FISH AND MEAT INTAKE DURING PREGNANCY

The effects on metabolism, bone and body composition

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Fish and meat intake during pregnancy

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“The doctor of the future will no longer treat the human frame with drugs, but rather will cure and prevent disease with nutrition.”

Thomas Edison

ABSTRACT

Fetal programming occurs when nutrients affect the DNA during fetal development and alters the fetus' genetic make-up. In connection with increasing obesity prevalence focus has been on dietary fats and fatty acids. Two important sources to these nutrients are red meat and fatty fish.

The aim of this thesis was to study the effects of fish and meat intake during pregnancy on maternal health and on the offspring's metabolic health, bones and body composition.

Herring or beef was provided to mice (C57Bl/6) during gestation and lactation. Later, after weaning, half of the offspring were crossed over to the other diet and followed to adult age. Weight gain, body composition, insulin sensitivity, tissue fatty acids and plasma triglycerides and cholesterol was investigated. In a sample of the animals bone mineral density, bone mineral content and bone strength was analyzed. It was found that maternal herring diet counteracted adiposity and postweaning herring diet increased insulin sensitivity and reduced plasma triglycerides and cholesterol at an early adult age, but the differences were no longer present when the tests were repeated later in life. The postweaning herring diet also gave a higher n-6/n-3 ratio in the tissues collected at adult age. Maternal beef intake also led to increased bone mineral density and content at an early age, but later the switch to a postweaning herring diet gave better bone strength (in tibia) and mineral density and content.

In a longitudinal randomized intervention study in pregnant women, effects of intake of fish and meat and gestational weight gain, fat mass and fat free mass and serum phospholipid fatty acids were investigated. Normal weight women were recruited during the 1st trimester and advised on fish intake (three portions per week), reduced sugar intake (<10 E %), 500 g vegetables and fruits per day and increase energy intake by 350 kcal in the 2nd trimester and by 500 kcal in the 3rd trimester. Meat intake during early pregnancy was associated with fat free mass gain during pregnancy. Serum phospholipid arachidonic acid was associated with meat intake and eicosapentaenoic acid and docosahexaenoic acid was associated with fish intake. The intervention group increased their fish intake.

As shown in this thesis, meat and fish intake may affect body composition both in the mother and in the offspring and also bone health in the offspring.

Meat and fish intake has beneficial effects on different tissues and health outcomes depending on time of intake. This thesis has added to the knowledge on the effect maternal meat and fish intake may have on maternal body composition and offspring metabolic health, bone health and body composition.

Keywords: Fatty fish, red meat, body composition, maternal diet, postweaning diet, metabolism, bone health, herring, beef

POPULÄRVETENSKAPELIG SAMMANFATTNING

Nära hälften av jordens vuxna befolkning är överviktiga eller lider av fetma. Fetma medför en ökad risk för att utveckla sjukdomar som exempelvis diabetes och hjärt-kärlsjukdom. Sjukdomar som leder till stort individuellt lidande samt höga kostnader för samhället. Risken att utveckla sjukdomar har av flera forskare blivit föreslagit att påverkas av miljöfaktorer under fosterlivet. Gravida kvinnors kost och dess effekt på barnens hälsa i vuxen ålder blir därmed ett viktigt forskningsområde.

Kostintaget av animalisk föda har ökat. Många forskare har undersökt skillnader i hälsoeffekter av intag av fet fisk och rött kött. Man vet dock inte hur mycket intaget av dessa födoämnen under graviditet påverkar individens hälsa båda tidigt och senare i livet.

Syftet med denna avhandling är att undersöka hur fisk- och köttintag hos gravida kvinnor påverkar deras och barnens hälsa. Studier båda på djur och människa ingår i denna avhandling.

Våra resultat visar att möss som äter nötkött under graviditeten och amningen får en avkomma med en högre andel kroppsfett jämfört med de mössen som får äta sill. De får också en högre bentäthet och benstyrka. Avkomman som äter nötkött efter avvänjning har en sämre metabol profil avseende insulin, fria fettsyror och kolesterol i blodet än de som äter sill. De får även en lägre benmassa och ett svagare skelett.

När vi undersöker fisk-och köttintag bland gravida kvinnor finner vi att de kvinnor som får kostråd av dietist ökar sitt fiskintag. Kvinnor som däremot äter mycket kött tidigt under graviditeten har en tendens att gå upp mer i vikt i jämförelse med de som har ett lägre köttintag. Denna viktökning tillskrivs en ökad andel muskelmassa men inte en ökad fettmassa.

Mer forskning kring intag av enskilda livsmedel och födoämnen behövs för att gravida kvinnor ska kunna få praktiska råd och på så sätt kunna påverka sin egen men även kommande generationers hälsa.

LIST OF PAPERS

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

I. **A maternal diet of fatty fish reduces body fat of offspring compared with a maternal diet of beef and a post-weaning diet of fish improves insulin sensitivity and lipid profile in adult C57BL/6 male mice** Aysha Hussain, Intawat Nookaew, Sakda Khoomrung, Louise Andersson, Ingrid Larsson, Lena Hulthén L, Nina Jansson, Robert Jakubowicz, Staffan Nilsson, Anne-Sofie Sandberg, Jens Nielsen and Agneta Holmäng. *Acta Physiol 2013, 209, 220-234*

II. **Maternal beef and postweaning herring diets increase bone mineral density and strength in mouse offspring** Aysha Hussain, Hanna Olausson, Staffan Nilsson, Intawat Nookaew, Sakda Khoomrung, Louise Andersson, Antti Koskela, Juha Tuukkanen, Claes Ohlsson and Agneta Holmäng. E-pub ahead of print 24th of October 2013 *Experimental Biology and Medicine 2011; 0: 1–8. DOI: 10.1177/1535370213506436*

III. **A longitudinal dietary intervention study during pregnancy: effects on fish and meat intake, gestational weight gain, body composition changes, and serum fatty acids** Marja Bosaeus[A], Aysha Hussain[A], Therese Karlsson, Louise Andersson, Lena Hulthén, Cecilia Svelander, Ann-Sofie Sandberg, Ingrid Larsson, Lars Ellegård, Agneta Holmäng. [A] Co-first authors. *Manuscript*

CONTENT

ABBREVIATIONS

aBMD	Areal bone mineral density
ADP	Air displacement plethysmography
ALA	α-linoleic acid
ARA	Arachidonic Acid, 20:4n-6
BAT	Brown adipose tissue
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
DEXA	Dual-energy X-ray absorptiometry
DHA	Docosahexaenoic Acid, 22:6n-3
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic Acid, 20:5n-3
FAs	Fatty Acids
FAME	Fatty acid Methyl Esters
FFQ	Food frequency questionnaire
FM	Fat mass
FFM	Fat free mass
GC-MS	Gas chromatography- mass spectrometry
GDM	Gestational diabetes mellitus
GWG	Gestational weight gain
iBAT	Interscapular brown adipose tissue
IGF-1	Insulin-like Growth Factor 1

LA	Linoleic Acid
LC n-3 PUFAs	Long-chain n-3 Polyunsaturated Fatty Acids
LGA	Large for gestational age
MUFA	Monounsaturated Fatty Acids
QUICKI	Quantitative Insulin Sensitivity Check Index
n-3 PUFAs	Omega-3 polyunsaturated fatty acids
n-6 PUFAs	Omega- 6 polyunsaturated fatty acids
PPARs	Peroxisome proliferator-activated receptors
pQCT	Peripheral Quantitative Computed Tomography
PUFA	Polyunsaturated Fatty Acids
SFA	Saturated Fatty Acids
SGA	Small for gestational age
vBMD	Volumetric bone mineral density
WAT	White adipose tissue

INTRODUCTION

The development of an individual starts in the mother's womb. The womb functions as a house that the fetus is not to leave until it is ready to face the outside world. While in the uterus, it is being prepared for this adventure by receiving signals from the world through its mother. The mother's blood vessels work like electricity, water pipes, TV-signals and Internet providing the fetus with information on the outside environment. The baby receives updates about the mother's situation and will in this way develop properties that will be advantageous for life. In fact, some of the genetic make-up of the child will be decided during this crucial period. The process whereby, for instance, nutrients change the expression of genes is called fetal programming.

It has been hypothesized that children born of obese and underweight mothers have an increased risk of developing adverse metabolic profiles (Barker, 1998, Catalano, 2003). Several non-communicable diseases (e.g. type 2 diabetes, heart and coronary diseases, cancer and osteoporosis) have been proposed to be partly caused by maternal health status during pregnancy (Gluckman and Mark Hanson, 2006).

The obesity trend has grown along with an increased intake of fats, sugars and animal protein (Drewnowski and Popkin, 1997, Popkin, 2001). The metabolic syndrome is a complex of several conditions in which low-grade inflammation may be involved (Warnberg and Marcos, 2008). This inflammatory state is believed to be rendered by factors such as different dietary fats. Also pregnancy is considered to be an inflammatory state(Sargent et al., 2006). Polyunsaturated fatty acids (PUFAs) especially have an anti-inflammatory effect and as such they may play an important part in reducing the risk of various diseases caused by a low state of inflammation (Robinson et al., 2007). n-3 and n-6 are important dietary fatty acids, n-3 being less inflammation-inducing than the n-6 (Calder, 2005). Important sources to fatty acids in the diet are fatty fish and meat.

In this thesis the link between fish and meat intake during pregnancy and the effect it has on offspring metabolism, adiposity and bone health and on maternal body composition is investigated.

BACKGROUND

PREGNANCY

Pregnancy is usually separated into different parts, either in two; embryonic and fetal (Gluckman and Hanson, 2004a) or into trimesters consisting of three months each (King, 2000), within which various developmental changes occur, both in the fetus and in the pregnant woman.

PHYSIOLOGICAL CHANGES

Increased blood volume, altered respiration and elevated renal blood flow are all part of normal physiological changes during pregnancy (Picciano, 2003, Weissgerber and Wolfe, 2006). It is an anabolic state, involving the development of a new endocrine organ, the placenta which secretes hormones that affect nutrient metabolism (King, 2000). Adjustments in nutrient metabolism are caused by the aforementioned hormonal changes, fetal demands and maternal nutrient supply (supported by altered maternal dietary habits). These metabolic adaptations take place to ensure fetal growth and development, provide the fetus with energy and substrates and in order to meet the mother's physiological needs to storage sufficient energy for the pregnancy, the labor and lactation period (Hadden and McLaughlin, 2009). In the first half of pregnancy the body is preparing for the demands of fetal growth that will take place later in pregnancy (King, 2000).

Only a couple of months after conception serum triglyceride levels increase (King, 2000), also other serum lipids increase, making pregnancy a hyperlipidemic period (Hadden and McLaughlin, 2009). In the later parts of pregnancy insulin sensitivity decreases, especially during the later parts of pregnancy (Catalano et al., 1991, Catalano, 2003). For some women the metabolic changes are of unfortunate character and lead to gestational diabetes (GDM), defined as glucose intolerance that starts during pregnancy (Persson, 2009), which increases the risk of later type 2 diabetes (Ekelund et al., 2010). Type 2 diabetes starts with a lower tolerance for glucose caused by reduced insulin sensitivity, meaning reduced response to insulin by the skeletal muscles which leads to a reduced uptake of glucose (Petersen and Shulman, 2006).

DIETARY CHANGES

Along with the physiological changes many pregnant women experience altered dietary habits caused by cravings, increased or decreased appetite, changed preferences, social pressure, and trends in the community, health beliefs, traditions and recommendations (Nobmann and Adams, 1970, Anderson et al., 1993, Anderson, 2001, Bayley et al., 2002). Also women may allow themselves to increase the intake of certain foods, such as sweets, snacks and other foods, considered unhealthy while being nonpregnant (Andersen et al., 2003). Energy requirements increase minimally in the 1st trimester and later with 350 kcal in the 2nd trimester and 500 in the 3rd (Butte et al., 2004).

WEIGHT CHANGES

In the beginning of pregnancy it is normal for maternal fat stores to increase (Hytten and Leitch, 1971, Lain and Catalano, 2007). There is a hypertrophy of fat cells accompanied with increased fat storage during the first two trimesters of pregnancy (Blackburn and Loper, 1992). The women gain weight, caused by fluid, fetus, developing mammary glands, increased uterus and deposition of fat mass (Hytten and Leitch, 1971). Adipose tissue gain is supposed to cover fetal and maternal needs later in gestation and lactation (Lederman et al., 1997, Nelson et al., 2010). Gestational weight gain (GWG) is affected by parity, weight before pregnancy, height, age, ethnicity, smoking and duration of gestation (Olafsdottir et al., 2006, Gaillard et al., 2013).

The Institute of Medicine (IOM) in the US have developed weight gain recommendations for pregnancy based on body mass index (BMI) at pregnancy start (Institute of Medicine. Subcommittee on Nutritional Status and Weight Gain during Pregnancy, 2009). These recommendations aim to reduce the risk of developing pregnancy complications such as GDM, hypertension, pre-eclampsia, as well as accelerated fetal growth.

An excess gestational weight gain (GWG) leads to higher risk of weigh retention after birth (Scholl et al., 1995) and may as such contribute to overweight and obesity. Overweight and obesity is often based on calculations of body mass index (BMI). Body mass index may be calculated in order to categorize people into underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2) and obesity (≥30 kg/m^2) of different degrees, first developed by Adolphe Quetelet and later named the body mass index by Ancel Keys (Eknoyan, 2008). Body mass index has limitations, being based on weight which is comprised of both fat and fat free mass (Garn et al., 1986, Frankenfield et al., 2001, Prentice and Jebb, 2001). Since obesity is defined as an excess of fat mass measurements of fat mass (FM) and fat free mass (FFM) are warranted (Coe and Naggert, 2001, Prentice and Jebb, 2001, Lamb et al., 2011, Poston, 2012). Also fat distribution is related to health and disease, visceral fat being more hormonally active and subcutaneous fat being less linked to adverse metabolic health (Wajchenberg, 2000, Wagenknecht et al., 2003). Body mass index is especially a poor measure during pregnancy because of the large changes in body composition during this period (Lindsay et al., 1997).

NUTRITION AND FETAL PROGRAMMING

As the prevalence of obesity and type 2 diabetes and other non-communicable diseases increase, reasons for these conditions are being explored. During the last decades researchers have become interested in how maternal health and nutrition can affect the offspring's future health.

It has been hypothesized that children born by obese and underweight mothers are at higher risk of developing an adverse metabolic profile (Barker, 1998, Catalano et al., 2003, Hanson and Gluckman, 2008). Forsdahl, a Norwegian researcher wrote about this topic during the 70's (Forsdahl, 1977). Also associations between data from men born by women pregnant during the Dutch famine (winter 1944-45) showed that they had greater BMI in adulthood (Gunther et al., 2007). In the 1990's the Barker's hypothesis was proposed (Barker, 1990, 1998); reduced fetal growth caused by intrauterine conditions is linked to chronic diseases later in life. This thought has later been elaborated upon and now it is believed that maternal health during pregnancy could affect the fetus' genes and thereby have an impact on the offspring's future health, especially their risk of developing non-communicable diseases (e.g. type 2 diabetes, heart and coronary diseases, cancer and osteoporosis) later in life has been suggested (Gluckman and Hanson, 2004b). This has been supported both by animal experiments and by studies in humans. Fetal programming might be caused by nutrients affecting the fetus' genetic make-up by DNA-methylation, histon modification and non-coding RNAs (Lillycrop and Burdge, 2012).

An individual's metabolism, bones and body composition might be affected by intrauterine programming. Body tissues and organs grow at different periods called critical periods (McCance and Widdowson, 1974), within which nutrition and epigenetics potentially could alter the characteristics of the fetus (Lucas, 1998). The fetus' adipose tissue can be detected already in gestation week 14 to 16 (Poissonnet et al., 1983), and in the 3rd trimester the fetus' fat depots are distinguishable (Poissonnet et al., 1983). Some studies suggest a direct relation between maternal BMI and the newborn's fat mass and obesity risk later in life, but also GWG and the offspring's BMI from infancy through adulthood (Hauner et al., 2013). Gestational weight gain is associated with childhood obesity (Moreira et al., 2007). A review shows that it is not the birth weight which is of greatest importance to later disease risk, but rather the fat mass of the infant (Kunz and King, 2007), and obesity during pregnancy is associated to higher birth weights and fat mass in the infant (Freeman, 2010). Maternal blood glucose affects fetal growth, insulin and adiposity (Metzger et al., 2008). Children born large for gestational age (LGA) are at an increased risk of developing metabolic syndrome in childhood (Boney et al., 2005). Even children with normal weight, born by mothers with gestational diabetes, have an increased fat mass as compared to fat free mass (Catalano et al., 2003). This may increase these children's risk of developing obesity later in life in is by Catalano described as "intergenerational obesity"(Catalano, 2003).

Fetal programming has long been linked to adverse metabolic health and cardiovascular diseases, but both animal and human studies also report that maternal diet during gestation have long-term effects on bone health in the offspring, animal studies, mostly in rodents show that maternal food intake also affects bone health of offspring in adult age (Lanham et al., 2010). It has been hypothesized that the peak bone mass is partly programmed in utero (Lanham et al., 2010). In humans long bones develop during the 2nd trimester (Cooper et al., 2000). The risk of poor bone health might therefore be modified by influences during early life. This is supported by four types of studies; bone mineral measurements from adults with detailed birth records, physiological studies on endocrine systems and age-related bone loss, studies on nutrition and pregnancy and bone mass in the offspring, studies relating childhood growth rates to later hip fracture risk (Cooper et al., 2000). A study on intrauterine programming and adult body composition found positive associations between birth weight and bone and lean mass at the age of 70-75 years (Gale et al., 2001), which supports that fetal life may affect later bone health. Protein deprivation during gestation in rats resulted in reduced bone mass (Bourrin et al., 2000, Mehta et al., 2002). Similar findings have been published from a study in rodents fed high-fat diets on skeletal growth (Liang et al., 2009).

FATTY ACIDS AND FETAL PROGRAMMING

The global obesity trend has grown along with an increased intake of fats, sugars and animal protein (Drewnowski and Popkin, 1997, Popkin, 2001). The metabolic syndrome is a complex of several conditions (e.g. reduced glucose tolerance, increased blood pressure, overweight and dyslipedemia) in which low-grade inflammation may be involved (Warnberg and Marcos, 2008). During pregnancy the body is in an increased inflammatory state (Sargent et al., 2006). Fatty acids have been a target of extensive research to combat the incidence of non-communicable diseases, which are associated with a low-grade inflammation (Robinson et al., 2007). Polyunsaturated fatty acids (PUFAs) are known to have an anti-inflammatory effect on many tissues in the body, and as such they are believed to play an important part in reducing the risk of various diseases associated with low state of inflammation, such as the metabolic syndrome (Robinson et al., 2007). n-3 and n-6 (found in fatty fish, such as herring and salmon) are important dietary fatty acids, n-3 being more anti-inflammatory than the n-6 (Calder, 2005, Dahl et al., 2006, Wall et al., 2010). Recommendations for increasing the intake of n-3 FAs come from their potential beneficial effect in cardiac and coronary diseases (Kris-Etherton et al., 2002). Certain fatty acids have also been linked to obesity and reduced insulin sensitivity, especially trans-fatty acids (Bray et al., 2002). The benefits of n-3 PUFAs is associated with the synthesis of eicosanoids (Abbas, 2009) and their ability to interact with nuclear receptor proteins that bind to DNA and thereby modulate transcription of regulatory genes involved in glucose and lipid metabolism (Benatti et al., 2004).

The fatty acid content of our diets have been pushed towards higher n-6/n-3 ratios, mostly due to increased consumption of vegetable oils, and this can also be seen in the composition of breast milk (Ailhaud et al., 2006).

Fatty acids may also have an effect on the fetus, based on a female's gestational intake. High-fat diets given to rodents during gestation resulted in increased body weight and fat mass gain in the offspring (Taylor and Poston, 2007). Also the type of fat might have an impact on the offspring's risk of obesity (Ailhaud et al., 2006). Arachidonic acid (ARA) has been shown to promote differentiation of adipose cells (Massiera et al., 2003, Ailhaud et al., 2006), whereas docosahexaenoic acid (DHA) and eicosapenaenoic acid (EPA) seem to counteract this process (Massiera et al., 2003, Azain, 2004, Madsen et al., 2005, Ailhaud et al., 2006). A review on fetal programming of fatty acid supply during gestation and offspring body composition concluded that it is to date insufficient evidence on effect of maternal n-3 intake and effect on offspring body composition (Muhlhausler et al., 2011). The results for humans studies involving supplements have been conflicting (Muhlhausler et al., 2010). Little is known about the role of long chain polyunsaturated fatty acids (LC-PUFA) in maternal plasma for adipose tissue growth in newborns (Hauner et al., 2013). However, maternal plasma phospholipid n-3 FA concentrations in early pregnancy might be positively related to birth weight (van Eijsden et al., 2008, Dirix et al., 2009). In rats a maternal high-fat diet leads to obesity in the adult offspring regardless of postnatal diet (Howie et al., 2009).

Gestational intake of saturated fatty acids gives negative effects on skeletal formation in mice offspring (Liang et al., 2009) and lower bone density in humans that have had a high intake of these fatty acids (Corwin et al., 2006).

FISH AND MEAT AS PART OF THE DIET

Fish is an important source of PUFAs, vitamin D, iodine and selenium (Swedish National Food Administration, 1994). Another protein and fat contributor in the diet is meat. Meat contains several nutrients (vitamin D, iron, zinc, selenium and vitamins A and B12)(Williamson et al., 2005).

Sixteen percent of the global consumption of animal protein comes from fish (Food and Agriculture Organization of the United Nations. Fisheries Department., 2010). Global annual fish consumption per capita is 17 kg/ year. In Sweden it is recommended to have an intake of 2-3 portions of fish per week, and the intake is 11 kg per year per person (Amcoff et al., 2012). Fish contains 0.1- 4.6 g lipids per 100 g of flesh(Venugopal and Shahidi, 1996). Fatty also called oily fish has 5% or more in the muscles (demersal fish, as cod, deposit fat in the liver)(Venugopal and Shahidi, 1996). Fatty fish is a good dietary source of polyunsaturated fatty acids (PUFA), especially EPA and docosahexaenoic acid DHA (Huynh and Kitts, 2009), which they get from phytoplankton and seaweed (Abbas, 2009). The intake of n-3 fatty acids has been low in comparison to n-6 FAs found in vegetable oils. Fish oils differ from vegetable oils and animal fats with respect to a wider range of chain length, degree of unsaturation and low levels of odd-numbered and branched chain fatty acids (Abbas, 2009).

In developing and developed countries there is a meat intake of 32 and 79 kilograms/person/year, respectively (FAO, 2012). In Sweden the average intake of meat and meat products/year/person is 50-55 kg (Amcoff et al., 2012). Meat is a source of all the essential amino acids (Williams, 2007), and also several minerals, such as iron and zinc (Williamson et al., 2005). Meat can be separated into red and white meat types (based on types of muscle fibers)(Daniels, 2006). The muscle fibers have different content of myoglobin giving it its color (Mrak et al., 1971). Red meat is beef, veal, lamb, mutton and goat (Williams, 2007). There is some disagreement in the literature on how to classify meat from pork, some classifying it as white and some as red (Bender et al., 1992, Williams, 2007, Wyness et al., 2011). Poultry (e.g. turkey, chicken) is usually classified as white meat (Bender et al., 1992). Animal foods are sources to micro- and macronutrients, but there are also environmental reasons to avoid overconsumption, such as carbon dioxide emissions and overfishing (Stehfest et al., 2009). This is however not an issue covered in this thesis, yet worth mentioning.

HERRING AND BEEF

Among fatty fishes is mackerel, anchovies, salmon and herring. Herring is a pelagic fish, found between 2-400 m below sea surface (Stroud and Station, 1979). The scientific name for this fish found in the north Atlantic is Clupea harengus. It is closely related to Pacific herring, Clupea pallasii. Herring is found on both sides of the north Atlantic. The most important fishing grounds are the North Sea, the Baltic Sea, and the coastal waters of Britain, Norway, Iceland and Canada (Stroud, 1979). Herring is also economically one of the most important fish species in Sweden (Öhrvik V. , 2012). There is a difference between herring fished in the Baltic sea compared to Atlantic herring (Swedish “sill”), Baltic herring (Clupea harengus membras, Swedish “strömming”) as the Baltic sea is more polluted and the fish there contains higher levels of PCB (polychlorinated biphenyls) than fish from the Atlantic sea (Burreau et al., 2006). The flesh of moderately fat herring contains 11% fat (Stroud and Station, 1979). Of its fat content 23 % is SFA and 27% is PUFA, whereof 25% is n-3 fatty acids and 2% is n-6 , but the composition varies and depends on season, fishing ground, environment and nutritional status (Murray et al., 1969, Venugopal and Shahidi, 1996). Fish meat also contains proteins, water, vitamin A, D, B12, niacin and pantothenic acid.

Of the meat eaten worldwide 24% is beef (FAO, 2012). In beef the fat is present in the membrane fat, intermuscular and subcutaneous fat depots (Abbas, 2009). Between 30-50% of the fatty acids in meat are saturated (Schmid, 2011). About 80% of the fatty acids in beef is composed of palmitic acid, stearic acid and oleic acid (Abbas, 2009). The most abundant SFAs in beef are myristic acid (14:0), palmitic acid (16:0) and stearic acid (18:0). Saturated fatty acids influence plasma cholesterol (especially palmitic, myristic acid and lauric acid). Major PUFAs found in beef are linoleic acid (LA), α-linoleic acid (ALA), ARA, EPA, docosapentaenoic acid (DPA), and DHA. Total amount of fat in meat from beef depends on cut and processing, and lean beef has low intermuscular fat content ranging from 2-5% in many countries (Abbas, 2009). Lean red meat is high in protein, niacin, vitamin B6 and B12, phosphor, zinc and iron (Williams, 2007).

POSSIBLE EFFECTS OF FISH AND MEAT INTAKE ON METABOLISM, BONE AND BODY COMPOSITION

People categorized as fish-eaters are often described as slimmer than meat-eaters. In the UK women’s Cohort Study oily fish-eaters (2-4 times per week) had a higher intake of energy than vegetarians, meat eaters and fish eaters (less than 2-4 times per week), yet had the lowest BMI (Cade et al., 2004). Also men with a high consumption of fish are less likely to be overweight (He et al., 2002). In the large scale EPIC study (European Prospective Investigation into Cancer and Nutrition) it was found that meat intake at baseline was associated with weight gain at the 5 year follow up (Vergnaud et al., 2010). Fish-oil has been shown to reduce weight gain (Cunnane et al., 1986, LeBoeuf and Veldee, 1993, Pan and Storlien, 1993, Clouet et al., 1995) fat mass (Hill et al., 1993, Su and Jones, 1993) and hypertrophy of fat depots in rodents (Parrish et al., 1990, Belzung et al., 1993). It has also been shown to reduce fat mass and stimulate lipid oxidation in human adults (Couet et al., 1997). In a randomized control study adult men that ate 3 portions of fish per week and had on energy restriction had a greater weight loss than the control group that were not eating any seafood but were supplemented with sunflower oil (Thorsdottir et al., 2007). Vergnaud et al. were later criticized for not distinguishing weight from body composition (Astrup et al., 2010), which is also a weakness of other studies that have only looked into weight and BMI. In a crossover study where meat was included or excluded from the diet in young women, the meat intake increased FFM (Petzke et al., 2011). This may be a cause to why oily fish, rather than red meat, can increase insulin sensitivity in young women (Navas-Carretero et al., 2009).

Both meat and fish intake can affect the cholesterol and triglycerides. Some of the fatty acids in beef are known to raise levels of “bad cholesterol” (LDL-cholesterol) which increases the risk of cardiovascular and coronary disease and stroke (Hu et al., 1999, Mozaffarian and Rimm, 2006, Abbas, 2009, Jakobsen et al., 2009). Fatty fish intake can increase HDL levels in overweight men (Lindqvist et al., 2009b).

The diet also plays an important role in bone health (Heaney et al., 2000, Palacios, 2006, Cashman, 2007, Rizzoli, 2008, Peters and Martini, 2010). The role of vitamin D in the development of rickets is well established (Mithal et al., 2009). Other vitamins, such as K and A, and minerals as calcium and phosphorus, have also been given attention in this research field (Feskanich et al., 1999, Booth et al., 2000, Cashman, 2002, 2007, Bonjour et al., 2009). At macronutrient level high fat diets have shown to reduce bone mineralization (Parhami et al., 2001), inhibit osteogenic differentiation (Parhami et al., 1999) and reduce bone mineral content in rodents (Macri et al., 2012). Specific fatty acids may have different properties, since n-3 fatty acids seem to reduce bone mass loss in ovariectomized mice, compared to n-6 fatty acids (Sun et al., 2003). Also in humans, a higher intake of n-3 fatty acids may have favorable effects on bone mineralization shown as decrease in a marker of bone resorption (Griel et al., 2007). Although, also opposite findings have been reported, at least in mice (Sirois et al., 2003), reviews have concluded that n-3 fatty acids are more beneficial for bone health than n-6 fatty acids (Watkins et al., 2001, Watkins et al., 2003). Combinations of different macronutrients, such as high-fat and sucrose diets have shown to affect mechanical properties of the bones (Salem et al., 1992, Zernicke et al., 1995). Mice fed more complex diets, such as “Western diets” have also been studied and showed that rodents fed a “Westernized” diet had lower bone mineral density and content in femur than those fed a standardized diet (Ward et al., 2003, Demigne et al., 2006). Fish intake was in a study associated with greater bone mass and lower risk of osteoporosis in women (Chen et al., 2010). Meat has also been associated with higher insulin like growth factor-1 (IGF-1) levels (Larsson et al., 2005, Takata et al., 2006) which is associated with increased bone mineralization and fewer fractures (Kanazawa et al., 2011). Diets with a high fish and seafood content and low red meat intake give higher bone mineral density (BMD) (Zalloua et al., 2007, Kontogianni et al., 2009).

There are challenges with interpretation of effects of fish and meat intake on metabolism and bone and body composition, as those eating mostly fish or meat might be part of larger dietary patterns and lifestyle differences. For instance consumption of a Mediterranean diet with high fish and olive oil and low red meat intake was positively related to bone mass (Kontogianni et al., 2009). The result from the different studies could differ because of type of fish and meat studied and whether the meat is processed or non-processed.

EFFECTS DURING PREGNANCY ON METABOLISM, BONE AND BODY COMPOSITION

Less is known about the impact a maternal fish and meat intake can have on maternal body composition and on the offspring's metabolism, bones and body composition. Here is a short review of the literature found on this.

Fish is considered to be an important part of the pregnant woman's diet (Dovydaitis, 2008). Due to pollutants there are precautions linked to intake of certain types of fish, during pregnancy (Grandjean et al., 2001, Budtz-Jorgensen et al., 2007, Dovydaitis, 2008, Genuis, 2008). For normal development of the neural system, long-chain n-3 fatty acids are essential (Cetin and Koletzko, 2008). Docosahexaenoic acid is an especially important component of neural and retinal membranes that accumulates in the brain during gestation and the postnatal period (Cetin and Koletzko, 2008). Both DHA and EPA have rapid uptakes into the fetus' brain during gestation and throughout the child's first years of life (Dovydaitis, 2008). Consumption of fish oils, during pregnancy and lactation may have beneficial effects on the intelligence quotient (IQ) of children (Helland et al., 2003).

A high intake of meat and fish increases the cortisol levels in the adult offspring (Reynolds et al., 2007), cortisol is again associated with decreased bone mineral density (Michelson et al., 1996) and in some studies a high intake of animal protein has been linked to increased blood pressure in the children (Shiell et al., 2001, Herrick et al., 2003). Studies have investigated the effect of fish or meat on birth size. The literature on fish seems to be parted in two; describing either negative effects of fish intake due to pollutants leading to smaller babies or positive effects on length of gestation and larger birth sizes. A moderate intake of fish during pregnancy is associated with a lower risk of preterm birth (Olsen et al., 2006, Guldner et al., 2007, Haugen et al., 2008). But also more negative associations have been found. In one study shell fish intake led to more babies being born small for gestational age (SGA) (Guldner et al., 2007). In another study an intake of 60 g fish per day or more was associated with SGA babies (Halldorsson et al., 2007). Some of the positive findings of fish intake are; moderate intake of fish per week was associated with a lower risk of preterm birth (Haugen et al., 2008, Klebanoff et al., 2011). Fish-oil supplementation in late pregnancy led to lower BMI in the infants at 21 months (Lucia Bergmann et al., 2007). Body length was not affected. Low meat protein intake during pregnancy is associated with lower birth weight (Godfrey et al., 1996). In a Danish study, factor analysis showed that a diet based on red, processed meat and high-fat dairy products was associated with an increased risk for SGA (Knudsen et al., 2008).

Meat is a rich source of ARA (Sinclair et al., 1994), which is important to the development of the brain (Crawford et al., 1976). Meat is often given attention as an important iron source for pregnant women, and some decades ago it was recommended by obstetricians to have a high intake of meat during pregnancy (Shiell et al., 2001). Red meat intake is now associated with cardiovascular disease, obesity, cancer (English et al., 2004) and diabetes (Pan and Storlien, 1993). It has also been linked to negative metabolic effects in pregnant women (Bao et al., 2013), and associated to adverse metabolism in the adult offspring (Shiell et al., 2001, Reynolds et al., 2007). A study from the US shows that consumption of red and processed meats is significantly associated with a higher risk of developing gestational diabetes (Zhang et al., 2006), which is associated to increased fat mass and less lean mass in new-borns (Durnwald et al., 2004). In one study pre-pregnancy red meat intake was associated with higher risk of GDM, whereas intake of fish was not (Bao et al., 2013).

We have only found one study investigating maternal meat intake and fat mass in child. Information on dietary intake from the 3rd trimester was in Tasmania used to study how maternal meat intake was positively associated with the fat mass in 16- year old children (Yin et al., 2010). Few intervention studies have been found that have looked into fish or meat intake in well-nourished populations.

AIMS

The aim of this thesis was to explore the effects of fish and meat intake on maternal and offspring health. Outcomes studied were metabolism, bone health and body composition. Paper I and II from the animal study were oriented towards the offspring's health, whereas paper III from the human study concerned maternal health.

PAPER I

To study whether the maternal and the offspring's intake of fatty fish and red meat (crossover design) affect the offspring when it comes to:

- Body composition
- Insulin sensitivity
- Fatty acid content and composition in the breast milk, fat depots, muscle and liver

PAPER II

To investigate potential effects of a fatty fish and red meat diet on bone tissue in a sample of the offspring from paper I. We studied:

- Bone mineral content and density (areal and volumetric) in subcranial area, lumbar area and in tibia
- Bone strength in tibia

PAPER III

To examine if:

- Fish and meat intake in normal weight women during pregnancy affects gestational weight gain, body composition and serum phospholipid fatty acids.
- A dietary intervention with emphasis on three servings of fish per week would affect fish intake, serum phospholipid fatty acids, and gestational weight gain and body composition changes during pregnancy.

METHODS AND METHODOLOGICAL CONSIDERATIONS

The papers in this thesis are based on experimental data from an animal study and one clinical study, both with a focus on fish and meat intake. Here the theoretical background for methods used will be presented. Details of how each method was performed (including manufacturers of equipment etc.) are given in each of the papers. Table 1 in the end of this section gives a short overview of the material and methods.

ANIMALS (PAPER I AND II)

Paper I and II involved animal experiments, approved by the ethical committee for animal research at the University of Gothenburg (No. 187/2008).

STRAIN

To test the effects of a fish and meat diet on offspring health, we used C57Bl/6 mice. The C57Bl/6 mouse is an inbred mouse (Peters et al., 2007) frequently used to study metabolic conditions and dietary manipulation (Feige et al., 2008).

BREEDING

Female C57Bl/6 mice were bred while fed test diets. Mice have a gestational period of approximately 19-20 days (Peters et al., 2007). The breeding process is a sensitive period for rodents and in addition to genetic reasons, after birth a stressed mother may if the surroundings are not perceived to be sustainable and safe, cannibalize her pups (Lane-Petter, 1968). Odors are very important to mice during mating (Doty, 1986). This was a concern for our study, as the diets were fresh food with a distinctive smell and therefore the diets were introduced on the 2nd day of the breeding process. Also cannibalism was tried to be minimalized by avoiding superfluous stress to the dams during gestation and in the lactation period. Two females and one male were placed in the same cage (in total 48 females and 24 males). The resulting pups were followed to adulthood.

DIETS AND DIETARY MANIPULATION (PAPER I AND II)

The two test diets consisted of traditional household ingredients (flour, sugar, butter, herring or minced beef), but also vitamins, minerals, casein (added protein) and cellulose (to add fiber) to make them suitable for a successful breeding and gestation in mice. The recipes were based on diets previously used in studies on male rats (Lindqvist et al., 2009a), but proved unsuitable for breeding dams and had to be adjusted for our project. Therefor pilot studies were performed to optimize the diets to ensure that both the dams and the offspring had a good health status. We added fiber, minerals and vitamins after correspondence with various researchers and animal keepers.

In our diets, the energy content was approx. 300 kcal/100g (calculated from data analyzed by Eurofins Food and Agro, Lidköping, Sweden). They contained high levels of protein (~27 E %) and fat (~10 E %) and was provided ad libitum to the mice. They were prepared by using defrosted herring and minced beef with the addition of the dry materials in a food processor and later heated up in a microwave oven. The diets can be seen in Figure 1. Afterwards the food was kept in a freezer and defrosted before being provided to the mice every 2^{nd} day. The n-6/n-3 ratio was 0.4 in the herring diet and 12.9 in the beef diet. The beef diet had considerably more stearic acid (18:0) and oleic acid (18:1n-9) compared to the herring diet, but almost the same levels of linoleic acid (LA), alpha-linolenic acid (ALA) and ARA, but non- detectable levels of DHA and EPA.

Normal lab chow is formulated to give the mice 10-15 kcal per day (Feige et al., 2008) and usually contains cereals as a source of carbohydrates, soy, yeast or fish extract as a fat and protein source and ash for its content of minerals. In addition more minerals and vitamins are added. The fat content is usually 50 % saturated fatty acids and 50 % mono-and polyunsaturated fatty acids. The fatty acids that are the most abundant in the chow are palmitic acid, oleic acid and linoleic acid. The caloric content of this chow is approximately 290 kcal/100g (which is comparable to our diets) of which 5-12 % is fats, 20-25 % is proteins and 65-70 %t is carbohydrates.

Due to lack of facilities the food intake per day could not be measured, but in a later study performed by our group the same diets were measured for intake. No differences in the intake were found.

Figure 1 Experimental diets based on herring and beef. The recipes were as follows: 500 g herring/minced beef, 205 g casein, 115 g of wheat flour, 90 g butter, 90 g white sugar, 50 g mineral mix, 50 g cellulose and 20 g vitamin mix.

EXPERIMENTAL DESIGN AND TIMELINE (PAPER I AND II)

After the lactation period, when the pups were 21 days old, half of the male offspring were put on the same diet as their dams and the rest where crossed over to the other diet. The experimental design is described in Figure 2 and a timeline is presented in Figure 3. Maternal diet was defined as diet provided to the dams during gestation and lactation, and the postweaning diet as diet provided to the offspring after weaning.

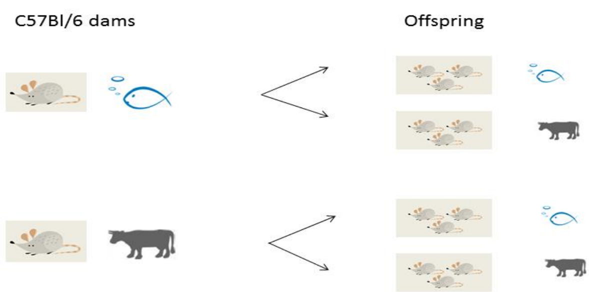

Figure 2 Experimental design with crossover after weaning

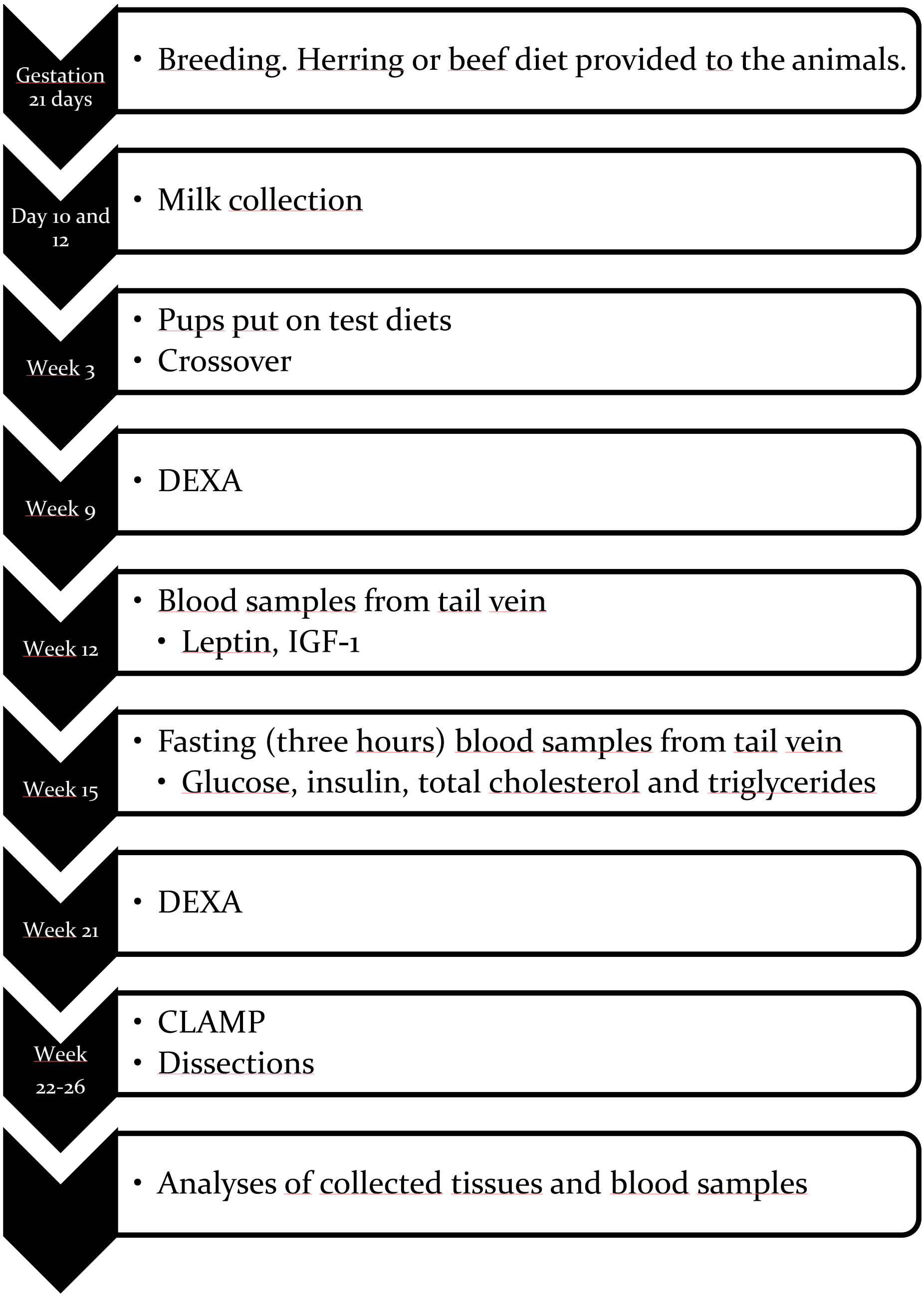

Figure 3 Experiment timeline

WEIGHT, FAT MASS AND FAT FREE MASS (PAPER I AND II)

There are several models of body composition. In the two-compartment model, that separates fat mass (FM) from fat-free mass (FFM). It is the most widely used approach to measure body composition in human adults (Lee and Gallagher, 2008). When using this division of body composition several methods are applicable; bioelectric impedance, dual X-ray absorptiometry (DEXA), quantitative computed tomography, dilution techniques, air displacement plethysmography, magnetic resonance spectroscopy and quantitative resonance, to mention some (Lee and Gallagher, 2008).

In mice, carcass analyses have been customary to perform in order to measure fat mass and bone mineral density, but this method is of course final (Nagy and Clair, 2000). An alternative non-invasive method is the DEXA. Dual X-ray assessment is a method used to investigate body composition. The measurement is fast to perform. The animal must however be sedated and therefore we anaesthetized the mice with isoflurane before performing sub-cranial scannings. The DEXA distinguishes between skeletal and non-skeletal tissue. The non-skeletal tissue is further separated into fat or fat free mass. The body composition is calculated by a ratio between attenuation of two X-ray energies (Plank, 2005). Estimations of body fat by DEXA correlate well with adipose tissue depots (Sjogren et al., 2001), but the PIXImus2 DEXA has been reported to overestimate the amount of total body fat (Brommage, 2003). The mice were weighed each week (week 4-16) by using a calibrated scale, but also in connection with all types of measurements (e.g. clamp, blood samples etc.).

Body fat is stored in several depots in the body (Cinti, 2005). White adipose tissue (WAT) is deposited both around the organs (visceral fat) and subcutaneous. Brown adipose tissue (BAT) is found several places, one depot is found in the interscapular region and is called interscapular brown adipose tissue (iBAT) (Cannon and Nedergaard, 2004). This type of fat tissue oxidizes fatty acids via uncoupled oxidation resulting in thermogenesis (heat production, rather than energy).

The mice were after performing the clamp procedure euthanized with an injection of pentobarbiturate and the gonadal, inguinal, peri-renal fat depots were dissected (Figure 4). Inguinal fat is a subcutaneous depot, whereas the peri-renal and gonadal fat is visceral fat tissue. Also iBAT, soleus muscle (red muscle from the hind leg), tibia and liver were dissected. Adipocyte size of gonadal fat depots was studied by computerized image analysis.

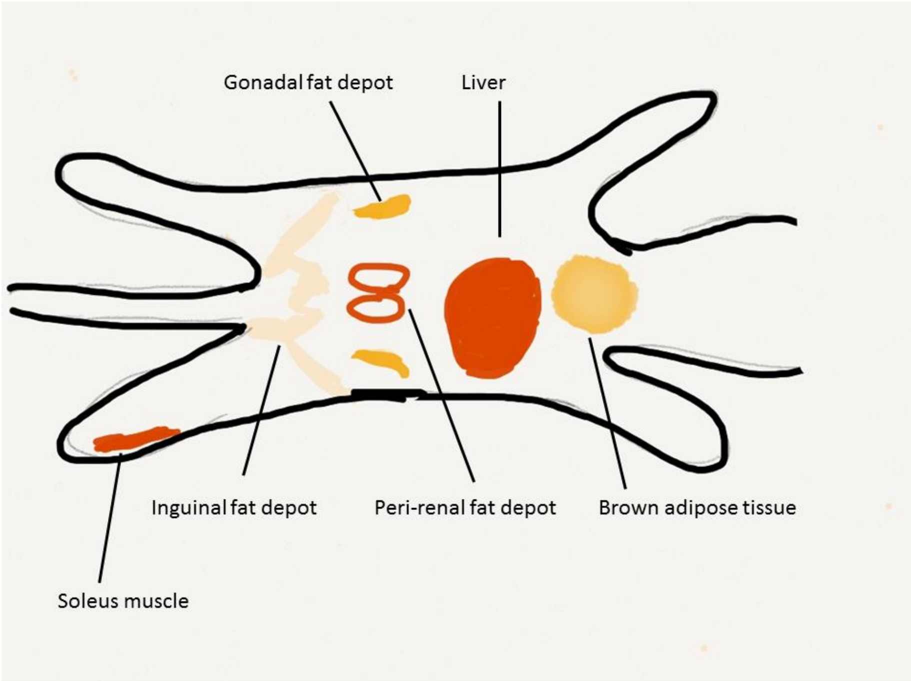

Figure 4 Dissected fat depots, soleus muscle and liver.

BONE COMPOSITION (PAPER II)

"Bone fragility is influenced by bone size, shape, architecture and tissue quality"(Turner, 2002).

DUAL X-RAY ABSORPTIOMETRY (DEXA)

Dual X-ray absorptiometry measurements were performed in week 9 and 21 as previously described. The data received together with FM and FFM was areal bone mineral density (aBMD) and bone mineral content (BMC). By using the software we later also gathered information on lumbar bone density and mineral content from lumbar disc 2 to 5. Lumbar disc measurements are used as a substitute for vertebrae measures done in humans as this area is susceptible to weight load (Rao and Draper, 1969). Bone mineral density is a measure of mineralization in the studied region and BMC bone mineral content.

PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY (PQCT)

Dual X-ray absorptiometry measurements do not give information on volumetric bone mineral density (vBMD) and cannot distinguish between trabecular and cortical tissue, therefor peripheral quantitative computed tomography (pQCT) was performed.

Peripheral quantitative computed tomography is a three-dimensional technique used to analyze the volumetric bone mineral density rather than aBMD and analyses cross-sectional slices of bones (Ferretti et al., 2001). It also distinguishes trabecular and cortical bone tissue (Engelke et al., 2008). This technique was carried out by biomedical laboratory technologist Anette Hansevi at the Centre for Bone and Arthritis Research at the Sahlgrenska Academy at Gothenburg University. Volumetric BMD reflects the amount of mineral in the selected bone region (Ferretti et al., 2001). The cortical bone is relevant to the bone structure, whereas trabecular bone is not suitable for biomechanical assessment of bone quality (Ferretti et al., 2001). The vBMD of trabecular bone is an indicator of the structural stiffness and strength.

The pQCT gives information on polar moment of inertia and polar moment of resistance, the first being calculations on the resistance of the bone to torsion or twisting forces and the second being the resistance of the structure to bending forces (Thakur, 1997).

BIOMECHANICS

Dual X-ray absorptiometry measured BMD accounts for 60-70% of the variation in bone strength and does not give information on geometry or microarchitecture of the bone tissue (Ammann and Rizzoli, 2003). In paper II biomechanics were used to measure the bone strength of the tibia samples. The biomechanical tests were performed by Antti Koskela at the Department of anatomy, internal medicine and cell biology at the University of Oulu. There three-point bending tests were carried out. Three-point bending tests are performed to measure the bending properties of bones (Beaupied et al., 2007). The bone sample is placed on two holders, one at each end and a third one on top in the middle of the sample, which explains the name of the method. The bone is loaded until failure.

Explanation of measures obtained (Thakur, 1997):

- *F (max):* maximal force resulting in breakage
- *E (max):* maximal energy absorption needed to cause fracture
- *Toughness:* resistance or the bone's ability to withstand sudden forces without fracture
- *Stiffness:* resistance to deformation

BLOOD SAMPLES (PAPER I AND II)

Blood samples were drawn in week 12, fasting samples in week 15 and blood collected in connection with the euglycemic clamp procedure. In paper I insulin, total cholesterol and triglycerides were analyzed from blood collected from the tail vein in fasting (three hours) animals. Insulin and leptin were measured with ELISA kits. Cholesterol and triglycerides were analyzed by spectrophotometry. In paper II leptin (also in paper I) and IGF-1 were measured in blood samples from week 12 (tail vein), analyzed with enzyme-linked immunosorbent assays (ELISA kits). Blood samples drawn in connection with the clamp procedure (described below) were also analyzed for adiponectin (paper I) using ELISA kits. Blood glucose was measured using microcuvettes and a HemoCue machine.

INSULIN SENSITIVITY (PAPER I)

EUGLYCEMIC CLAMP TECHNIQUE (CLAMP)

Euglycemic clamp technique is considered a golden standard for measuring insulin sensitivity (Ayala et al., 2006).

The mice were sedated with isoflurane, and kept on a heating blanket (37°C). By surgery, a catheter was inserted into the left carotid artery (for blood sampling, every 5th minute) and in the jugular vein for glucose and insulin infusions. Breathing was facilitated by tracheotomy. Prior to the clamp 5µl blood was drawn into a microcuvette and analyzed for glucose (HemoCue Glucose, HemoCue, Ängelholm, Sweden).

Human insulin (Actrapid Penfill) was diluted in physiological saline containing 4% human albumin (200 g/liter; Baxter Medical, Kista, Sweden). After a bolus injection, insulin infusion was continued at 30 mU/min/ kg. To maintain plasma glucose at 5 mmol/L a 30% glucose solution in physiological saline was administered at a rate guided by blood samples. Stable glucose and insulin levels were reached after approximately 60 min and maintained for the remainder of the clamp. At 60 and 90 min, blood samples (50 µl) were drawn to determine plasma concentrations of insulin. After the clamp the mice were killed with pentobarbiturate injections. Since this method is terminal it was done as the last step of the study when the mice were 22-26 weeks old. We had facilities to perform the clamp method in four mice per day, which led to the time span. Glucose infusion rate (GIR) could then be calculated as: (Glucose x 0.3)/body weight (kg).

QUICKI

However, since the clamp method is terminal, surrogate insulin sensitivity measures are often used. One of them is quantitative insulin sensitivity check index (QUICKI) (Katz et al., 2000). Fasting glucose (G_0) and fasting insulin (I_0) are used to calculate the index:

$$QUICKI = 1/ [\log (I_0) + \log (G_0)]$$

This surrogate insulin sensitivity measure is convenient to use when an experiment is still ongoing (Katz et al., 2000). Calculations of QUICKI were done based on fasting (for three hours) glucose and insulin values collected when the mice were 15 weeks of age.

FATTY ACIDS (PAPER I)

Fatty acids from the maternal circulation can affect the breast milk fatty acid content. Fatty acids were therefore analyzed in the milk from the dams. Fatty acids were also measured in selected fat depots, liver and muscle from the offspring.

MILK SAMPLING (PAPER I)

At the 10^{th} and 12^{th} day after delivery, dams were separated from their pups 1 hour before milk collection. Oxytocin (Syntocinon 8.3 µg/mL) was injected 15 minutes before the milking procedure to support milk production. Meanwhile the pups were kept warm on a heating blanket. The dams were sedated with isoflurane, and mammary glands were stimulated by gentle massaging. Milk was collected for 15 minutes with a milking device modified from a previously described milking method involving vacuum suction (Oskarsson and Moller, 2004), later adjusted by Lager (Lager, 2010), thereafter the milk was frozen and stored at -80°C. Because of the small amounts collected the samples were pooled for several mice in each group.

FATTY ACIDS IN THE DIET, MILK, LIVER, SOLEUS MUSCLE AND FAT DEPOTS (PAPER I)

Diet samples, milk and tissues were analyzed for fatty acids by Intawat Nookaew and Sakda Khoomrung at Systems Biology at the Chalmers University of Technology. They performed gas chromatography-mass spectrometry (GC-MS) by using freeze-dried samples of milk, soleus muscles, liver, gonadal, inguinal, peri-renal and brown adipose tissue. After preparation unknown fatty acid methyl esters (FAMEs) were identified by comparison of their retention times and mass spectrum profiles with known standards.

STUDY POPULATION (PAPER III)

The study was approved by the Ethics Committee at the University of Gothenburg (no. 402-08). The participants were given oral and written information. Written informed consent was obtained from the participants.

PARTICIPANTS

Participants for the Pregnancy Obesity Nutrition and Child Health (PONCH) - study were recruited by help of maternal care centers, public bill boards and ads on a website for pregnant women in the Västra Götaland region of Sweden. The PONCH-study is a randomized, longitudinal intervention study. Participants were included early during pregnancy (less than 13 weeks of gestation). The study was conducted at the Sahlgrenska University Hospital. Participants were randomized into four groups (normal weight interventions/normal weight controls/obese interventions/obese controls). Randomization to the intervention group or the control group was done using software developed at the department created to minimalize differences in age, BMI and parity between the groups.

Exclusion criteria were:

- BMI <18.5
- BMI: 25.0-29.9
- Smokers
- Diabetics
- Taking medicines for epilepsy (neuroleptics)
- Vegetarian/vegan diet
- Non-European decent
- Twin pregnancy
- Age <20 years

SELECTION OF PARTICIPANTS IN PAPER III

As the PONCH-study is an ongoing study, in paper III we analyzed data from women included between 2009 and 2012. We only used data from normal weight women as at this time point there were not enough obese women included. Data was analyzed for those that attended all three visits but also for a baseline population (those attending the 1st trimester visit) where women in the control group and the intervention group were pooled.

DIETARY INTERVENTION AND ASSESSMENT (PAPER III)

The aim of the dietary intervention was to follow recommendations for pregnant women stated by the Swedish National Food Agency (The Swedish National Food Agency, 2013) and in the Nordic Nutrition Recommendations (NNR) 2004 (Nordiska ministerrådet, 2004). The intervention group was advised to:

- Consume three meals of fish/week
- Lower sugar intake to <10 E%
- Eat 500 g of vegetables and fruits/day
- Increase daily energy intake by 350 kcal in the 2nd trimester and by 500 kcal in the 3rd trimester.

In addition, trained dieticians gave dietary advices during the study, individualized for each woman. The dietary advices included fat quality, food frequency, fiber intake, and nutrient density.

After the 1st visit (gestational week 8-12), the women were repeatedly called (three times between the 1st and 2nd visit and two times between the 2nd and the 3rd visit) in order to remind them of the recommendations and followed-up at visits to the university hospital in the 2nd (gestational week 24-26) and 3rd trimester (gestational week 35-37).

A self-administered dietary questionnaire was used to assess energy intake during the 3 previous months (Lindroos et al., 1993), to give information on each trimester. In addition, before all study visits, participants completed a food frequency questionnaire to assess their weekly intake of fish and meat during the past trimester. For the results in paper III, we specifically analyzed two questions:

1. "How many hot meals of fish do you have per week?" and
2. "How many hot meals of meat do you have per week?"

The frequency reported was then converted into grams by assuming that a serving of fish was equal to 150 g and a serving of meat equal to 175 g, based on serving sizes recommended by the Norwegian Health Authorities (The Norwegian Directorate of Health, 2011).

Also questions on supplements were asked; did you use supplements the last three months? Which one? Later the information was analyzed as yes/no for supplements containing fish oil or n-3 fatty acids.

BODY COMPOSITION (PAPER III)

We needed a method without radiation (as there is in the DEXA method) for studying body composition in the pregnant women. Air displacement plethysmography (ADP) is a quick, comfortable, non-invasive and safe method for measuring body composition (Fields et al., 2002). It measures an individual's volume, by measuring the volume of air displaced in the air chamber of the machine when the person is seated in the chamber while lightly clothed. The BodPod (Cosmed inc., Rome, Italy) was used to perform this technique. The procedure took approximately 10-15 minutes. Using software fat mass and fat free mass is calculated. A Swedish study showed that this method can be used to measure body fat in pregnant women (32 weeks of gestation) (Henriksson et al., 2013).

SERUM PHOSPHOLIPID FATTY ACIDS (PAPER III)

In humans fat in the serum or plasma mirrors the fatty acid intake the last weeks (Arab, 2003). Polyunsaturated fatty acids are most abundant in the blood in phospholipids. Venous blood samples were obtained after overnight fast at each visit. Serum phospholipid fatty acids (ARA, EPA and DHA) were analyzed by GC-MS by Cecilia Svelander at Division of Life Sciences/Food Science at Chalmers University of Technology.

STATISTICS

All statistical analyses were done in SPSS (SPSS Inc., Chicago, IL, USA). In paper I and II data is expressed as mean ± standard error of mean (SEM) and in paper III as median (25^{th} and 75^{th} percentile) In paper I and II two-way ANOVA was conducted. Differences were considered significant at $p<0.05$. When the two diets were compared for FA composition Student's t-test were performed (paper I and II). The effects of the preweaning and postweaning diets and potential interactions were analyzed by two-way ANOVA. In paper I Student's *t*-test was used to compare the mean body weights before crossover and differences in cell size in white adipose tissues. Weekly weight gain was estimated by linear regression. In the breast milk FA composition was analyzed with Student's t-test. In paper III Friedman test for repeated measurements (a non-parametric alternative to ANOVA for repeated measures) and Spearman correlations were performed.

Table 1 Overview of methods and materials in paper I, II and III

PAPER I AND II			PAPER III		
Material	Method	Results on	Material	Method	Results on
Animals	C57Bl/6, dams Breeding Crossover	Offspring metabolism Offspring bone health	Pregnant women	Longitudinal intervention study	Maternal nutrition
Diets	Herring Beef		Dietary assessment	Food frequency questionnaire (FFQ)	Fish, meat and energy intake
Feeding	Ad libitum		Dietary inter-vention	Dietary counseling in each trimester	
Milk	Oxytocin injections Vacuum pump GC-MS	Fatty acids			
Blood samples	ELISA GC-MS	TAG Cholesterol Leptin Glucose Insulin Fatty acids IGF-1	Blood samples	GC-MS	Serum phospholipid fatty acids (ARA, EPA and DHA)
In vivo, animals	Clamp QUICKI calculations DEXA	Insulin sensitivity Body composition Subcranial body and lumbar bone mineral density and content			
Liver iBAT Gonadal fat Inguinal fat Peri-renal fat	Dissection GC-MS	Fatty acid content Adipocyte size Body composition			
Tibia	pQCT Three-point bending	Thickness Cortical and trabecular BMC and BMD Bone strength			
Data	Statistical analyses: Two-way ANOVA		Data	Statistical analyses Friedman test Spearman correlations	

KEY RESULTS AND DISCUSSION

In this part of thesis key results will be presented and discussed . The complete results can be read in each paper. Paper I and II are from the same animal experiment and therefor some parts will concern both papers. An overview of the results in paper I and II are presented in Table 2.

PAPER I

Summary of findings

Maternal herring diet counteracted weight gain and adiposity in the adult offspring

A postweaning herring diet counteracted insulin resistance and dyslipidemia in the offspring.

n-6/n-3 ratio was higher in the breast milk of dams fed beef and significantly lower in milk from the herring fed dams.

The fat depot's fatty acid profile was affected by the postweaning diet; herring-based diet lead to a lower n-6/n-3 ratio and the opposite was evident in the beef-fed offspring.

Herring fed mice had larger interscapular brown adipose tissue mass.

WEIGHT GAIN AND BODY COMPOSITION

Average weight gain was significantly higher among the mice with dams fed beef than the ones fed a maternal herring diet. When they reached the age of 9 weeks these mice also had more body fat than the mice with dams fed herring, even though they had no significant difference in body weight. Twelve weeks later they weighed more than the maternal herring groups, but the fat percent was no longer significantly higher. Yet no differences were found in adiponectin and leptin levels, however these hormones were not measured at the same time point as the body composition measurements (week 22-26 and week 12, respectively).

Fat depots were dissected and weighed in connection with the clamp procedure. The weight was adjusted for body weight (mg/g) and it was found that mice with a postweaning herring diet had significantly larger interscapular brown adipose tissue depots compared to mice with a postweaning beef diet. Brown adipose tissue produces warmth instead of storing energy (Himms-Hagen, 1990), and has therefore caught the attention of obesity researchers as an upregulation of fat tissue's amount and activity could be possibly counteract obesity (Enerback, 2010). No difference was found in white fat adipose tissue, soleus muscle or liver or in gonadal adipocyte size (white fat tissue). This is in accordance with the findings from the body composition measurements done by DEXA in week 21 where fat percentage did not differ.

INSULIN SENSITIVITY

At 15 weeks of age, mice fed herring after weaning had lower total cholesterol and triglyceride levels than those fed beef, which is in accordance with known effects of the fatty acids in the diets. The fasting insulin levels were lower in the mice fed herring after weaning than in those fed beef. At 15 week of age, mice fed herring had higher insulin sensitivity, as shown by QUICKI analysis of plasma. However, at 22–26 week of age, the groups did not differ in insulin sensitivity measured by the hyperinsulinemic euglycemic clamp. Because the clamped mice were randomly selected, the body composition results from week 21 were reanalysed for these mice; no differences in body composition were found.

The clamp procedure is a challenging method, especially in mice due to their small size. We did have a loss of mice during the experiment and as such had few mice in each group with completed clamp (n=6-8), when the aim was ten. Another suggestion for the lack of difference is that the mice at this time point where at such an age that the protective effects of herring diet could not counteract the process of age-related decline of insulin sensitivity.

FATTY ACIDS IN BREAST MILK, FAT DEPOTS AND LIVER

Breast-milk from herring fed dams had higher levels of n-3 PUFAs and from the beef dams more monounsaturated fatty acids (MUFA) and n-6 PUFA. The effects of fish intake on the increased n-3 PUFAs in breast milk have previously been reported in women (Urwin et al., 2012). Total amount of fatty acids in fat depots and liver did not differ between the groups of mice, but the fatty acid profile did. n-6/n-3 ratios were calculated for the collected fat depots and tissues (Figure 5). All fat depots and liver had similar trend of n-6/n-3 ratio mirroring their intake from the post-weaning diet. Fatty acids influence biological systems by being involved in eicosanoid synthesis, altering substrate specificity, lipid peroxidation, affecting membrane flexibility, acylation of proteins and by working on transcription factors (Rustan and Drevon, 2001). The lower n-6/n-3 fatty acids in the fat depots of mice fed herring could lead to higher amounts n-3 FAs being incorporated in membrane phospholipids, which in turn could alter membrane protein functions. The insulin receptor's affinity has been reported to be increased in rats fed a diet with a high PUFA/SFA- ratio (Field et al., 1990, Liu et al., 1994). As we found the herring-fed mice to have better insulin sensitivity in week 15 this could have been caused by increased incorporation of n-3 fatty acids in cell membranes, but we did not find differences in results from the clamp procedure which were done at the same time as fat depots were dissected. This could be an age-related result or a result of a small sample size, as mentioned in the section on insulin sensitivity.

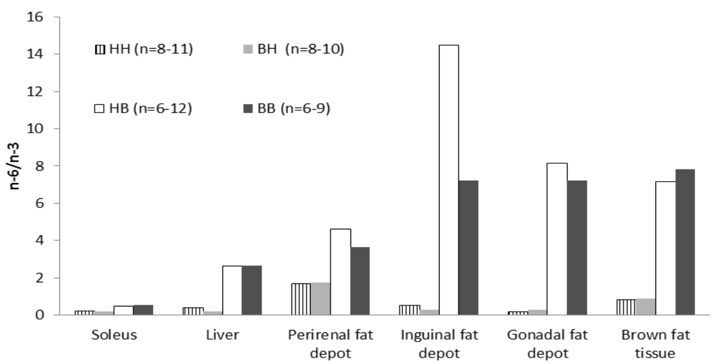

Figure 5 n-6/n-3 ratios in tissues

Animal experiments performed to study human diseases always raise the question of generability and transferability. Mice have not only many of the same genes as humans, but also similarities in anatomy and physiology (Peters et al., 2007). Although, when it comes to metabolism there are some differences. Mice have low levels of total cholesterol and LDL but high levels of HDL, which is opposite of the case in humans (Gajda et al., 2012). This was also a reason to why we did measure total cholesterol rather than LDL and HDL.

Here we used herring to represent fatty fish, the fatty acid composition in different types of fish differ and is also affected by the waters they have been fished from (Abbas, 2009), in addition also farmed and wild fish might differ in health effects (Mozaffarian and Rimm, 2006). Beef was used to represent red meat intake, but there might be other effects of meat from e.g. lamb and goat.

PAPER II

Summary of findings

Maternal beef diet gives stronger bones with higher mineral content and density at an early age.

On the contrary in the postweaning period a beef diet decreased plasma IGF-1, bone mineral density in the lumbar spine and lower subcranial and lumbar bone mineral content.

Taken together a maternal beef diet in combination with a postweaning herring diet gave higher tibia cortical volumetric bone mineral density, bone mineral content and thickness

OFFSPRING DEXA MEASUREMENTS AT 9 WEEKS OF AGE

At 9 weeks of age the offspring's BMD and content BMC was measured by DEXA. Sub-cranial measurements (here also called whole body measurements) showed that the mice which dams had been fed beef during gestation and lactation had a higher aBMD and BMC, both at the whole body level and also in the lumbar spine. The group differences are believed to be induced by the beef or herring in the diet, as the diets were made to be similar on energy content, ingredients (except from beef and herring) and macronutrient content.

IGF-1 IN WEEK 12

Insulin-like growth factor 1 (IGF-1) is considered to be an important growth factor for bone tissue (Delany et al., 1994). It was higher in the postweaning herring group than in the beef group. This is in accordance with the positive effect seen by this postweaning diet in bone tissue in week 21 and later. However, as the measurements if IGF-1 (week 12) is closer in time to the DEXA measurements in week 9, the results are somewhat surprising. A study on adolescent boys given fish-oil, the IGF-1 levels increased yet there was found no association with bone mass (Damsgaard et al., 2012). This resembles our results. The authors themselves suggested there to have been a too short of an intervention period. It might be that the increased IGF-1 levels we found gave a later effect on the bones, which we then could measure after the mice were 21 weeks old.

DUAL X-RAY ABSORPTIOMETRY MEASUREMENT IN WEEK 21

In week 21 the groups with a maternal beef diet still had a higher aBMD and BMC both at the whole body level and in the lumbar region (L2-L5). However, now also the postweaning diet showed to have an effect on the bone quality, postweaning herring diet increased whole body BMC, aBMD and BMC in the lumbar region. These results suggest that different diets can affect bone health during different times of development. Saturated fatty acids have been reported to affect the absorption of calcium and by such mechanisms lover bone mineralization (Salem et al., 1992). It might be that there are compounds in the diet affecting the uptake of the offspring's calcium uptake. There might also be differences in the dietary vitamin D content, which is another factor important for bone health.

PQCT IN WEEK 22-26

Tibia tests were done on dissected (week 22-26) bone in connection with the clamp procedure. The pQCT testing showed that cortical bone volumetric BMD and BMC was increased in mice with beef-fed dams. Cortical area and thickness was also increased, as well as polar moment of inertia and resistance. There were interaction effects between preweaning beef diet and postweaning herring diet, when it came to volumetric BMD (vBMD), BMC and cortical area and thickness. Tibia length and trabecular BMD did not show any differences. That tibia length did not differ strengthens the results, as bone strength is dependent also on the bone length in addition to other structural and material properties (Leppanen et al., 2008).

BIOMECHANICAL TESTING WEEK 22-26

The maximal breaking load, F (max) of the tibia was higher in the maternal beef diet groups compared to the maternal herring groups. Stiffness and F (max) of the tibia were higher in the crossover diet groups than in groups remaining on their dams' diet. No differences were found in toughness.

The results show that at an early age, the offspring's BMD and BMC were dependent on the diet of the dams. Dams with a beef diet had offspring with higher mineral density and content. There are no good references to how to convert the age of 9 weeks in mice to human age, but they have at this point reached sexual maturity, which happens at 6-8 weeks.

Bone development in mice is different form in humans. For instance, rodents have growth plates until adult age and cortical bone thickness increase until 6 months of age, where after it declines (Halloran et al., 2002). There are as there is in humans differences in age-related bone loss in male and female C57Bl/6 mice (Jilka, 2013). One of the strengths of the study was its longitudinal design and with repeated measures results the bones could be reported for two time points.

The effects of the diets on the bone parameters are interesting as both maternal diet and postweaning diet may have effects on the offspring's bones. Even more intriguing was the finding that there were interactions between a maternal beef diet and the postweaning herring diet. Taken together it seemed as though the intake of beef during the gestation and lactation period was beneficial for the pups in young age, whereas later it was more beneficial for them to have a herring diet. The positive effects of the postweaning herring diets are in accordance with effects on n-3 PUFAs and fish-oil on bone quality (Li et al., 1999) and osteogenesis (Sun et al., 2003) found in the literature.

Also, mice from the same group (maternal beef group) had, as described in paper I, more body fat in week 9. As the mice in paper II was a selection of the mice included in paper I we did analyses for body composition in the mice we had tibia samples from and found no differences in fat percent. In adult humans adiposity has been linked to higher bone density, this has recently come into question and remains discussable (Reid, 2010). However, it is interesting that we saw both adiposity and higher aBMD and BMC in week 9 in offspring from dams fed beef.

Maternal diet can be parted into the preconception period, the gestation period and the lactation period (also called the perinatal period). In our studies we have not distinguished between these periods. As seen in paper II the maternal diet and the postweaning diet may interact. The lactation period in itself may play a role in offspring health as many nutrients are being provided by the breast milk and in our study cannot be distinguished from the gestational period.

In this paper we investigated the effect on the male offspring, and did not explore gender differences. Female mice might be different in their response to maternal and postweaning diet.

Table 2 Overview of results from paper I and II

Maternal herring diet	Postweaning herring diet	Maternal beef and postweaning herring diet (interaction effect)
↓Weekly weight gain	↓ Plasma TAG	↑ Cortical vBMD, BMC, area and thickness, F(max), stiffness
↓Body fat percent	↓ Plasma total cholesterol	
↓aBMD and BMC (subcranial and lumbar)	↑ Insulin sensitivity	
↓Cortical area and thickness	↓ n-6/n-3 ratio in soleus muscle, iBAT, liver, gonadal-, inguinal and peri-renal fat depots	
↓ Cortical vBMD and BMC	↑ iBAT	
↓Polar moment of inertia and resistance	↑IGF-levels	
	↑ Whole body BMC and lumbar aBMD and BMC	

PAPER III

Summary of findings:

The intervention group increased their fish intake from the 1st trimester to the 2nd and 3rd trimester.

They also had a lower meat intake in the 3rd trimester compared to the control group. The meat intake in the 1st trimester correlated positively with fat free mass gain.

In the 1st trimester phospholipid EPA and DHA were associated with fish intake and ARA with meat intake.

DHA levels in the serum phospholipids increased in both groups from the 1st trimester to the 2nd.

Arachidonic acid increased in both groups from the 1st to the 2nd trimester, but increased only in the intervention group from the 2nd to the 3rd trimester.

PARTICIPANTS

Almost half of the women were primiparous. The mean age was close to 30 years. Comparisons showed no differences in age, parity, and BMI between drop-outs and completers, and as such we believe the drop-out to not have caused any biased selection of participants. Reasons that were given for drop-out were either not given or often stated to be due to lack of time. The participants had a BMI of 22kg/m^2. The analyses done at baseline (1st trimester) included 36 control women and 37 women in the intervention group, whereas among the completers there were 17 women in the control group and 18 in the intervention group. Education level was high in the women that participated and as such there might be a selection bias. The study required follow-ups and visits at the university hospital in addition to filling out forms before each visit. This may have caused women with lower educations to refuse participation. It is also not uncommon for a selection of health conscious participants to participate in nutrition studies (Geissler and Powers, 2011). Statistical analyses showed no difference in age, parity or BMI between the drop-outs and the women that completed the study. The number of drop-outs and exclusions after recruitment are shown in Figure 6.

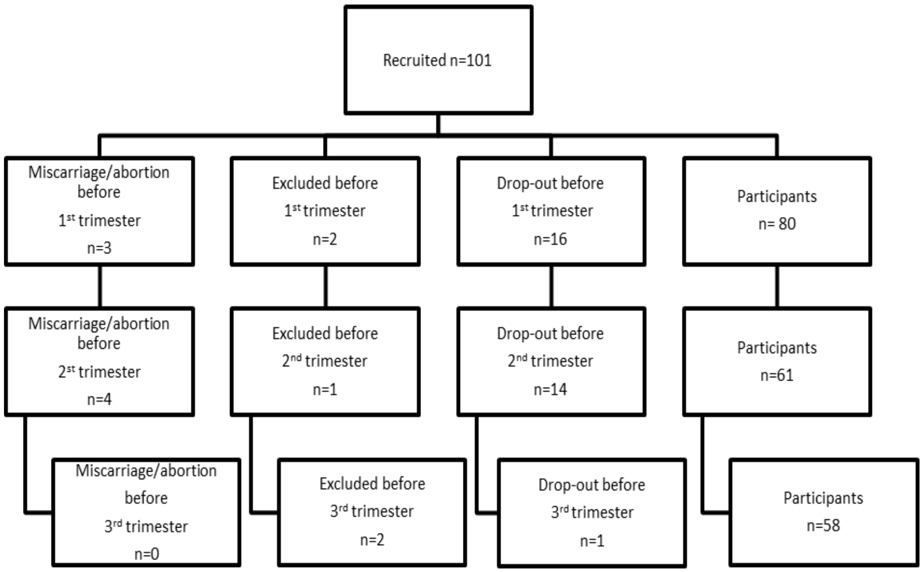

Figure 6 Drop-out and exclusions

FIRST TRIMESTER

First trimester data from the two groups were pooled for some analyses. Seventy-seven percent reached the recommendation of three servings of fish per week. In the 1st trimester EPA and DHA correlated positively with fish intake that was reported. Serum ARA correlated positively with meat intake in the 1st trimester.

In the 1st trimester FFM gain correlated positively with the meat intake. FM gain did not correlate with fish or meat intake in the 1st trimester. These findings are interesting and emphasize the need to distinguish between FM and FFM, rather than only investigating weight changes. Gestational weight gain had a tendency towards being positively correlated to meat intake (p=0.053).

The importance of gaining fat free mass during pregnancy is unknown. As FFM is both water retention and muscle mass the interpretation of the results are somewhat difficult to make. Measures of weight after birth would give a clear answer to if FFM is mostly water or muscles. If most of this were weight from water retention it is likely that these women would drop faster in weight after giving birth and as such have reduced weight retention.

MEAT, FISH AND ENERGY INTAKE

The intervention group significantly increased their fish intake from the 1st to the 2nd trimester and from the 1st to the 3rd trimester. In the 1st trimester, five women in the intervention group and nine in the control group reached the recommendation of three servings of fish per week, as did nine women in each group in the 2nd trimester and eight women in the intervention group and nine in the control group in the 3rd trimester. The increase in reported fish intake in the intervention group could be due to two possible reasons: 1. Actual increased intake 2. Over-reporting to please the researchers. Biased self-reporting is a known phenomenon in dietary intervention studies (Hebert et al., 1995).

Reported fish intake (Figure 7) in the 1st trimester was non-significantly lower in the intervention group than in the control group. The reason for this is considered to be by chance, as the women were randomized at inclusion. This could also have made it more likely for these women to follow advices on fish intake, as they could improve the fish intake.

Reported meat intake (Figure 7) was not found to change significantly during pregnancy in any of the groups. Meat intake was lower in the intervention group than in the control group in all three trimesters, but the difference was not statistically significant. In both groups, meat intake increased from 1st to the 3rd trimester, however not statistically significant.

Energy intake did not differ between the groups in any of the trimesters or within the groups between trimesters, but rose in the intervention group in each trimester, whereas in the control group it increased in the 2nd trimester and decreased in the 3rd trimester. The energy levels were within reasonable levels and the questionnaire is therefore believed to be suitable for our study population (Figure 7).

All dietary assessments come with biases. In this study dietary intakes were self-reported. It is known that socially desirable food groups lead to report bias (Hebert et al., 1995). Fish and meat intake was reported in a questionnaire on intake of these food groups, whereas energy intake was calculated based on a larger questionnaire. As such the women may have understood that the researchers were specifically interested in these food groups. As the groups were of limited size individual variations in biased reporting may affect the group differences seen.

All participants had been to high school or had higher education, which may have caused their baseline fish intake to be quite close to the recommendations, as a high fish intake has been associated to higher education (Mozaffarian et al., 2004).

The recommendations in the PONCH-study were three servings of fish per week. The Swedish recommendations are two to three servings per week. It was decided to set the recommendations to the upper limit. If we had set it to two the intake looked like this:

In the 1st trimester, 10 women (56%) of the intervention group and 15 women (88%) in the control group reached the recommendation of two servings of fish per week (p=0.060). In the 2nd trimester these percentages was 12 women (67%) in the intervention group and 15 women (88%) in the control group (p=0.228), whereas the percentages were 16 women (89%) for the intervention group and 15 (88%) for the control group in the last trimester (p=1.000). This shows that the intervention group became more similar to the control group in the 3rd trimester.

SERUM PHOSPHOLIPID FATTY ACIDS

The median concentration of ARA increased significantly from the 1st to the 2nd and from the 1st to the 3rd trimester in both groups, and also from the 2nd to the 3rd trimester in the intervention group. The DHA increased in both groups from the 1st to the 2nd and from the 1st to the 3rd trimester. EPA increased from the 1st trimester to the 3rd trimester in the intervention group, and was higher than the control group in the 3rd trimester, but the difference was not significant. The fatty acid levels in the two groups did not differ significantly between the trimesters. The EPA concentration correlated positively with reported fish intake during the 1st trimester in both groups. DHA correlated positively with fish intake in the 1st trimester in the intervention group. Meat intake was correlated negatively with s-EPA in the control group in the 2nd trimester. ARA did not correlate with the meat intake in any of the trimesters.

The lack of correlation between s-EPA and s-DHA to fish intake in the 2nd and the 3rd trimester could be a result the active transport of PUFA s, particularly DHA, across the placenta to the fetus, or that the PUFAs are used to synthesize prostaglandins. In the British “Salmon in Pregnancy Study”, did not find two portions of salmon per day to affect the inflammation that happens during pregnancy (Garcia-Rodriguez et al., 2012b), yet might affect antioxidant defences in the pregnant woman (Garcia-Rodriguez et al., 2012a). The women increased their circulatory EPA and DHA and also in the child’s circulation (Miles et al., 2011). The fish intake also changed the fatty acids in breast milk (Urwin et al., 2012). This shows that a fish intake might be beneficial both for maternal and offspring health. Also s-ARA might not be the best biomarker for meat intake as also fish is a source of this fatty acid. Serum EPA and DHA are markers for fatty fish; we did not separate these in this paper. The lack of correlations between the fatty acids and fish and meat intake might also have been caused by the lower number in women analyzed in each group in the 2nd and the 3rd trimester.

There was some concern that supplements could be disturbing the results. Use of supplements containing fish oil or n-3 fatty acids was reported only by women in the control group. In the 3rd trimester, significantly more women in the control group reported supplement use, however serum levels of EPA and DHA in the 1st, 2nd, and 3rd trimesters were not statistically different among users and nonusers of supplements. In the baseline population serum levels of EPA and DHA in the 1st trimester were not statistically different among users of fish-oil or n-3 supplements and non-users.

GESTATIONAL WEIGHT GAIN AND BODY COMPOSITION

Gestational weight gain was similar in the intervention and control group. In neither group did GWG or gains in FM or FFM correlate significantly with fish or meat intake in any of the trimesters. The gains in FM and FFM from the 1st to the 3rd trimester did not differ significantly between the two groups. To our knowledge, previous studies have not examined the associations between fish- and meat intake and changes in maternal body composition during pregnancy.

BIRTH WEIGHT AND BIRTH LENGTH

There were no significant differences in birth weight or birth length between the two groups. Fish- and meat intake was not associated with birth length or weight. Similarly, GWG, FM gain, and FFM gain did not correlate with birth weight or length. We did not have body composition measurements of the children, there might have been differences here. No significant correlations were found between baseline meat and fish intake and either weight or length at birth. Previous studies have linked fish intake to birth weight, but here it also was found that fish intake may increase the gestation length (Olsen and Secher, 2002).

Nordic studies have investigated whether the maternal intake of marine fats could have an effect on birth weight. The results are somewhat conflicting, but the possible increased birth weight might be caused by increased length of gestation, and there might also be negative associations with high intake of this type of fats (Olsen et al., 1993, Grandjean et al., 2001, Thorsdottir et al., 2004). The studies looking into the effect of maternal diet on birth weight have been of larger scale then our study, which may have influenced the results. We also did not distinguish between kinds of meat or fish, which could give different outcomes.

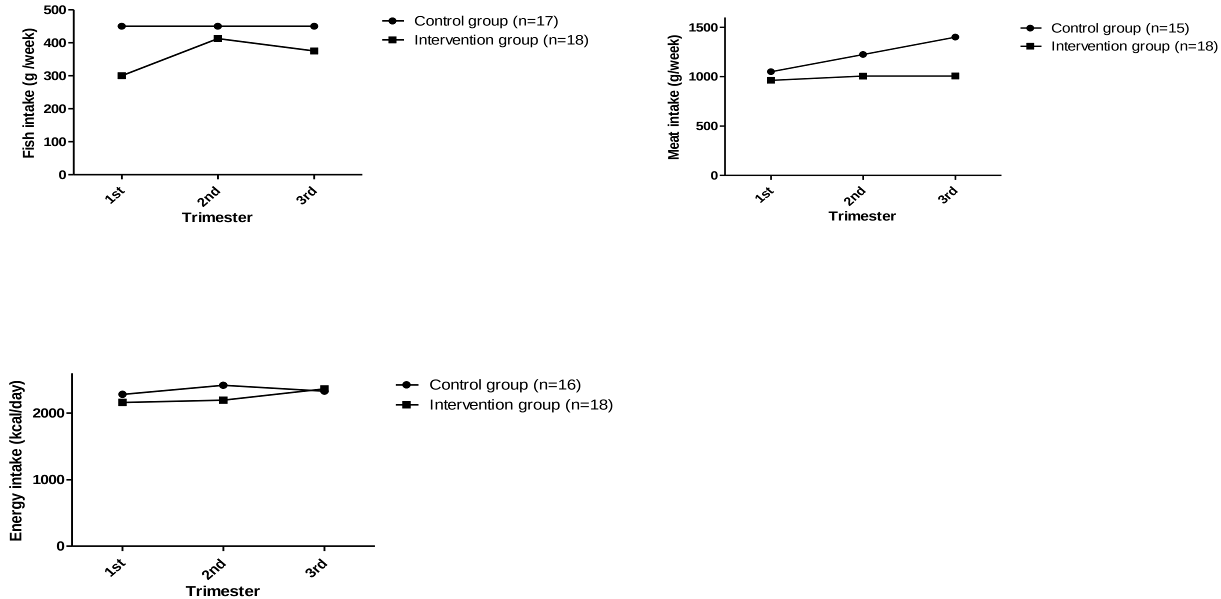

Figure 7 Median self-reported fish, meat and energy in each trimester

GENERAL DISCUSSION AND CONTRIBUTIONS TO CURRENT KNOWLEDGE

Often nutritionists are asked: "What shall we eat?" Unfortunately the answer "Eat a balanced diet "is perceived as somewhat boring. While doing the research included in this thesis the same question was raised. "So what shall we eat? Fish or meat?"

A WHOLE FOODS AND A WHOLE BODY APPROACH

As mentioned research has been conducted on fatty acids, but whole foods contain nutrients that interact and have both synergistic and antagonistic effects (Hoffmann, 2003, Jacobs and Steffen, 2003, Liu, 2003, Massey, 2003, Jacobs and Tapsell, 2007). More knowledge on victuals is needed to give recommendations on healthy food consumption. Recent literature is scarce on about health effects of victuals, rather than single nutrients. Although research on specific food groups was done during the early 20th century, it was not done with the same advanced techniques that are available today. As such there might be time to return from a reductionist approach and back to a more holistic one. In this thesis whole foods were investigated with a multidisciplinary approach including lab techniques, animal research, clinical research, nutrition, endocrinology, physiology, body and bone density and strength assessments. When research is done on one specific disease, tissue or cell types, the information is confined. A nutrient that may cause cancer in a cell type, might as a part of diet counteract negative effects of other compounds and as such protect the body from harm.

Whole foods research also raises the question of: how much? How much fish and meat should we consume? Considering only health aspects (e.g. not environmental or animal right aspects), this question should be investigated more thoroughly. There are national recommendations for adults on this issue, and some also for pregnant women, but they may not be based on considerations of the offspring's future risk for non-communicable diseases.

Both fish and meat contribute to important nutrients and to the energy intake. Their compositions are different and may play various roles in the development of tissues, which could also depend on period in life they are consumed. As it is morally ethically challenging to defend intervention studies with extreme diets in humans during pregnancy, animal research is preferred for this purpose. The clinical study involved women that ate both meat and fish. As normal diets involve several food groups and dietary patterns, this thesis has contributed to information on some aspects of fish and meat intake and opened up for research on other food groups and dietary patterns as well and added information to what was known about some nutrients (e.g. fatty acids). If one thinks of nutrients as the basis for current knowledge, this thesis has contributed to the current knowledge by going one step further and studied whole foods (Figure 8). The next level would be to study dietary pattern.

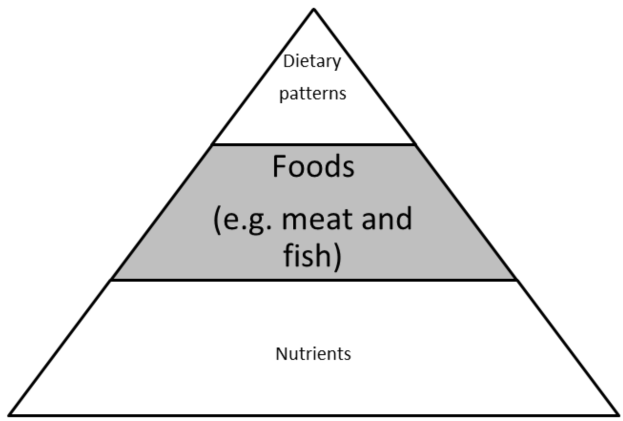

Figure 8 Level of contribution of this thesis to nutrition research

PREGNANCY ASPECT AND LONGITUDINAL STUDY DESIGN

In addition the studies on whole foods have rarely been done with a pregnancy/fetal programming aspect. Also the studies presented in this thesis were longitudinal with repeated measures which contribute to knowledge on effects at several time points. We found effects in pregnant women (paper III) and in young offspring and adult offspring (paper I and II).

A longitudinal design with repeated measurements gives information about the intervention effect at several life stages. This raises the question about positive and negative findings in cross-sectional studies. Studies that only investigate outcomes at one time point misses out important information and knowledge. In this thesis we have added the aspect of maternal diet and offspring diet (Figure 9). In paper III we described how concentrations of serum phospholipid fatty acids change during pregnancy. Many studies focus solemnly on one specific gestational week. The participants were healthy normal weight women and could for other studies serve as a “control” or “reference” for women with other characteristics in each trimester.

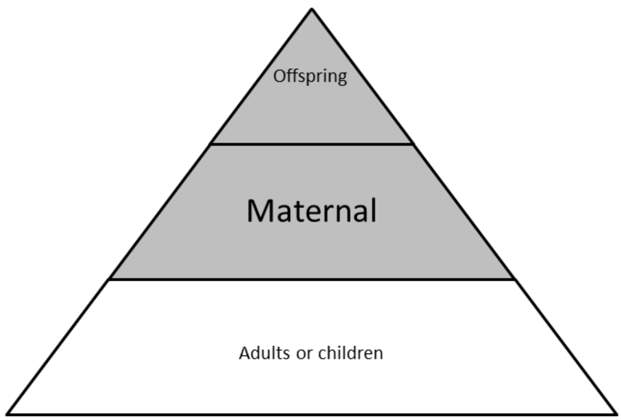

Figure 9 Level of information in this thesis on diet in different life stages

WEIGHT, BMI AND BODY COMPOSITION

The findings related to maternal and offspring body composition indicates the importance of separating weight and BMI into fat mass and fat free mass. The papers also show that through a maternal fish diet similar effect were found in the offspring as previous studies on n-3 fatty acids and fish-oil in adult humans or animals. Interestingly, maternal meat intake had metabolic potentially beneficial effects on maternal body composition, but lead to more adiposity in the mice offspring. Although there are issues with drawing conclusions based on data from both animal and clinical studies, this indicates that there might be very different effects of the same foods in different time points of life. Whereas meat intake might be positive for the pregnant woman's body composition it might not be beneficial for a nonpregnant adult person's own metabolic health to have a high intake of this. Also the quite opposite effects of the maternal beef diet and postweaning fish diet on bone health in paper II support this. We lifted the knowledge from weight and BMI by including body composition (Figure 10).

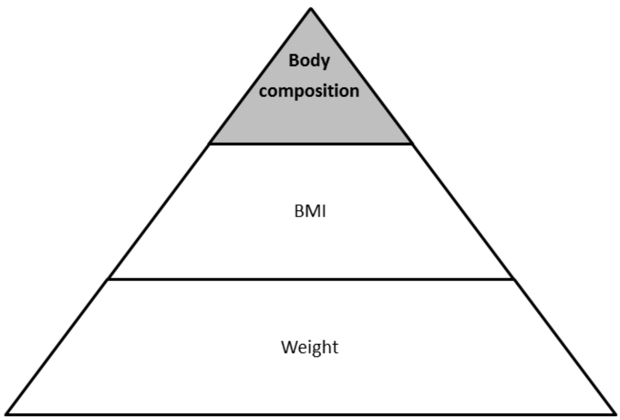

Figure 10 Level of information on body size in this thesis

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

As the gestational period is a crucial part in the development of an individual, nutrition interventions targeting this life period would benefit both mother and child health. Both maternal diet and postweaning diet affects metabolism, body composition and the bones of the offspring. Foods may have different health outcomes depending on which phase of development we are exposed to them. The interplay between nutrients and foods is complex.

In the animal studies we found that in a mouse model a maternal herring diet could reduce adiposity in the adult offspring. A maternal beef diet gave offspring with a more beneficial bone status. The postweaning herring diet lead to mice with better insulin sensitivity, more brown adipose tissue and a healthier levels of triglycerides and cholesterol in the blood. They also had better bone strength than mice fed beef in this period of life.

In the intervention study, it was found that meat intake in early pregnancy might be associated to a larger increase in fat free mass during pregnancy. We also found that the intervention group increased their fish intake during the study period.

This makes it possible to suggest that fish intake is a healthier food choice than meat for adult individuals, but that also meat intake may be beneficial for pregnant women, both for the development of the offspring and for the female's body composition during pregnancy.

Studies that elucidate differences between whole foods and single nutrients give valuable information on which foods to include as part of a healthy diet. Challenges of whole food studies are that they do not give clear answers to mechanisms but rather to descriptive health effects and are therefore not considered fulfilling serious medical scientific demands. We need to open up for accepting another level of uncertainty and scientific methodology when considering nutrition. Nutrition is a large component of our everyday life and is affected by several aspects of lifestyle. Fish and meat are important food components in the diet and their health effects should be explored beyond the effects of their fatty acid content. Studies that also look into the diet before pregnancy/gestation and during the lactation period should be conducted, and in all weight groups. A step further would be to look at dietary patterns rather than single whole foods.

Suggestions for future animal studies:

- Include both male and female offspring in the studies, as the results may differ with gender
- Distinguish between gestation, lactation and postweaning period by exposing the animals to the test diets in each of these periods and then put them on normal lab chow in the other periods
- That also looks into the health effects the diets have in the dams

Suggestions for human studies:

- Larger epidemiological studies on meat and fish intake during pregnancy and body composition, both in mother and child
- Intervention studies (if ethically approved) with increased fish or meat intake to compare the effect of these food groups
- Comparative studies which look at effects of fatty acid supplements and the food source they were extracted from
- Studies that distinguish between different types of fish and meat
- Include all weight groups (underweight, normal weight, overweight and obese of different degrees)

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The research animals I do not take lightly on using animals for research as animals cannot give their consent. However, recognize that it might be necessary to experiment on animals to contribute to scientific knowledge. This thesis is therefor also dedicated to the animals that were part of the research.

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