighed food records before baseline (habitual diet) and during the two last weeks of the intervention trial (compliance to meal plans). At baseline, information on physical activity pattern during the last year, smoking habits and alcohol consumption were collected using a questionnaire [19]. Subjects were instructed not to change their physical activity level during the eight-week intervention period.

Dietary intervention

Basal metabolic rate (BMR) was calculated using Harris-Benedict equations [20] and a correction factor due to the overweight and obesity of the subjects [21]. To estimate total energy expenditure the physical activity level was set to 1.3, because a low physical activity level was reported by all subjects [22]. Each subject was instructed to follow an energy-restricted diet for eight consecutive weeks (- 30% from calculated total energy expenditure; -633 ± 88 kcal/day), and was allocated in one of three diet groups: group 1 = control group, which consumed no seafood during these eight weeks; group 2 = 150 g cod three times a week; and group 3 = 150 g cod five times a week. Cod in group 2 provided 0.26 g LC n-3 PUFA and 11.4 g fish protein per day, in group 3 these numbers were 0.43 g and 19.1 g per day, respectively. The macronutrient composition of the three diets was designed to be identical in total fat (30% of total energy), carbohydrate (50% of total energy), protein (20% of total energy) and dietary fibre (20-25 g/day). Each subject received a detailed diet plan and instructions were given to standardize sources of fat, fruit and vegetable consumption and meal frequency.

Anthropometric measurements

Weight was measured in light underwear on a calibrated scale (SECA 708, Hamburg, Germany). Subject's height was measured at baseline with a calibrated stadiometer, and waist circumference was measured with a tape measure following standardized methods. Body fat mass and fat-free mass were assessed by bioelectrical impedance analysis (BIA) (Bodystat 1500; Bodystat, Douglas, Isle of Man, British Isles).

Serum lipids, glucose, insulin and blood pressure

Participants were instructed to avoid strenuous exercise and alcohol consumption the day before the drawing of blood samples. Blood sampling (after overnight fasting) was conducted at baseline and at endpoint of the study. Total cholesterol, triglycerides and glucose were analysed using an enzymatic colorimetric assay and an automated analyzer (Hitachi 911; Roche Diagnostics), HDL-cholesterol was determined in serum using PEG-modified enzymes and dextrane sulphate. Levels of low density lipoprotein LDL- cholesterol were calculated using the Friedewald formula (levels below 3.95 mmol/l). Insulin was measured with electrochemiluminescence immunoassay (ECLIA) on a Modular Analytics E170 system from Roche Diagnostics (Manheim, Germany). A strict routine for BP measurements was adhered to, which was defined in the research protocol.

Subjects were defined as having the metabolic syndrome (according to the definition from the International Diabetes Federation [23]), if they had central obesity (waist circumference female ≥ 80 cm and male ≥ 94 cm; or if body mass index is > 30 kg/m^2) plus any two of the four additional factors below:

- raised TG level: ≥ 1.7 mmol/l
- reduced HDL-cholesterol: < 1.03 mmol/l in males and < 1.29 mmol/l in females
- raised blood pressure (systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg)
- raised fasting plasma glucose (FPG ≥ 5.6 mmol/l)

Statistical analysis

The data were analyzed using the SPSS statistical package, version 11.0 (SPSS, Chicago, IL, USA). Values are reported as mean ± standard deviation (s.d.). Distributions of the investigated variables were estimated using the Kolmogornov Smirnov test. Differences between baseline and endpoint were calculated using Wilcoxon Signed Ranks Test. Baseline characteristics and dietary intake were compared using 1-way ANOVA. In order to assess the effects of cod consumption (group 1 = control, group 2 = cod three times a week, group 3 = cod five times a week) on anthropometrical changes and improvements of blood variables (Δy) during the intervention, linear models including fixed factors (gender, diet group) and covariates (y at baseline) were constructed. Results from the linear models are shown as parameter estimates where the two cod groups were compared separately with the control group. Linear trend analysis was performed to investigate a possible dose-response relationship between cod consumption and outcome variables. The quality of the models was checked using Levene's test of homogeneity and the residuals of the model were checked for normality using Kolmogornov Smirnov test. The significance niveau was set at $P \leq 0.05$.

Results

The baseline characteristics of the subjects are shown in Table 1. The reported energy intake at baseline was higher than the calculated energy requirement (men: 2707 ± 860 vs. 2427 ± 143 kcal/day, P = 0.023; and women: 2081 ± 512 vs. 1935 kcal/day, P = 0.016). Reported mean energy intake during the intervention in men was lower than the prescribed energy intake according to the meal plans (1465 ± 356 vs. 1703 ± 101 kcal/day, P < 0.001), but not in

women (1322 ± 216 vs. 1359 ± 62 kcal/day, P = 0.221). Nutrient intakes at baseline and during the intervention were not different between the three intervention groups, with exception of a higher protein intake in group 3 compared to group 1 during the intervention (77.7 vs 66.5 g/d, Bonferroni post hoc test = 0.012).

The average reduction in body weight after the eight-week intervention was 5.0 ± 2.9 kg, or 5.4 ± 3.4 kg for men and 4.6 ± 2.6 kg for women. The percentage of subjects classified as obese (BMI ≥ 30.0 kg/m^2) declined from 54% at baseline to 13% at endpoint.

Figure 2 show the mean unadjusted decrease in body weight and other anthropometric measurements in the three diet groups. The linear models for anthropometric measurements are shown in Table 2. There was 1.7 kg greater weight loss (P = 0.015) in group 3 than in group 1 after the eight-week intervention, and a 0.7 kg non-significantly greater weight loss in group 2 compared to group 1. Trend analysis showed a significant dose-response relationship (P = 0.007) for weight loss between diet groups. Addition of a gender*diet group interaction term to the multivariate analysis did not turn out significant (male*cod 3x /w: P = 0.839; male*cod 5x /w: P = 0.126).

Reductions in BMI and waist circumference were significantly greater in group 3 than in group 1 (P = 0.015 and P < 0.001, Table 2). Trend analysis showed a significant dose-response relationship between diet groups for BMI reduction and waist circumference reduction (P = 0.007 and P < 0.001).

The intervention resulted in significant reductions in systolic and diastolic blood pressure, serum triglyceride and insulin concentrations (Figure 3). Changes for total cholesterol and HDL cholesterol were borderline significant (P = 0.067 and P = 0.083, respectively). The prevalence of the metabolic syndrome declined during the diet intervention from 28.6% at baseline to 21.0% at endpoint, meaning a 26.6% decrease among participants diagnosed with the metabolic syndrome at baseline. According to multivariate statistics, changes in blood lipids, insulin and glucose were not significantly affected by cod consumption (results not shown). There was a significant effect of diet group 3 on systolic blood pressure reduction compared to group 1 (less reduction, B = 4.808, P = 0.009), and a significant effects of group 2 on diastolic blood pressure reduction in group 2 compared to group 1 (less reduction, B = 2.960, P = 0.034).

Discussion

Our study is one of the first interventions in humans to test the effects of different amounts of lean fish consumption in combination with energy restriction on weight loss and risk factors for cardiovascular disease. The most important finding of our study is that inclusion of lean fish to an energy-restricted diet for eight weeks results in significantly more weight loss than an isocaloric diet without seafood in young overweight or obese individuals. In the multivariate analysis there was on average 1.7 kg significantly more weight loss among subjects consuming 150 g cod five times a week compared to the control group receiving no seafood. The trend analysis clearly supports a dose-response relationship between cod consumption and weight loss. Similar dose-response relations were also observed between cod intake and reductions in waist circumference and body mass index.

According to the statistical analysis cod consumption accounts for more weight loss in both genders than the diet without seafood. The SEAFOODplus YOUNG study [6] showed significantly greater weight loss only in male subjects receiving lean fish (150 g cod three times a week) compared to control group, but not in women. The mechanism of the observed gender difference in weight loss is not known. However, gender differences in obesity related hormones [24] and metabolic pathways [25] with relevance for dietary interventions and body weight control [6] have been reported.

Currently it is not clear whether fish proteins are responsible for increased weight loss observed in our study. The weight loss effects of cod protein have only been studied to a limited degree. It has been suggested that the body weight reduction with fish protein consumption could be related to lower fat deposition, since rats fed fish protein diet had lower total lipids in adipose tissue than rats fed casein diet [14].

Particular amino acids in fish protein can have beneficial effects on body weight, and taurine is a candidate therefore. Taurine (2-amino-ethane-sulfonic acid) is a free amino acid that is concentrated in fish and seafood and has been used as a marker of seafood intake [26]. An inverse association has been observed between taurine excretion and ischemic heart disease mortality in the WHO-CARDIAC study [26]. Several studies have also shown that taurine might promote weight loss. An early study by Fujihira et al. [27] showed that taurine decreases body weight in obese mice, mainly due to inhibition of excess fat deposition in the

body. A human study by Zhang et al. [28] also found that seven-week supplementation with 3 g taurine per day resulted in weight loss.

An additional mechanism which can explain the positive influence of fish protein on body weight is its possible effect on satiety, which has been reported to be greater compared to other animal proteins, such as beef and chicken [29]. Frequent lean fish consumption can also help people to decrease meat consumption with a simultaneous reduction in saturated and total fat intake while maintaining an adequate intake of high quality protein and other nutrients.

During the intervention systolic and diastolic blood pressure, serum triglycerides and serum insulin concentration decreased significantly. The prevalence of subjects diagnosed with the metabolic syndrome dropped from ~29% to ~21% during the intervention. In the present study cod consumption did not affect the improvements of blood parameters during the intervention, although in the SEAFOODplus YOUNG study significant effects of cod consumption (3 x 150 g/week) on total cholesterol and triglycerides were observed [8]. Several other animal and human studies have shown positive effects of fish protein or taurine supplementation on blood lipids [15,28].

In our study the amount of cod consumed seemed to negatively affect the blood pressure reduction during the intervention. However, these effects were not consistent, because, e.g., the negative effect on diastolic blood pressure seen in the cod three times a week was not observed in the cod five times a week group. The effect might be explained by unequal distribution of relevant factors or covariates between the diet groups, nevertheless, these findings deserve further investigations.

Limitations

A limitation of the current study is that it was not fully randomized, because group 3, receiving cod five times a week, was investigated after the two other diet groups. However, the same inclusion and exclusion criteria, recruitment area, research facilities, meal plans, procedures and laboratories were used for group 3. Additionally, there were no significant differences in age, body weight, BMI and waist circumference between the groups at baseline. Habitual dietary intake of the groups was not significantly different, which all together makes the three groups fully comparable.

A limitation of each dietary intervention trial is the uncertainty whether dietary intakes of subjects during the study period were as reported or prescribed. As there was an intense support of the study participants by our staff, frequent contact via phone and in person visits, and as compliance was tested during the intervention trial using a FFQ, validated for assessing frequency of seafood consumption [18], this risk was minimized.

As indicated by our study, lean fish consumption may promote weight reduction in humans, which is an important public health message, because the consumption of lean fish has been reported to be more common than consumption of fatty fish [30]. Further studies are needed to verify the weight loss effects of lean fish consumption in general, as well as mechanisms behind that process.

Conclusion

Our study indicates a dose–response relationship between cod consumption and weight loss during an eight-week period of energy restriction. Inclusion of 5 times 150 g of lean fish per week results in 1.7 kg significantly greater weight loss in young overweight or obese individuals than an isocaloric diet without seafood. Blood parameters improve during an eight-week intervention and the prevalence of the metabolic syndrome decreases, although these changes are most likely attributed to weight loss and not cod consumption.

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Table 1: Baseline characteristics; anthropometric measurements and blood parameters. Values shown as means ± s.d.

	Control group		Cod three times a week		Cod five times a week		P-value[1]		post-hoc[2]	
	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
Anthropometric measurements	mean ± s.d.	mean ± s.d.	mean ± s.d.	mean ± s.d.	mean ± s.d.	mean ± s.d.				
Body weight (kg)	98.4 ± 8.4	82.7 ± 7.8	98.8 ± 7.4	86.8 ± 7.5	101.8 ± 9.2	85.4 ± 8.9	0.441	0.266		
Height (m)	1.81 ± 0.07	1.67 ± 0.06	1.81 ± 0.05	1.70 ± 0.06	1.82 ± 0.07	1.69 ± 0.07	0.698	0.417		
BMI (kg/m^2)	30.2 ± 1.6	29.7 ± 1.4	30.3 ± 1.0	30.2 ± 1.5	30.6 ± 1.2	30.0 ± 1.4	0.544	0.500		
Waist (cm)	101.5 ± 5.9	91.7 ± 5.5	100.6 ± 4.6	93.2 ± 5.5	103.8 ± 5.8	95.5 ± 6.9	0.255	0.094		
Fat mass (kg)	23.9 ± 4.4	31.7 ± 5.2	23.7 ± 3.6	32.9 ± 4.2	28.6 ± 4.0	34.1 ± 5.2	0.001	0.234	3 > 1, 2	
Fat-free mass (kg)	74.6 ± 5.3	51.0 ± 3.4	75.1 ± 5.1	54.1 ± 4.3	73.2 ± 6.2	51.0 ± 4.8	0.620	0.023		2 > 3, 1
Blood parameters										
Total cholesterol (mmol/L)	5.1 ± 0.9	5.1 ± 0.6	5.0 ± 0.8	4.9 ± 0.7	4.7 ± 1.0	4.6 ± 1.0	0.502	0.121		
HDL cholesterol (mmol/L)	1.2 ± 0.3	1.5 ± 0.2	1.0 ± 0.2	1.5 ± 0.3	0.9 ± 0.2	1.3 ± 0.4	0.002	0.049	1 > 3	3 < 1, 2
LDL cholesterol (mmol/L)	3.2 ± 0.9	3.1 ± 0.5	3.2 ± 0.9	3.0 ± 0.7	3.1 ± 0.9	2.8 ± 0.9	0.938	0.305		
Triglyceride (mmol/L)	1.5 ± 0.9	1.0 ± 0.4	1.5 ± 0.6	1.1 ± 0.4	1.5 ± 0.9	1.2 ± 0.6	0.816	0.495		
Glucose (mmol/L)	5.0 ± 0.3	4.6 ± 0.4	4.8 ± 0.4	4.6 ± 0.4	4.8 ± 0.5	4.5 ± 0.5	0.383	0.374		
Insulin (mU/L)	12.9 ± 8.2	9.8 ± 5.0	9.0 ± 2.5	10.5 ± 4.4	8.6 ± 6.3	6.1 ± 5.1	0.075	< 0.001		3 < 2, 1
Systolic blood pressure (mmHg)	133.4 ± 7.5	124.2 ± 7.6	133.9 ± 7.2	123.7 ± 9.1	139.3 ± 11.2	127.0 ± 10.7	0.113	0.361		
Diastolic blood pressure (mmHg)	73.1 ± 8.0	73.0 ± 5.7	73.4 ± 5.9	74.0 ± 5.8	73.1 ± 8.5	71.3 ± 8.2	0.996	0.329		

[1] 1-way ANOVA, [2] LSD post hoc test

Table 2: Estimated changes[1] in anthropometric variables relative to the control group.

	Decrease	P- value	95% CI	
Body weight (kg)				
Lean fish 3x/w	-0.666	0.396	-2.218	0.885
Lean fish 5x/w	-1.729	0.015	-3.115	-0.344
BMI (kg/m2)				
Lean fish 3x/w	-0.161	0.527	-0.663	0.341
Lean fish 5x/w	-0.555	0.015	-1.001	-0.108
Waist (cm)				
Lean fish 3x/w	-0.994	0.199	-2.520	0.532
Lean fish 5x/w	-3.419	< 0.001	-4.830	-2.007
Fat mass (kg)				
Lean fish 3x/w	-0.191	0.793	-1.633	1.251
Lean fish 5x/w	-0.873	0.209	-2.242	0.496
Fat free mass (kg)				
Lean fish 3x/w	-0.333	0.533	-1.388	0.722
Lean fish 5x/w	-0.529	0.254	-1.444	0.387

[1] Variables included in the linear model: anthropometric measurement at baseline, gender, diet group

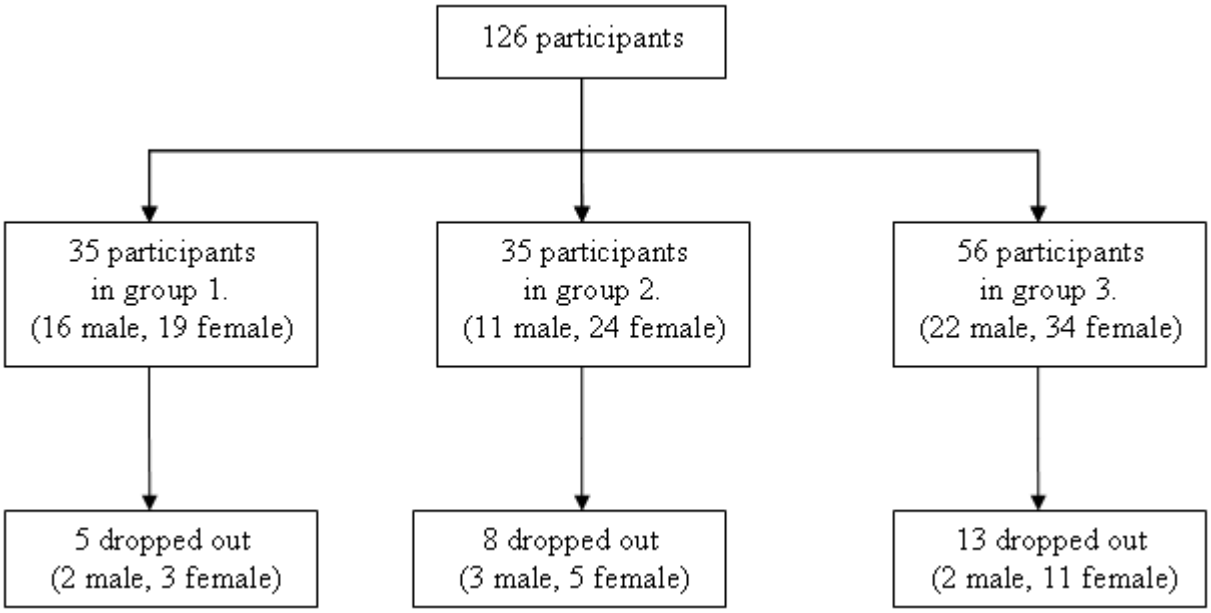


Figure 1: Participants, groups and drop outs

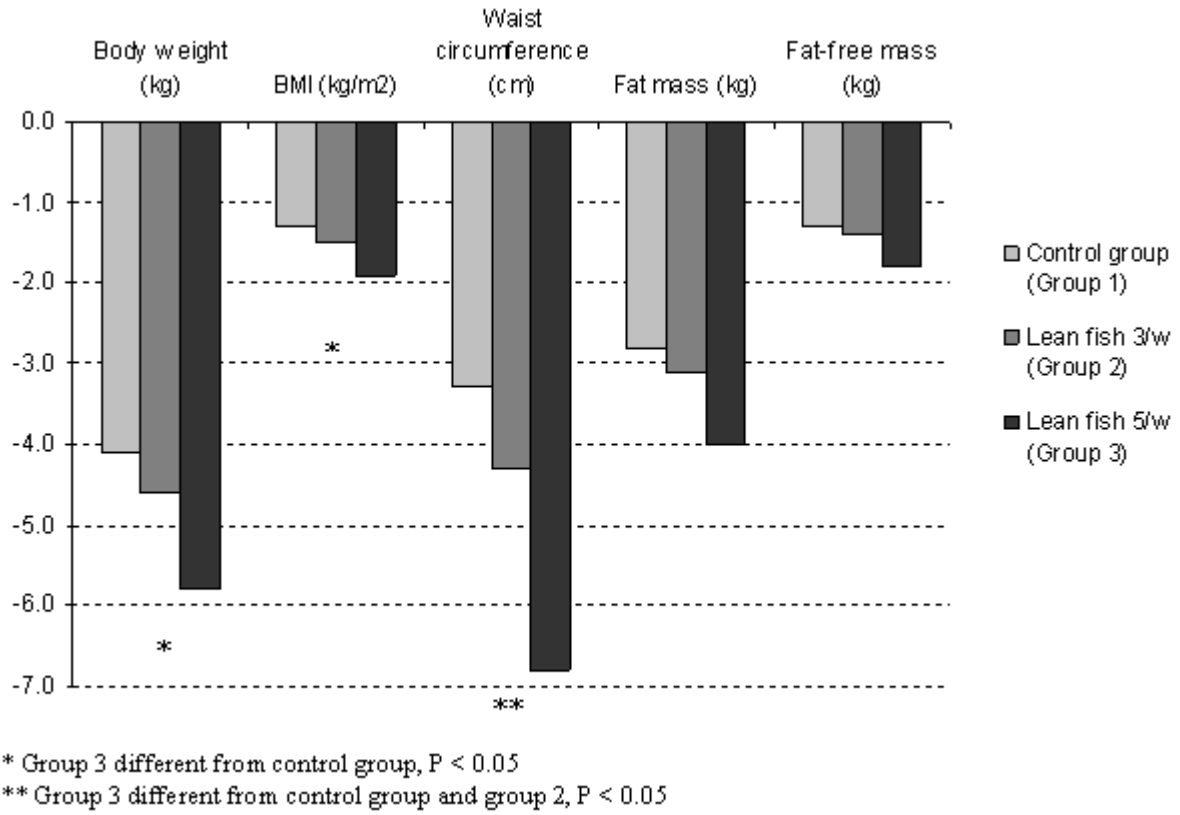
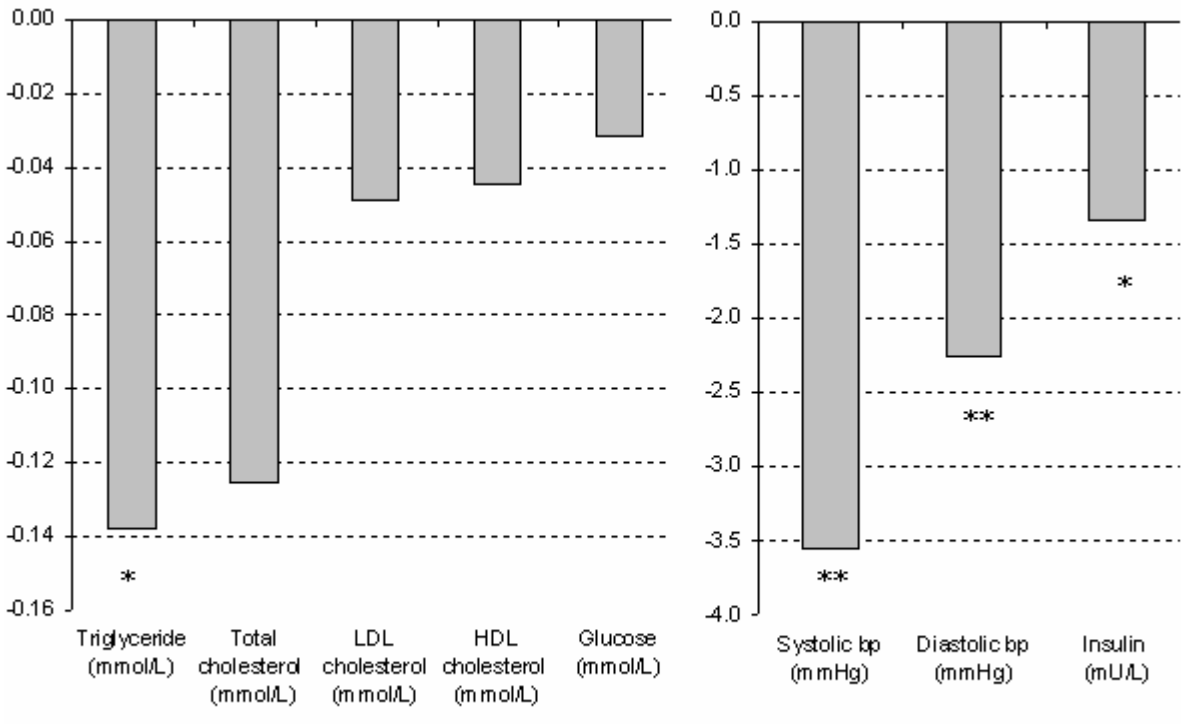


Figure 2: Decreases in anthropometric measurements after the eight-week intervention (unadjusted data).



* P < 0.05 (Wilcoxon Signed Ranks Test); ** P < 0.01 (Wilcoxon Signed Ranks Test).

Figure 3: Changes in anthropometric measurements after the eight-week intervention

Hefur þú áhuga á að taka þátt í RANNSÓKN og GRENNAST í leiðinni?

Rannsóknastofa í næringarfræði við Landspítala Háskólasjúkrahús og Raunvísindadeild Háskóla Íslands óskar eftir þátttakendum í rannsókn sem hlotið hefur samþykki Vísindasiðanefndar.
Rannsóknin ber heitið „Lífvirk efni í fiski – heilsufarsleg áhrif“.

Þátttakendur þurfa að vera

- **á aldrinum 20-40 ára**
- **með líkamsþyngdarstuðul (BMI) milli 28 og 32 kg/m^2 (sjá töflu hér að neðan) og**
- **hafa mikla löngun til þess að fylgja ákveðnu mataræði og grennast.**

Áhugasamir eru beðnir um að hafa samband sem fyrst við Margréti Þóru Jónsdóttur, meistaranema í næringarfræði, í síma 543-3329 eða senda tölvupóst á margrtho@landspitali.is og tilgreina nafn, símanúmer, aldur og BMI.

Tafla fyrir líkamsþyngdarstuðull (BMI, kg/m^2). Gráa svæðið sýnir alla sem eru með BMI milli 28-32 kg/m^2

Hæð í m																																						
2,00	15	15	16	16	17	17	18	18	19	19	20	20	21	21	22	2	23	23	24	24	25	25	26	26	27	27	28	28	29	29	30	30	31	31	32			
1,98	15	15	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25	26	26	26	27	27	28	28	29	29	30	30	31	31	32	32		
1,96	15	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25	26	26	27	27	28	28	29	29	30	30	31	31	32	32	33			
1,94	15	16	16	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25	26	26	27	27	28	28	29	29	30	30	31	31	32	32	33	33			
1,92	16	16	17	17	18	18	19	20	20	21	21	22	22	23	23	24	24	25	25	26	27	27	28	28	29	29	30	30	31	31	32	33	33	34	34			
1,90	16	17	17	18	18	19	19	20	20	21	22	22	23	23	24	24	25	25	26	27	27	28	28	29	29	30	30	31	32	32	33	33	34	34	35			
1,88	16	17	18	18	19	19	20	20	21	22	22	23	23	24	24	25	25	26	27	27	28	28	29	29	30	31	31	32	32	33	33	34	35	35	36			
1,86	17	17	18	18	19	20	20	21	21	22	23	23	24	24	25	25	26	27	27	28	28	29	29	30	31	31	32	32	33	34	34	35	35	36	36			
1,84	17	18	18	19	19	20	21	21	22	22	23	24	24	25	25	26	27	27	28	28	29	30	30	31	31	32	32	33	34	34	35	35	36	37	37			
1,82	18	18	19	19	20	21	21	22	22	23	24	24	25	25	26	27	27	28	28	29	30	30	31	31	32	33	33	34	34	35	36	36	37	37	38			
1,80	18	19	19	20	20	21	22	22	23	23	24	25	25	26	27	27	28	28	29	30	30	31	31	32	33	33	34	35	35	36	36	37	38	38	39			
1,78	18	19	20	20	21	21	22	23	23	24	25	25	26	27	27	28	28	29	30	30	31	32	32	33	33	34	35	35	36	37	37	38	39	39	40			
1,76	19	19	20	21	21	22	23	23	24	25	25	26	26	27	28	28	29	30	30	31	32	32	33	34	34	35	36	36	37	37	38	39	39	40	41			
1,74	19	20	20	21	22	22	23	24	24	25	26	26	27	28	28	29	30	30	31	32	32	33	34	34	35	36	36	37	38	38	39	40	40	41	42			
1,72	20	20	21	22	22	23	24	24	25	26	26	27	28	28	29	30	30	31	32	32	33	34	34	35	36	37	37	38	39	39	40	41	41	42	43			
1,70	20	21	21	22	23	24	24	25	26	26	27	28	28	29	30	30	31	32	33	33	34	35	35	36	37	37	38	39	39	40	41	42	42	43	44			
1,68	21	21	22	23	23	24	25	26	26	27	28	28	29	30	30	31	32	33	33	34	35	35	36	37	38	38	39	40	40	41	42	43	43	44	45			
1,66	21	22	22	23	24	25	25	26	27	28	28	29	30	30	31	32	33	33	34	35	36	36	37	38	38	39	40	41	41	42	43	44	44	45	46			
1,64	22	22	23	24	25	25	26	27	28	28	29	30	30	31	32	33	33	34	35	36	36	37	38	39	39	40	41	42	42	43	44	45	45	46	47			
1,62	22	23	24	24	25	26	27	27	28	29	30	30	31	32	33	34	34	35	36	37	37	38	39	40	40	41	42	43	43	44	45	46	46	47	48			
1,60	23	23	24	25	26	27	27	28	29	30	30	31	32	33	34	34	35	36	37	38	38	39	40	41	41	42	43	44	45	45	46	47	48	48	49			
1,58	23	24	25	26	26	27	28	29	30	30	31	32	33	34	34	35	36	37	38	38	39	40	41	42	42	43	44	45	46	46	47	48	49	50	50			
1,56	24	25	25	26	27	28	29	30	30	31	32	33	34	35	35	36	37	38	39	39	40	41	42	43	44	44	45	46	47	48	48	49	50	51	52			
Þyngd í kg	*58*	*60*	*62*	*64*	*66*	*68*	*70*	*72*	*74*	*76*	*78*	*80*	*82*	*84*	*86*	*88*	*90*	*92*	*94*	*96*	*98*	*100*	*102*	*104*	*106*	*108*	*110*	*112*	*114*	*116*	*118*	*120*	*122*	*124*	*126*			

Leiðbeiningar: Finndu hæð þína í dálkinum lengst til vinstri og þyngd þína í neðstu línunni og kannaðu hvar þessar tvær tölur skarast í töflunni. Ef þú lendir inni á gráa svæðinu þá ert þú með BMI innan þeirra marka sem hentar til þátttöku í rannsókninni.

Appendix III – Introduction letter for participants

Rannsóknastofa í næringarfræði
Landspítali-Háskólasjúkrahús
Eiríksgata 29, 1. hæð
101 Reykjavík
Sími: 543 8417
Fax: 543 4824
ingathor@landspitali.is

Kynningarbréf fyrir þátttakendur í rannsókninni „Lífvirk efni í fiski – heilsufarsleg áhrif“

Kæri viðtakandi,

Rannsóknarverkefni þetta er styrkt af AVS, Rannsóknarsjóði í sjávarútvegi. Megnimarkmið verkefnisins er að auka þekkingu á tengslum heilsu og næringar sem nýst gæti í heilsueflingu og forvörnum sjúkdóma meðal ungs fólks. Meginmarkmið rannsóknarinnar er að kanna áhrif fiskneyslu á ýmsar heilsufarslegar breytur, sem meðal annars tengjast ofþyngd og fitudreifingu í líkamanum. Þessi rannsókn er hluti af námsverkefni Margrétar Þóru Jónsdóttur sem er í doktorsnámi í næringarfræði við Háskóla Íslands.

Ábyrgðarmaður rannsóknarinnar:	Prófessor Inga Þórsdóttir Rannsóknarstofa í næringarfræði Landspítali Háskólasjúkrahús Eiríksgötu 29, 1 hæð 101 Reykjavík Símar: 543-8414 / 543-8410 Tölvupóstur: ingathor@landspitali.is

Rannsókn þessi hefur fengið leyfi vísindasiðanefndar og hefur verið tilkynnt til persónuverndar. Þér er frjálst að hafna þátttöku eða hætta í rannsókn á hvaða stigi sem er, án útskýringa og án afleiðinga. Ábyrgðarmaður rannsóknar er til aðstoðar við framkvæmd viðtals ef þess þarf. Þátttakendur geta ávallt haft samband við ábyrgðarmann rannsóknarinnar eða aðstoðarmann hans. Auglýst var eftir þátttakendum í þessa rannsókn.

Hvað felst í þátttöku – og hvers er ætlast til af þáttakanda?
Þátttaka í þessari rannsókn felur í sér að við þú skuldbindur þig til að fylgja ákveðnu mataræði í 8 vikur samfleytt en þetta mataræði miðar að því að þú léttist um 0,5-1 kg á viku. Nánari útskýringar á mataræði verða gefnar af næringarfræðingi í fyrsta viðtali. Þessi minnkun í hitaeiningainntöku hefur ekki í för með sér neina hættu fyrir þig. Að auki verða lagðir fyrir spurningalistar í byrjun og í lok rannsóknar.

Þátttakendur koma inn til mælinga þrisvar sinnum en að auki verður haft samband í gegnum síma tvisvar sinnum til að athuga með framgang rannsóknarinnar og hvort þú hafir einhverjar

spurningar. Tekin verður blóðprufa við upphaf rannsóknar og í lokin til að kanna m.a. blóðfitur, insúlín-næmni, fitusýrusamsetningu og heilbrigði beina ásamt fleiru.

Fyrsta heimsókn. Í byrjun rannsóknar koma þátttakendur FASTANDI inn að morgni. Þá verður tekin blóðprufa og gerðar verða ýmsar mælingar á viðkomandi (hæð, þyngd, mittisummál, fitusamsetning líkamans og blóðþrýstingur). Þátttakendur svara einnig spurningarlistum um mataræði og lífsstíl (hreyfingu, lyfjanotkun, reykingar, áfengisnotkun o.fl.). Þátttakendur fá einstaklingsviðtal við næringarfræðing sem útskýrir hvernig ná skuli markmiðum rannsóknarinnar og hvernig viðkomandi eigi að fylgja matseðlum sem gefnir verða.

Önnur heimsókn. Um miðbik rannsóknarinnar, þ.e. eftir 4 vikur, koma þátttakendur aftur inn FASTANDI að morgni. Þá verður viðkomandi mældur aftur (þyngd, mittisummál, fitusamsetning líkamans). Þátttakandi svarar einnig spurningalista um mataræði.

Þriðja heimsókn. Í lok rannsóknar koma þátttakendur FASTANDI inn að morgni. Þá verður tekin blóðprufa og viðkomandi verður mældur aftur (þyngd, mittisummál, fitusamsetning líkamans og blóðþrýstingur). Þátttakendur svara einnig spurningalista um mataræði, svengd og hreyfingu.

Lífsýni (þ.e. blóð) verða greind erlendis og verður þeim eytt fimm árum eftir lok rannsóknar. Öll gögn frá þátttakendum verða ópersónutengd (kóðuð) þannig að ekki er hægt að rekja þau til viðkomandi.

Áhætta og ávinningur af þátttöku
Hugsanlegur ávinningur er að þú léttist og bætir þar með lífslíkur þínar með því að lækka blóðþrýsting og minnka kólesteról og þríglýseríð í blóði. Þannig getur þú jafnvel minnkað líkur á því að fá króníska sjúkdóma eins og insúlínóháða sykursýki, hjarta- og æðasjúkdóma og krabbamein.
Hugsanleg hætta af rannsókninni verður að teljast hverfandi.

Ábyrgðarmaður rannsóknar: Prófessor Inga Þórsdóttir, sími: 543-8414
Tölvupóstur: ingathor@landspitali.is

Aðstoðarmaður ábyrgðarmanns: Margrét Þóra Jónsdóttir, sími: 543-8417
Tölvupóstur: margrtho@landspitali.is

Ef þú hefur spurningar um rétt þinn sem þátttakandi í vísindarannsókn eða vilt hætta þátttöku í rannsókninni getur þú snúið þér til Vísindasiðanefndar, Laugavegi 103, 105 Reykjavík. Sími: 551-7100, fax: 551-1444.

Appendix IV – Informed consent

Rannsóknastofa í næringarfræði
Landspítali-Háskólasjúkrahús
Eiríksgata 29, 1. hæð
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UPPLÝST SAMÞYKKI fyrir þátttöku í vísindarannsókninni „Lífvirk efni í fiski – heilsufarsleg áhrif“

Þátttakandi samþykkir að fylgja þeim leiðbeiningum um mataræði sem næringarfræðingur gefur í alls 8 vikur. Þátttakandi samþykkir einnig að koma inn til mælinga fyrir rannsókn, á miðju tímabili rannsóknar (eftir 4 vikur) og í lok rannsóknar og svara um leið tveimur spurningalistum. Tvisvar sinnum verður tekin blóðprufa.

Niðurstöður rannsóknarinnar munu verða sendar til birtingar í virtum erlendum vísindatímaritum.

Þátttakandi hefur lesið kynningarbréf um rannsóknina „Lífvirk efni í fisk – heilsufarsleg áhrif“.

Undirskrift þátttakanda:

Ég, _____
lýsi því hér með yfir að ég gef samþykki mitt af fúsum og frjálsum vilja fyrir því taka þátt sem sjálfboðaliði í þessari íhlutandi rannsókn „Lífvirk efni í fiski – heilsufarsleg áhrif“ sem tilheyrir SEAFOODplus YOUNG Evrópurannsókninni. Ég samþykki að lífsýni megi flytja úr landi til greiningar. Ég hef fengið nauðsynlegar upplýsingar og lesið þær yfir.

Mér hefur verið kynnt eðli og umfang þessarar vísindarannsóknar og ég er samþykk(ur) þátttöku og skrifa því undir þessi tvö eintök:

_____
Dagsetning og staður:

_____
Undirskrift þátttakanda

_____
Undirritun þess sem aflar samþykkis

UPPLÝST SAMÞYKKI ÞETTA ER Í TVÍRITI, ÞÁTTTAKANDI HELDUR EFTIR EINU EINTAKI, SÁ SEM AFLAR SAMÞYKKIS HELDUR EFTIR ÖÐRU EINTAKI

Appendix V – Instructions for participants

Rannsóknastofa í næringarfræði
Landspítali-Háskólasjúkrahús
Eiríksgata 29, 1. hæð
101 Reykjavík
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Þátttaka í rannsókninni „Lífvirk efni í fiski – heilsufarsleg áhrif“

Leiðbeiningar fyrir þátttakendur

Næstu þrjár heimsóknir á LSH verða að morgni til

- Fitumæling verður framkvæmd í hverri heimsókn, þetta er viðkvæm mæling og því er mikilvægt að þú farir eftir þessum leiðbeiningum fyrir hverja heimsókn:
    1) Vera fastandi í 12 tíma fyrir hverja heimsókn, þú mátt drekka vatn en þú verður að hætta því 4 klst. áður en þú kemur til okkar. Vinsamlegast slepptu morgunmatnum, við munum veita þér morgunmat (í heimsókn 1 og 3) um leið og allar mælingar hafa farið fram. Þú matt hvorki drekka te né kaffi a.m.k. 24 klst fyrir hverja heimsókn.
    2) Ekki drekka áfengi 24 klst fyrir hverja heimsókn.
    3) Slepptu öllum erfiðum æfingum daginn áður og rétt áður en mælingarnar fara fram.
    4) Mikilvægt að þú breytir ekki út af hefðbundnum neysluvenjum daginn áður en þú kemur til okkar (þ.e. átt að borða eins og venjulega).

- Við upphaf og í lok rannsóknarinnar (heimsókn 1 og 3) mun hjúkrunarfræðingur taka blóðsýni hjá þér, og verður það sent til greiningar.

- Mælt verður þyngd, hæð, mittismál, fituprósenta og blóðþrýstingur.

- Í fyrstu heimsókn mun næringarfræðingur einnig spyrja þig nokkurra spurninga um þitt venjulega mataræði og aðstoða þig við að fylla út spurningalista er varða mataræði og heilsu.

- Ef þú þarft að taka verkjalyf þá vinsamlegast (nema fyrirmæli komi frá lækni um annað) takið lyf sem eru ekki bólgueyðandi (bólgueyðandi lyf eru t.d. magnyl, aspirin og ibufen). Verkjalyf sem eru ekki bólgueyðandi eru t.d. paracetamol.

LEIÐBEININGAR FYRIR FÆÐUSKRÁNINGU

- Skrá á allan mat og drykk í **2 daga samfleytt**, einn virkan dag og einn helgardag.
- Fyllið í matardagbókina um leið og þið borðið svo ekkert gleymist.
- **Reynið að borða eins og þið eruð vön og ekki láta fæðuskráninguna hafa áhrif á hvað þið borðið.**
- Hefjið skráningu á fyrstu máltíð (t.d. morgunmat) daginn sem skráningin hefst.

Fyrir hverja máltíð á að:

- Skrifa niður dagsetningu og u.þ.b. klukkan hvað (t.d. þri. 07.09. kl. 12). Best að byrja á nýrri blaðsíðu fyrir hvern dag og skrifa þá dagsetningu efst á hvert blað. Hafið línubil á milli máltíða. Skrifið bara eina fæðutegund í hverja línu (t.d. brauðsneið og smjör í sitt hvora línu).
- Lýsið mat, drykk og magni eins nákvæmlega og hægt er.
- Gefið upp tegund af matvælum og jafnvel framleiðanda. T.d. undanrenna, léttmjólk, nýmjólk, heilhveitibrauð, brauðostur 17% gouda, appelsínu Trópí, kjöttegund; t.d. steikt ungnautahakk 8-12% fita og fisktegund; t.d. soðin lúða.
- Lýsið matreiðsluaðferðum. T.d. steikt, soðið eða grillað. Hrátt, soðið eða niðursoðið grænmeti. Ferskir eða niðursoðnir ávextir.
- Ef um er að ræða heimatilbúinn mat, látið þá uppskrift fylgja með, þ.e. hvaða hráefni voru notuð. T.d. u.þ.b. 2 msk olía, 300 g nautakjöt steikt, 1 laukur steiktur, 200 dl tómatar í dós, hvítlaukur og krydd, látið malla í smástund.
- Ekki gleyma að skrá drykki, millibita, sælgæti, vítamín, lýsi og fæðubótarefni.

Hvernig á að vigta?

1. dæmi: *Vigtun á brauði með smjöri og osti*

Brauðið sett á vogina og þyngdin skráð. Stillt aftur á núll (reset) með brauðsneiðina á voginni. Smjörið sett á brauðsneiðina og þyngdin skráð. Stillt aftur á núll með brauðsneiðina á voginni. Osturinn settur á og þyngdin skráð.

2. dæmi: *Vigtun á kvöldmat; fiskur með kartöflum og salati.*

Diskurinn settur á vogina og stillt á núll (reset). Vigtið síðan hverja fæðutegund fyrir sig. Fiskurinn settur á diskinn og þyngdin skráð. Stillt aftur á núll með diskinn og fiskinn enn á voginni. Kartöflurnar næst settar á diskinn og þyngdin skráð. Stillt aftur á núll eins og áður. Salatið sett á diskinn og þyngdin skráð. Tilgreinið helstu hráefni í salatinu t.d. salatblöð, gúrka og tómatar með olíu og edik dressingu.

ATH! Ef ekki er klárað af disknum vigtið hann þá eftir máltíðina og skráið það sem er afgangs. Bein (í kjöti eða fiski) og hýði (t.d. af banana eða appelsínu) og aðra óæta hluta þarf að vigta og skrá sem afgang.

Dæmi:

Dags. *Þriðjudagur 7 .september*				
Kl.	Matur eða drykkur (heiti, vörutegund)	Skammtað magn (g)	Magn afgangs (g)	Magn borðað eða drukkið (g)
7	Cheerios	50		50
	Nýmjólk	200	10	190
10	Nesti:			
	Heilhveitibrauð	40		40
	Smjör	7		7
	Ostur, brauðostur 17%	25		25
	Epli	98	10	88
	Vatn	200		200

Appendix VI – Food frequency questionnaire (FFQ)

Rannsóknastofa í næringarfræði
Landspítali-Háskólasjúkrahús
Eiríksgata 29, 1. hæð
101 Reykjavík
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Fæðuvenjur: Nokkrir fæðuþættir

Spurningar vegna rannsóknarinnar „Lífvirk efni í fiski – heilsufarsleg áhrif"

Raðnúmer:

Hvernig á að fylla út spurningalistann

Vinsamlega lestu spurningarnar og valmöguleikana vandlega áður en þú svarar spurningunum. Flestar spurningarnar eru þannig að það á bara að merkja við einn reit. Í nokkrum spurningum má merkja við fleiri möguleika ef við á. Í nokkrum spurningum biðjum við þig um að skrifa svarið.

Þú ert beðinn að svara með tilliti til þess hvernig þú varst að borða að meðaltali síðustu 3-4 vikurnar.

Fiskur – hvað borðar þú vanalega?

Aðalréttur

F1. Hvað borðar þú oft fisk sem aðalrétt?

- ☐ Aldrei
- ☐ 1-3 sinnum í mánuði
- ☐ 4-6 sinnum í mánuði
- ☐ 2 sinnum í viku
- ☐ 3 sinnum í viku
- ☐ 4-6 sinnum í viku
- ☐ Á hverjum degi
- ☐ Annað (skrifaðu):

_____

F2. Hvaða gerðir/tegundir fiska borðar þú oftast sem aðalrétt?
(merktu við með tölum 1-4 ef við á)

- ☐ Ýsu
- ☐ Þorsk
- ☐ Lúðu
- ☐ Lax
- ☐ Annað (skrifaðu):

_____

F3. Þegar þú borðar fisk sem aðalrétt, hve mikið borðar þú í hverjum matmálstíma?
(veldu að segja frá eins og þér hentar best að lýsa því)

_____ grömm

_____ flak (t.d. 1 flak, ½ flak, ⅓ flak o.s.frv.)

Fékk mér_____ sinnum á diskinn

Annað (skrifaðu):

_____

Brauðálegg, forréttur

F4. Hvað borðar þú oft fisk sem brauðálegg eða sem forrétt?

- ☐ Aldrei (ef aldrei farðu beint í spurningu F7)
- ☐ 1-3 sinnum í mánuði
- ☐ 4-6 sinnum í mánuði
- ☐ 2 sinnum í viku
- ☐ 3 sinnum í viku
- ☐ 4-6 sinnum í viku
- ☐ Á hverjum degi
- ☐ Annað (skrifaðu):

_____

F5. Hvaða gerðir/tegundir fiska borðar þú oftast sem álegg eða sem forrétt?
(merktu við með tölum 1-6 ef við á)

- ☐ Síld
- ☐ Reyktan/grafinn lax
- ☐ Kavíar
- ☐ Sardínur
- ☐ Makríl
- ☐ Rækjur
- ☐ Annað (skrifaðu):

_____

F6. Hve mikið borðar þú í hvert sinn? **(t.d. hve margar sneiðar, bita eða matskeiðar á eina brauðsneið og hve margar brauðsneiðar)**

_____

Lýsi

(Merktu bara einu sinni við hverja spurningu)

F7. Tekur þú inn lýsi eða lýsisperlur? (Ef nei þá máttu sleppa þeim spurningum sem eftir eru)

- ☐ Nei
- ☐ Já

F8. Hvaða tegund?

- ☐ Þorskalýsi
- ☐ Ufsalýsi
- ☐ Lúðulýsi
- ☐ Hákarlalýsi
- ☐ Annað (skrifaðu):

_____

F9. Hve oft?

- ☐ Á hverjum degi
- ☐ 4-6 sinnum í viku
- ☐ 2-3 sinnum í viku
- ☐ Annað (skrifaðu):

_____

F10. Hve mikið?

- ☐ Teskeið
- ☐ Barnaskeið
- ☐ Matskeið
- ☐ Fjöldi lýsisperla (skrifaðu):

_____

Appendix VII – Life style questionnaire (LSQ)

Rannsóknastofa í næringarfræði
Landspítali-Háskólasjúkrahús
Eiríksgata 29, 1. hæð
101 Reykjavík
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ingathor@landspitali.is

Númer þátttakanda _____

Spurningalisti fyrir þátttakendur

<u>Spurningar um reykingar (spurningar 1-4)</u>

1) Hefur þú einhvern tíma reykt?
- ❑ já
- ❑ nei (ef svarað neitandi farðu beint að spurningu 5)

2) Reykir þú núna?
- ❑ já (ef svarað játandi farðu beint að spurningu 4)
- ❑ nei

3) Ef þú ert hættur að reykja, hvað ertu búinn að vera hætt/hættur lengi?
- ❑ < 1 ár
- ❑ 1-5 ár
- ❑ 6-10 ár
- ❑ >10 ár

4) Ef þú reykir eða hefur reykt, hve margar sígarettur/vindla reykir þú/reyktir þú að meðaltali?
- ❑ "Social smoker" – minna en 10 stk að meðaltali á viku
- ❑ <10 stk á dag
- ❑ 11-15 stk á dag
- ❑ 16-20 stk á dag
- ❑ > 20 stk á dag

<u>Spurningar um áfengisneyslu (spurningar 5-6)</u>

5) Drekkur þú áfengi?
- ❑ já
- ❑ nei (ef svarað neitandi farðu beint að spurningu 7)

6) Ef þú drekkur áfengi hve marga drykki af áfengi drekkur þú <u>að meðaltali</u> í viku?
(einn drykkur er 1 lítill bjór, 1 léttvínsglas, 1 einfaldur sterkur drykkur)
- ❑ 1 drykk eða minna á viku
- ❑ 2-5 drykki á viku
- ❑ 6-10 drykki á viku
- ❑ >10 drykki á viku

Spurningar um heilsu (spurningar 7-11)

7) Ertu almennt heilsuhraustur?

- ❑ já
- ❑ nei – hvaða heilsubrestir ? _____

_____

8) Hefur þú gengist undir skurðaðgerð/ir síðustu 5 ár?

- ❑ já
- ❑ nei (ef svarað neitandi farðu beint að spurningu 10)

9) Hvernig skurðaðgerð/ir gekkst þú undir?

_____

10) Hefur þú fæðuofnæmi og/eða fæðuóþol fyrir einhverju svo þú vitir?

- ❑ já (fæðuóþol _____ fæðuofnæmi ____)
- ❑ nei (ef svarað neitandi farðu beint að spurningu 12)

11) Fyrir hverju hefur þú ofnæmi/óþol (merktu við fleiri en eitt svar ef við á)?

- ❑ Fiskofnæmi
- ❑ Skelfiskofnæmi
- ❑ Eggjaofnæmi
- ❑ Mjólkurofnæmi
- ❑ Mjólkursykursóþol (laktósaóþol)
- ❑ Hnetur og/eða möndlur
- ❑ Jarðhnetur og/eða ertur
- ❑ Glútenóþol
- ❑ Sojaofnæmi
- ❑ Annað ofnæmi – hvaða: _____

Spurningar um lyf (spurningar 12-13)

12) Tekur þú núna einhver lyf , þ.m.t. náttúrulyf og getnaðarvarnalyf?

- ❑ já
- ❑ nei (ef svarað neitandi sleppa spurningu 13)

13) Hvaða lyf tekur þú að staðaldri (merkið við fleiri en eitt svar ef við á)?

- ❑ Getnaðarvarnalyf (nafn lyfs:_____)
- ❑ Þunglyndislyf (nafn lyfs:_____)
- ❑ Blóðþrýstingslækkandilyf (nafn lyfs:_____)
- ❑ Blóðfitulækkandilyf (nafn lyfs:_____)
- ❑ Blóðþynningarlyf (nafn lyfs:_____)
- ❑ Ofnæmislyf (nafn lyfs_____)
- ❑ Lyf við sykursýki (nafn lyfs_____)
- ❑ Sýklalyf (nafn lyfs_____)
- ❑ Verkjalyf (nafn lyfs_____)
- ❑ Önnur lyf – hvaða: _____

Spurning um hreyfingu (spurningar 14-16)

14.) Hreyfir þú þig reglulega (vinsamlegast lestu yfir listann fyrir neðan áður en þú svarar) ?

- ❑ Já
- ❑ Nei (ef svarað er neitandi farðu beint að spurningu nr 16)

15) Hve miklum tíma eyðir þú í eftirfarandi athafnir (miðað við síðasta ár)?

Tíðni hverrar athafnar að meðaltali í viku merkja einnig við hægra megin (<3 / 3-6 ef árstíðabundið, annars >6)													
	Aldrei	1-4	5-19	20-59	<1	1-1,5	2-3	4-6	7-10	>11	<3	3-6	>6
		mín/ viku	mín/ viku	mín/ viku	klst/ viku	klst/ viku	klst/ viku	klst/ viku	klst/ viku	klst/ viku	mán/ári	mán/ári	mán/ári
Gönguferð / Spila golf													
Hlaup / hægt skokk													
Hratt hlaup													
Hjólreiðar													
Sund													
Tennis, badminton, veggtennis													
Körfubolti, blak, handbolti													
Dans, þolfimi													
Ganga upp brekku/fjall Fjallaklifur													
Leikfimi													
Garðvinna													
Skíði, skauta													
Júdó, karate, aðrar bardagaíþróttir													
Siglingar													
Hjólað á kyrrstæðu hjóli (t.d í líkamsræktarstöð)													
Knattspyrna													
Annað (vinsamlegast takið fram hvað)													

16) Hve miklum tíma eyddir þú um það bil daglega við eftirfarandi athafnir síðasta árið annars vegar fyrir dæmigerðan virkan dag og hins vegar fyrir dæmigerðan helgardag.
(Ef klukkustundir merkja þá við hve margar klukkustundir per dag, annars merkja við með x)?

	Dæmigerður virkur dagur				Dæmigerður helgardagur			
	Aldrei	< 30 mínútur	30 – 60 mínútur	klst / dag	Aldrei	< 30 mínútur	30 – 60 mínútur	klst / dag
Að horfa á sjónvarp / Myndband / DVD								
Sitja fyrir framan tölvu / læra / vinna								
Aka								
Sitja (heildartími yfir daginn, leggja saman)								
Sofa (nótt)								
Blunda / Leggja þig								
Sólbað (yfir sumartímann)								
Sólbað(yfir vetrartímann)								
Ganga til og frá vinnu/skóla								
Standa við vinnu								
Heimilisstörf								
Aðrar erfiðari athafnir en að standa við vinnu								

-Takk fyrir

Appendix VIII – Menu: An example in diet group 3

MATSEÐILL, 1500 KCAL – Yfirlit yfir daginn

Morgunverður og morgunbiti:
1 sk.[1] morgunkorn
1 sk. léttmjólk *eða* annað skv. skiptilista
1 sk. brauð með 6 g Létt og laggott með ólífuolíu (L&L grænt)
1 sk. af áleggi I
Grænmeti að minnsta kosti 50 g
1 sk. ávöxtur
1 tafla Ein á dag án A- og D-vítamína *eða* 1 tafla Vítamínus

Hádegisverður:
1 sk. léttjógúrt *eða* annað skv. skiptilista
2 sk. brauð með L&L
1 sk. af áleggi I
1 sk. af áleggi II
Grænmeti að minnsta kosti 150 g

eða rétti úr flokknum "ýmislegt" skv. blaði um kvöldverði.

Eða
Morgunverður:
2 sk. morgunkorn
1 sk. léttmjólk
Grænmeti a.m.k. 50 g
1 sk. ávöxtur
1 Vítamíntafla

Hádegisverður:
1 sk. léttjógúrt
2 sk. brauð m/L&L
2 sk. álegg I
1 sk. álegg II
Grænmeti a.m.k. 150 g
6 g L&L

Síðdegisbiti:
1 sk. brauð með L&L
1 sk. af áleggi I
1 sk. ávöxtur

Kvöldverður:
150 g þorskur
150 g kartöflur *eða* annað skv. skiptilista
Grænmeti a.m.k. 150 g
6 g Olíuedikssósa
12 g ólífuolía *eða* annað skv. skiptilista

eða aðrir réttir samkvæmt blaði um kvöldverði 2svar í viku.

Drykkir:
*vatn að vild
*kolsýrt vatn að vild
*svart og sykurlaust kaffi og te að vild

Kvöldbiti:
1 sk. ávöxtur
½ sk. brauð með L&L
½ sk. álegg I

---

[1] sk. = skammtur (sjá skiptilista).

ATH:

- Það má ekki borða skelfisk (t.d. rækjur, humar, krækling o.þ.h.) eða annan fisk (harðfisk, túnfisk, síld, ýsu o.þ.h.) nema þorsk í ákveðnu magni meðan á rannsókn stendur. Einnig má ekki borða neinar fisksósur eins og t.d. ostrusósu.
- Einnig má ekki taka inn lýsi, D-vítamín eða kalk.
- Notið eingöngu ólífuolíu við matreiðslu og Létt og laggott með ólífuolíu (L&L í grænum umbúðum) sem viðbit á brauð.
- Má ekki nota rapsolíu (Canola oil), sojaolíu og Ísío4 matarolíu.

Appendix IX – Exchange list (an example)

AVS

SEAFOOD plus

SKIPTILISTI
fyrir 1400-1600 kcal matseðill

Álegg I á 1 brauðsneið

Magurt	*g*	*Skammtur*
Ostur 26%	16	2 skornar sn
Ostur 17%	16	2 skornar sn
Smurostur 18%	15	1 msk
Rjómaostur	15	1 msk
Kotasæla	30	2 msk
Kotasæla m/ananas	30	2 msk
Fetaostur	15	5 stk
Mozzarella kúlur	15	
Mysingur	15	1 msk
Mjúkur mysuostur	15	1 msk
Egg	30	½ egg
Lifrarkæfa	15	1 msk
Parmesanostur	10	

Álegg II á 1 brauðsneið

Magurt kjötálegg/skinka	18	1 sneið
Smurostur, létt 6%	15	1 msk
Létt Kotasæla	30	2 msk

Brauð

Tegund	*g*	*Skammtur*
Rúgbrauð	40	1 sneið
Heilhveitibrauð	35	1 sneið
Hrökkbrauð	25	2 stk
Finn Crisp	25	4 stk
Bruður	25	2 stk
Kringla	23	1/3 stk
Beyglur/pítur	35	¼ stk / ½ stk
Tekex/vatnskex	18	2½ stk
Vatnskex, litlar	18	5 stk

Morgunkorn

Cheerios	22	1¾ dl
Kornflex	22	1¾ dl
All bran	30	1 dl
Special K	22	1½ dl
Fitness	22	> 1 dl
Weetabix	24	1 1/3 kaka
Hunangscheerios	22	1¾ dl
Múslí	22	< ½ dl
Granóla/Morgungull/Axa múslí	17	2 msk
Haframjöl	24	4 msk
Hafragrautur	200	2 dl

Ávextir (án hýðis)/safi

Tegund	*g*	*Skammtur*
Appelsína	130	½ - 1
Banani	80	1 lítill
Epli	140	½ - 1
Kíví	120	1 stórt
Melóna, hunangs	170	2½ dl
Melóna, vatns	220	3 dl
Pera	140	½ - 1
Vínber	90	13 stk
Plómur	170	2-4 stk
Ferskjur	150	1 stór
Nektarínur	140	1 stór
Mangó	100	1½ dl
Ávaxtasafar ekki oftar en einu sinni á dag		
Ávaxtasafi	150	1½ dl
Skyr.is drykkur	165	½ flaska
Léttvín/bjór, mest 2 skammtar einu sinni í viku		
Léttvín	100	1 dl
Bjór	165	½ flaska/lítil dós

Mjólk/Jógúrt/Súpa

Tegund	*g*	*Skammtur*
Léttmjólk	175	1¾ dl
Létt súrmjólk ósæt	175	1¾ dl
Létt ab-mjólk ósæt	175	1¾ dl
Létt súrmjólk epla/peru	180	1¾ dl
Léttjógúrt ferskjur/ástaraldin	180	1 dolla
Létt jógúrt jarðarberjum	180	1 dolla
Létt jógúrt vanillu	180	1 dolla
Létt jógúrt kíví/perum	180	1 dolla
Kraftsúpa/grænmetissúpa	200	1 bréf/2 dl
Tær Kraftsúpa + 1 sk L&L m/ólífuolíu	200 6	2 dl > 1 tsk

Hrísgrjón/kartöflur/pasta

Tegund	*g*
Soðnar kartöflur	150
Soðin hrísgrjón	85
Soðið pasta	100
Brauð	45

Sósur með kvöldverði		
Tegund	***g***	***Skammtur***
Edikssósa (salatdressing), 65%	6	1 ½ tsk
Olía, ólífuolía 100%	4	1 tsk
Viðbit, L&L grænt, 40%, 1 tsk=5 g	9	< 2 tsk
Pestó	7	1½ tsk
Sósur (vatn/léttmjólk/jógúrt) 1-5%	60	> ½ dl
Sýrður rjómi 10%/Sósur 6-14%	30	2 msk
Kaldar sósur 15-24%	16	1 msk
Kaldar sósur, 25-39%	11	> 2 tsk
Kaldar sósur, 40-59%	7	1½ tsk
Kaldar sósur, 60-80%	5	1 tsk
Majónes, smjör 80%	5	1 tsk
Smjör, 80%	5	1 tsk
Hnetusmjör, 1 tsk = 5 g	6	> 1 tsk

Viðbit á brauð		
Tegund	***g***	***Skammtur***
Viðbit, L&L grænt, 40%, 1 tsk=5 g	6	> 1 tsk
Edikssósa (salatdressing), 65%	4	1 tsk
Olía, ólífuolía 100%	3	½ tsk
Pestó	5	1 tsk
Til tilbreytingar af og til		
Hnetusmjör, 1 tsk = 5 g	4	< 1 tsk
Sýrður rjómi 10%/Sósur 6-14%	20	1 msk+1 tsk
Kaldar sósur 15-24%	10	2 tsk
Kaldar sósur, 25-39%	7	1½ tsk
Kaldar sósur, 40-59%	5	1 tsk
Kaldar sósur, 60-80%	3	½ tsk
Majónes, smjör 80%	3	½ tsk
Heit sósa	45	< ½ dl

Afbrigði:

- 1 skammtur morgunkorn með 1 skammti af léttmjólk samsvarar 1 brauðsneið með viðbiti og áleggi I.
- 1 skammtur léttmjólk samvarar ½ brauðsneið með viðbiti og áleggi I.
- Má stundum fá sér 1 skammt ávöxt og 2 skammta af osti (álegg I) í staðinn fyrir 1 skammt af brauði með viðbiti og áleggi I.
- Má stundum fá sér 15 g af grófu kexi (t.d. hafrakex, heilhveitikex) í staðinn fyrir ½ skammt af brauði með viðbiti og áleggi I.
- 1 tortillas hveitikaka samsvarar 1½ brauðsneið.
- Má fá sér mest 2svar í viku 35 g af léttu poppkorni í staðinn fyrir 1 brauðsneið með viðbiti og áleggi

ATH:

- Það má ekki borða skelfisk (t.d. rækjur, humar, krækling o.þ.h.) eða annan fisk (harðfisk, túnfisk, síld, ýsu o.þ.h.) nema þorsk í ákveðnu magni meðan á rannsókn stendur. Einnig má ekki borða neinar fisksósur eins og t.d. ostrusósu.
- Einnig má ekki taka inn lýsi, D-vítamín eða kalk.
- Notið eingöngu ólífuolíu við matreiðslu og Létt og laggott með ólífuolíu (L&L í grænum umbúðum) sem viðbit á brauð.
- Má ekki nota rapsolíu (Canola oil), sojaolíu og Ísío4 matarolíu.

Appendix X – Result tables

Table A1: *Endpoint characteristics; anthropometric measurements and blood parameters. Values are unadjusted, shown as means ± s.d.*

	Group 1 - Control		**Group 2 - Cod 3x /w**		**Group 3 - Cod 5x /w**		**All subjects**		**P-value**[1]		**post-hoc**	
	Men (n=14)	**Women** (n=16)	**Men** (n=8)	**Women** (n=19)	**Men** (n=20)	**Women** (n=23)	**Men** (n=42)	**Women** (n=58)	**Men**	**Women**	**Men**	**Women**
Anthropometric measurements:												
Body weight (kg)	91.7 ± 5.3	78.8 ± 6.7	93.5 ± 8.7	82.5 ± 8.7	95.4 ± 10.5	79.7 ± 10.0	93.8 ± 8.7	80.4 ± 8.8	0.479	0.423		
BMI (kg/m^2)	28.5 ± 1.9	28.3 ± 1.6	28.7 ± 0.9	28.6 ± 1.6	28.9 ± 1.6	27.8 ± 1.4	28.7 ± 1.6	28.2 ± 1.5	0.771	0.194		
Waist circumference (cm)	96.3 ± 5.0	88.7 ± 5.2	94.8 ± 5.2	89.4 ± 6.5	97.7 ± 7.6	88.2 ± 6.0	96.7 ± 6.4	88.7 ± 5.9	0.537	0.802		
Fat mass (kg)	20.2 ± 3.3	28.6 ± 4.1	20.1 ± 4.3	29.6 ± 5.0	25.1 ± 5.0	29.9 ± 5.7	22.5 ± 5.0	29.4 ± 5.0	0.003	0.716	3 > 1, 2	
Fat-free mass (kg)	71.5 ± 4.4	50.2 ± 3.8	73.4 ± 5.3	52.9 ± 5.0	70.3 ± 6.6	50.0 ± 6.2	71.3 ± 5.7	51.0 ± 5.3	0.221	0.160		
Body fat percentage (%)	22.0 ± 3.0	36.2 ± 3.0	21.3 ± 3.1	35.7 ± 3.2	26.2 ± 2.9	37.3 ± 4.0	23.8 ± 3.7	36.5 ± 3.5	<0.001	0.343	3 > 2, 1	
Blood parameters:												
Total cholesterol (mmol/L)	5.0 ± 0.9	4.9 ± 0.6	4.5 ± 0.8	4.7 ± 0.6	4.6 ± 1.0	4.6 ± 1.1	4.7 ± 0.9	4.7 ± 0.8	0.256	0.533		
HDL cholesterol (mmol/L)	1.2 ± 0.2	1.4 ± 0.2	1.1 ± 0.3	1.3 ± 0.3	0.9 ± 0.2	1.3 ± 0.3	1.1 ± 0.3	1.3 ± 0.3	0.006	0.360	1 > 3	
LDL cholesterol (mmol/L)	3.2 ± 0.9	3.0 ± 0.5	2.9 ± 0.7	3.0 ± 0.6	3.0 ± 0.7	2.9 ± 1.1	3.1 ± 0.8	3.0 ± 0.8	0.681	0.892		
Triglyceride (mmol/L)	1.3 ± 0.6	1.1 ± 0.6	1.0 ± 0.5	0.9 ± 0.3	1.5 ± 1.2	1.1 ± 0.5	1.3 ± 0.9	1.0 ± 0.5	0.532	0.847		
Glucose (mmol/L)	4.8 ± 0.4	4.6 ± 0.4	4.4 ± 0.3	4.6 ± 0.4	4.8 ± 0.5	4.5 ± 0.4	4.7 ± 0.5	4.6 ± 0.4	0.075	0.308		
Insulin (mU/L)	8.8 ± 5.2	9.2 ± 4.9	7.1 ± 3.1	9.7 ± 4.2	8.8 ± 6.1	5.7 ± 3.2	8.5 ± 5.3	7.9 ± 4.4	0.736	0.004		3 < 2, 1
Systolic BP (mmHg)	128.4 ± 6.6	119.8 ± 5.2	129.4 ± 7.2	119.9 ± 9.2	136.8 ± 10.3	124.3 ± 9.2	132.6 ± 9.4	121.6 ± 8.5	0.017	0.138	3 > 1, 2	
Diastolic BP (mmHg)	69.5 ± 6.3	70.6 ± 6.3	71.1 ± 5.2	74.1 ± 5.3	69.8 ± 5.7	67.0 ± 5.0	70.0 ± 5.7	70.3 ± 6.2	0.811	<0.001		3 < 2, 1

[1] One-way ANOVA / Kruskal-Wallis H-test

Table A2: *Correlations[1] between BMI at baseline and other parameters at baseline.*

BMI at baseline correlations:	**All subjects**		**Men**		**Women**		**Group 1**		**Group 2**		**Group 3**	
	r-value[1]	**P-value**	**r-value**	**P-value**	**r-value**	**P-value**	**r-value**	**P-value**	**r-value**	**P-value**	**r-value**	**P-value**
Body weight	0.514*	*< 0.001*	0.517*	*< 0.001*	0.582*	*< 0.001*	0.530*	*0.001*	0.450*	*0.007*	0.537*	*< 0.001*
Waist circumference	0.555*	*< 0.001*	0.605*	*< 0.001*	0.563*	*< 0.001*	0.597*	*< 0.001*	0.378*	*0.025*	0.629*	*< 0.001*
Fat mass	0.443*	*< 0.001*	0.648*	*< 0.001*	0.702*	*< 0.001*	0.455*	*0.006*	0.344(*)	*0.050*	0.475*	*< 0.001*
Fat-free mass	0.272*	*0.002*	0.279	*0.055*	0.264*	*< 0.001*	0.290	*0.091*	0.184	*0.305*	0.336*	*0.013*
Body fat percentage	0.110	*0.229*	0.574*	*< 0.001*	0.644*	*< 0.001*	0.126	*0.470*	0.124	*0.493*	0.035	*0.799*
Total cholesterol	0.054	*0.549*	0.174	*0.230*	0.070	*0.548*	-0.146	*0.402*	0.097	*0.581*	0.185	*0.173*
HDL cholesterol	-0.186*	*0.037*	-0.159	*0.275*	-0.117	*0.309*	-0.238	*0.169*	-0.009	*0.961*	-0.233	*0.084*
LDL cholesterol	0.034	*0.710*	0.005	*0.972*	0.025	*0.828*	-0.235	*0.174*	0.098	*0.576*	0.182	*0.183*
Triglyceride	0.183*	*0.040*	0.130	*0.371*	0.253*	*0.027*	0.359*	*0.034*	-0.023	*0.894*	0.231	*0.087*
Glucose	0.091	*0.311*	0.210	*0.147*	-0.043	*0.709*	0.319(*)	*0.062*	-0.272	*0.114*	0.171	*0.207*
Insulin	0.006	*0.943*	-0.123	*0.402*	0.050	*0.668*	0.150	*0.391*	-0.263	*0.127*	0.152	*0.265*
Systolic BP	0.165	*0.065*	0.091	*0.532*	0.114	*0.323*	0.222	*0.200*	0.096	*0.584*	0.166	*0.220*
Diastolic BP	0.111	*0.216*	-0.082	*0.573*	0.226*	*0.048*	0.066	*0.707*	0.075	*0.670*	0.167	*0.218*
Energy intake	0.150	*0.096*	0.136	*0.352*	0.072	*0.534*	0.273	*0.118*	0.327(*)	*0.056*	0.010	*0.944*
Fat intake	0.163	*0.069*	0.175	*0.230*	0.136	*0.241*	0.339(*)	*0.050*	0.319(*)	*0.062*	0.039	*0.778*
Added sugar intake	0.057	*0.526*	0.143	*0.325*	-0.073	*0.531*	0.078	*0.662*	0.114	*0.514*	0.040	*0.770*

[1] Pearson correlation / Spearman correlation coefficient

* Significant r-value (P > 0.05)