Body composition in women of reproductive age and during pregnancy

Method comparisons and gestational changes

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Gothenburg 2018

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ISBN 978-91-629-0442-5 (PRINT)
ISBN 978-91-629-0443-2 (PDF)

Printed in Gothenburg, Sweden 2018
Printed by BrandFactory

To my family

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ABSTRACT

Body composition measurements can contribute to assessment of nutritional status, both for clinical use and for research purpose. During pregnancy, body composition measurement is complicated by decreased density of the fat-free mass (FFM). Body composition assessment during pregnancy can be valuable for studies in unbeneficial gestational weight gain (GWG), as a large weight gain during pregnancy is associated with complications. In addition, nutritional intake during pregnancy is hypothesized to affect the growing foetus, and some polyunsaturated fatty acids (PUFA) are important for foetal development.

In these studies, body composition measurements by quantitative magnetic resonance (QMR) and bioelectrical impedance analysis (BIA) were respectively compared with air displacement plethysmography (ADP) in normal weight and obese non-pregnant women of reproductive age and during pregnancy. Non-pregnant women were analyzed in a cross-sectional study. Pregnant women were measured in each trimester, and ADP measurements were adjusted for changed FFM density. Also, effects of a dietary intervention study in normal weight women during pregnancy were analyzed, with focus on fish intake, serum phospholipid (s-) PUFAs (arachidonic acid, ARA; docosahexaenoic acid, DHA; eicosapentaenoic acid, EPA), and body composition changes. Additionally, effects of fish intake and meat intake in early pregnancy were analyzed, with focus on s-PUFAs, GWG, and body composition changes.

In non-pregnant normal weight women, fat mass (FM) estimates by QMR and by BIA were biased 1 kg compared with ADP. In non-pregnant obese women, FM estimates by QMR and by BIA were underestimated 2 kg and 9 kg respectively, compared with ADP. Total body water estimates by BIA were

larger compared with QMR estimates in both normal weight and obese non-pregnant women.

Reported fish intake increased from the first trimester to the second and third, respectively, in the group of normal weight women that received the dietary intervention. In early pregnancy, reported fish intake correlated with s-DHA and s-EPA, and reported meat intake correlated with s-ARA. In addition, reported meat intake in early pregnancy correlated with the subsequent maternal FFM gain.

GWG was 12 kg with 4 kg FM in normal weight women, and 9 kg with 2 kg FM in obese women. Cross-sectional FM and FM changes measured by QMR and by pregnancy-adjusted ADP were similar in obese women during pregnancy. BIA underestimated FM in obese pregnant women, compared with pregnancy-adjusted ADP. FM measured by QMR and FM changes measured by BIA yielded higher values than pregnancy-adjusted ADP in normal weight women during pregnancy.

In conclusion, dietary counselling during pregnancy may help women to increase fish intake. FM measurements by QMR and by BIA were biased in non-pregnant normal weight and obese women. QMR and the BIA equipment used here would need validation against gold standard methods during pregnancy, but the results indicate that the present software specific BIA equipment is unsuitable for FM measurements during pregnancy.

Keywords: body composition, pregnancy, women, polyunsaturated fatty acids, fish intake

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SAMMANFATTNING PÅ SVENSKA

Mätning av kroppssammansättning kan användas för att undersöka en individs näringsstatus, både inom sjukvård och för forskningsändamål. Under graviditet förändras vikten ofta, och även densiteten i den så kallade fettfria massan. Eftersom en stor viktuppgång under graviditet är förknippad med risker för kvinna och barn, så är det önskvärt att med korrekta metoder kunna undersöka om viktuppgångens kroppssammansättning har betydelse för komplikationer. Dessutom påverkas det växande fostret av näringsintaget under graviditeten, och vissa fleromättade fettsyror anses vara viktiga för fosterutvecklingen.

I denna avhandling jämfördes två metoder för kroppssammansättning (bioelektrisk impedansanalys (BIA) samt kvantitativ magnetresonans (KMR)), mot en tredje metod (luftpletysmografi) hos normalviktiga och feta icke gravida kvinnor i fertil ålder, samt hos gravida kvinnor. Hos de gravida kvinnorna korrigerades luftpletysmografimätningarna för den fettfria massans ändrade densitet, och kvinnorna mättes i varje trimester av graviditeten. Vidare, så utvärderades effekten av att ge normalviktiga gravida kvinnor kostråd. Utvärderingen fokuserade på fiskintag, förändring i kroppssammansättning och koncentration i serum av de fleromättade fettsyrorna arakidonsyra (ARA), dokosahexaensyra (DHA) och eikosapentaensyra (EPA). Dessutom undersöktes det om fiskintag eller köttintag i tidig graviditet hade ett samband med den kommande viktuppgången under graviditeten, förändring i kroppssammansättning, samt serumkoncentrationer av ARA, DHA och EPA.

Hos de icke gravida normalviktiga kvinnorna skiljde sig fettmassan mätt med BIA respektive KMR 1 kg jämfört med luftpletysmografi. BIA underskattade fettmassan med 9 kg hos de icke gravida feta kvinnorna, och KMR underskattade fettmassan med 2 kg.

Normalviktiga kvinnor som fick kostråd rapporterade ett ökat fiskintag under graviditetens andra och tredje trimester, jämfört med tidig graviditet. I tidig graviditet korrelerade rapporterat fiskintag med serumkoncentration av DHA och EPA, och rapporterat köttintag med serumkoncentration av ARA. Rapporterat köttintag i tidig graviditet korrelerade också med den kommande ökningen av fettfri massa under graviditeten.

Normalviktiga och feta kvinnor gick upp 12 kg respektive 9 kg under graviditeten, varav 4 kg respektive 2 kg fettmassa. Fettmassa i varje trimester samt förändring av fettmassa under graviditeten skiljde sig inte åt med KMR

och luftpletysmografi hos feta kvinnor. BIA underskattade fettmassa hos feta gravida kvinnor, jämfört med luftpletysmografi. Hos normalviktiga gravida kvinnor var fettmassa enligt KMR, samt förändring av fettmassa enligt BIA, större än motsvarande mätningar med luftpletysmografi.

Sammanfattningsvis, så antyder resultaten att kostrådgivning kan hjälpa kvinnor att öka fiskintaget under graviditet. Mätningar av fettmassa med KMR respektive BIA skiljde sig från luftpletysmografi hos icke gravida normalviktiga och feta kvinnor. KMR och den BIA-utrustning som användes i dessa studier skulle behöva utvärderas mot referensmetoder under graviditet. Resultaten indikerar dock att den specifika BIA som användes här inte är lämplig att använda för att mäta fettmassa under graviditet.

LIST OF PAPERS

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

I. Bosaeus M, Karlsson T, Holmäng A, Ellegård L.
Accuracy of quantitative magnetic resonance and eight-electrode bioelectrical impedance analysis in normal weight and obese women.
Clinical Nutrition 2014; 33: 471-7.

II. Bosaeus M, Hussain A, Karlsson T, Andersson L, Hulthén L, Svelander C, Sandberg AS, Larsson I, Ellegård L, Holmäng A.
A randomized longitudinal dietary intervention study during pregnancy: effects on fish intake, phospholipids, and body composition.
Nutrition Journal 2015; 14:1.

III. Bosaeus M, Andersson L, Karlsson T, Ellegård L, Holmäng A.
Body composition during pregnancy: longitudinal changes and method comparisons.
Manuscript under review.

CONTENT

ABBREVIATIONS

ADP	air displacement plethysmography
ARA	arachidonic acid
BIA	bioelectrical impedance analysis
BIS	bioelectrical impedance spectroscopy
BMI	body mass index
BW	body weight
D_B	body density
D_{FFM}	density of fat-free mass
D_{FM}	density of fat mass
DHA	docosahexaenoic acid
DXA	Dual-energy X-ray Absorptiometry
ECF	extracellular fluid
ECW	extracellular water
EPA	eicosapentaenoic acid
FFM	fat-free mass
FFQ	food frequency questionnaire
FM	fat mass
GDM	gestational diabetes mellitus
GWG	gestational weight gain
HDL	high density lipoprotein
ICF	intracellular fluid
ICW	intracellular water
IOM	Institute of Medicine
LBW	low birth weight
LCPUFA	long chain polyunsaturated fatty acid
LDL	low density lipoprotein
LGA	large for gestational age
NMR	nuclear magnetic resonance
PONCH	Pregnancy Obesity Nutrition and Child Health study
PUFA	polyunsaturated fatty acid
QMR	quantitative magnetic resonance
SGA	small for gestational age
TBW	total body water
VLDL	very low density lipoprotein

1 INTRODUCTION

1.1 MATERNAL OBESITY DURING PREGNANCY

Obesity is a global health problem, and a very large risk factor for noncommunicable diseases such as diabetes mellitus, cancer, and cardiovascular diseases (1). The World Health Organization (WHO) defines overweight and obesity in adults as a body mass index (BMI) ≥ 25 kg/m^2 and ≥ 30 kg/m^2 respectively, in both women and men (1). Furthermore, WHO reports that 15 % of adult women worldwide were obese in 2016 (1). Thirty-four percent of women of reproductive age (20-39 years) were obese in 2011-2014 in the United States (2). The Swedish Medical Birth Register reported that 25 % of Swedish pregnant women were overweight and 13 % were obese in 2014 (3).

Maternal obesity during pregnancy entails risks for a compromised health in both women and fetuses. Obese women have increased risk for developing gestational diabetes mellitus (GDM) (4-10), and pre-eclampsia (4-6, 8-10), to need delivery by caesarian section (4-9), for preterm delivery (5, 11, 12), post-partum infections (5), and shoulder dystocia during labour (5).

Babies to obese women are more likely to be large for gestational age (LGA) (4, 7, 8, 13, 14), have increased risk for macrosomia (4, 6), intrauterine fetal death (9), stillbirth (15), and higher perinatal mortality (5). Additionally, maternal obesity is associated with foetal malformations, such as congenital heart defects (16), increased risk for having babies with orofacial cleft (17), and maternal obesity is also pointed out as a risk factor for having babies with neural tube defects (18).

Also, maternal obesity brings increased risk for problems while initiating breastfeeding (19, 20) and for not breastfeeding at all (21). Furthermore, obese women were more likely to stop breastfeeding before six months (19).

High maternal BMI before pregnancy is linked to higher offspring childhood BMI and fat mass (FM), (22). Also, maternal obesity during pregnancy increased the risk for offspring childhood metabolic syndrome (23).

1.2 WEIGHT GAIN DURING PREGNANCY

The total energy expenditure for a singleton pregnancy in "well-nourished" women has been suggested to use approximately 370 MJ, with a maternal weight gain of 13.8 kg, of which 4.3 kg is fat (24). BMI range specific gestational weight gain (GWG) recommendations were revised and published in 2009 by the Institute of Medicine (IOM, United States) (25), with the aim to optimize both maternal and foetal health (Table 1). As a comparison to these GWG recommendation ranges; in a compilation of 245 526 pregnancies from 1994 to 2002, mean GWG was 14 kg in Swedish normal weight women, 11 kg in obese women, and 9 kg in morbidly obese women (26), i.e. a rather large mean gain in obese women compared with the present IOM recommendations. Furthermore, 30 % of the normal weight women gained > 16 kg while 30 % of the obese women and 45 % of the morbidly obese women gained < 8 kg (26). In 2011-12, 64 % of healthy nulliparous obese US women gained more than the IOM recommendations (27).

Table 1. Gestational weight gain recommendations in different pre-pregnancy body mass index classes.

BMI class	BMI range (kg/m^2)	GWG (lbs)	GWG (kg)
Underweight	< 18.5	28 – 40	12.7 – 18.1
Normal weight	18.5 – 24.9	25 – 35	11.3 – 15.9
Overweight	25.0 – 29.9	15 – 25	6.8 – 11.3
Obese	≥ 30.0	11 – 20	5.0 – 9.1

GWG, gestational weight gain. BMI, body mass index. From the Institute of Medicine guidelines (2009) (25).

1.2.1 LARGE GESTATIONAL WEIGHT GAIN

Large GWG is associated with adverse health outcomes. A large GWG increases the risk for developing pre-eclampsia in normal weight, overweight (28), and obese women (26). Also, a large GWG raises the risk for emergency ceasarian section in all BMI classes (29). Furthermore, GWG is positively associated with post-partum weight retention (30). However, the association between large GWG and risk for developing GDM is rather inconclusive and it appears that the timing of GWG is of importance (31-33).

Excessive GWG entailed increased risk for LGA babies in all pre-pregnancy BMI classes when BMI classes were analyzed separately (26), increased risk for macrosomia in normal weight women (34), and for macrosomia after adjusting for BMI before pregnancy (35). Also, GWG above the IOM guidelines from 2009 resulted in heavier babies at birth (36) and increased risk for having babies classified as LGA in normal weight, overweight (37), and obese women (28).

1.2.2 SMALL GESTATIONAL WEIGHT GAIN

Contrary, a small GWG is linked to increased risk for having babies that weigh < 3500 g at delivery (38) or are small for gestational age (SGA) (39), also in obese women (26, 37). A GWG under the IOM recommendations was also associated with slightly shorter Swedish neonates (40).

Consequences of large and small GWG have recently been neatly summarized by Goldstein *et al.* (41). A comprehensive meta-analysis and review over GWG and health outcomes included 23 cohort studies dated from 1999-2017, resulting in > 1 300 000 women, and women were categorized and analyzed in pre-pregnancy BMI groups (41). In total, 47 % gained more and 23 % gained less than the IOM recommendations (41). A GWG below IOM guidelines increased the risk for SGA and preterm delivery in each pre-pregnancy BMI group. A GWG higher than IOM guidelines increased the risk for LGA and macrosomia in each pre-pregnancy BMI group. Furthermore, Goldstein *et al*. reported that no GDM conclusions could be drawn (41).

In conclusion, both large and small GWG can be harmful. The model by Butte and King (24) suggested a GWG of 13.8 kg in "well-nourished" women. The updated IOM GWG recommendations from 2009 were developed to assure health of both women and fetuses / neonates. However, lower beneficial GWG ranges for specific BMI groups have been suggested, developed from almost 300 000 Swedish pregnancies (42), and a GWG < 7 kg in obese women was considered as safe (43).

1.2.3 GESTATIONAL WEIGHT GAIN INFLUENCES MATERNAL BODY COMPOSITION

Weight gain during pregnancy is naturally composed by both maternal and foetal tissues, and the separation of GWG into different tissues or components, i.e. body composition, can indicate if the type of tissue gains are connected with unbeneficial or even severe outcomes. Some previous studies investigated if maternal body composition in late pregnancy or body composition changes during pregnancy is associated with any outcome. The main outcome in these studies is birth weight, but also maternal post-partum weight retention;

Birth weight was associated with maternal fat-free mass (FFM) (44), FM (44, 45), and total body water (TBW) (46) near term, but absence of association with FFM (45) and FM (46) has also been shown. Maternal FFM gain and TBW gain (30) correlated with birth weight, whereas no association was detected for FM gain (30, 45, 47). Maternal post-partum weight retention was

associated with gain in FM but not with FFM gain (30). Also, maternal FM gain was associated with post-partum FM retention (30).

1.3 BODY COMPOSITION

1.3.1 CONCEPTS IN BODY COMPOSITION

Some basic concepts are useful knowledge in the body composition field. First, body composition theories, measurements and methods use assumptions of fixed relationships and densities (48), i.e. that the interindividual variation is considered low enough and therefore constants can be used. Some used densities are; water 0.9937 kg/L (49), fat 0.900 kg/L (50), protein 1.34 kg/L (49, 50), bone mineral 2.982 kg/L (49). Second, concepts that may be interchangeable in other settings need to be distinguished and defined in the body composition field;

LIPIDS, FAT, AND ADIPOSE TISSUE

Lipids, fat, and adipose tissue are not equal concepts in body composition methods. Lipids are extractable with lipid solvents such as chloroform, and is constituted by triglycerides, phospholipids, and structural lipids (51). Fat refers to triglycerides only (52). Adipose tissue contains adipocytes, extracellular fluid (ECF), nerves and blood vessels, and is the main location for fat (51).

LEAN BODY MASS AND FAT-FREE MASS

Lean body mass and FFM are not always equal components. Nowadays, the expression lean body mass is seldom used, and there is no uniform definition (51). FFM is defined as “the actively metabolizing component at the molecular level of body composition” (51).

TOTAL BODY WATER, INTRACELLULAR WATER, EXTRACELLULAR WATER, INTRACELLULAR FLUID, AND EXTRACELLULAR FLUID

TBW is the sum of intracellular water (ICW) and extracellular water (ECW) (52). Additionally, total body fluid is the sum of ECF and intracellular fluid (ICF). ECF, with approximately 94 % water, is located as interstitial fluid and plasma (52).

1.3.2 BODY COMPOSITION LEVELS

The body is conceptually separated in different *levels* in body composition theory; either in an atomic, molecular, cellular, tissue-system level, or whole body level (Figure 1) (52). Each level further result in the body weight (BW) (52).

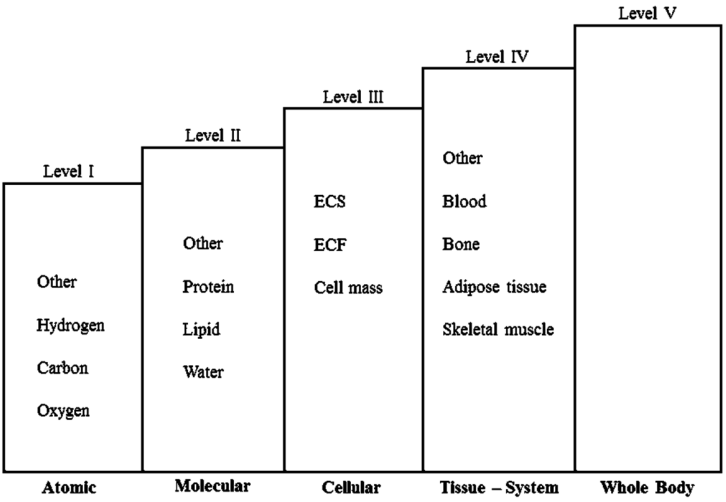

Figure 1. The five level model. Human body composition levels, figure drawn from original by Wang et al. (52). ECS is extracellular solids, and ECF is extracellular fluid.

Furthermore, body composition measurements in humans can be analyzed and organized into different number of components;

1.3.3 BODY COMPOSITION COMPONENT MODELS

The basic two-component model separates the body into FM and FFM, and is based on the assumption of fixed densities of FM and FFM, although it is well known today that the FFM density can vary in different conditions. The two-component model with hydrodensitometry was initially introduced by Behnke *et al*. in 1942, using Archimedes' principle; "low values for specific gravity indicate obesity and, conversely, high values denote leanness" (53). Later, William Siri proposed the commonly used two-component equation based on a FM density of 0.90 kg/L and a FFM density of 1.1 kg/L (50). Brožek *et al.* published a different two-component formula in 1963, based on a FM density of 0.9007 kg/L (49).

A three-component model separates the FFM into water and "residual" (54). A constant ratio of protein to mineral is assumed (50), and measurements of body density and TBW are combined (54).

Four-component models further add bone mineral content measurements to minimize errors from interindividual variation in bone mineral, which is part of the FFM (54). These models use a combination of BW, body volume, TBW and bone mineral content (54).

1.4 BODY COMPOSITION METHODS

There are several methods to measure body composition, where two or more methods also can be combined to yield different models. The best method choice for a certain measurement depends on factors such as equipment availability, economy (55), if radiation exposure is acceptable, demand on accuracy and precision, and mainly obviously on which tissue / component that is in focus. E.g. the two-component model yield FM and FFM, but cannot separate skeletal muscle mass from the FFM, or separate the foetus from the mother. In this thesis, air displacement plethysmography (ADP), bioelectrical impedance analysis (BIA), and quantitative magnetic resonance (QMR) were used for body composition assessment. Below follows an introduction to the methods used here, and also a brief description of other important body composition methods. When body composition studies by others are referred to in this thesis, mainly gold standard or at least reference methods were used, unless noted otherwise.

1.4.1 AIR DISPLACEMENT PLETHYSMOGRAPHY

ADP measures body volume by air displacement, and uses gas laws, such as Boyle's (56) and Poisson's law (57). That is, the relationship between volume and pressure given a constant temperature or an isolated system, respectively. The ADP technique uses two chambers (58). In the commercially used ADP system, there is one reference chamber of 300 L, and the subjects are seated in another chamber of 450 L (59). A movable diaphragm situated between the chambers is oscillated by the equipment, giving rise to complementary disturbances in the chambers (58). The volume disturbance causes minimal fluctuations in pressure, which first is analyzed for the estimation of the empty chamber volume (58). Thereafter, the procedure is done with the subject inside the large chamber, and body volume can thereafter be derived as the difference between the empty chamber volume and the chamber volume with the subject inside (58). During measurement, thoracic gas volume should preferably be measured, and the skin surface area is estimated by a formula, as these contribute to so called nonadiabatic conditions, i.e. loss or gain of heat (58). Correction for surface area and thoracic gas volume is accounted for in the calculation of body volume (59). As an alternative, thoracic gas volume can be predicted instead of measured (59). Another possible source of error, besides

the complexity of skin surface area and thoracic gas volume, is hair that may trap air and then result in falsely large body volume (60).

Body density is calculated as the ratio of BW to body volume (56), and the two-component body composition is thereafter estimated using densitometric equations, such as the Siri equation (50).

The densitometric two-component model is based on the following equations and relationships. BW is body weight (kg), D_B is body density (kg/L), D_{FM} is the density of fat mass (kg/L), and D_{FFM} is the density of fat-free mass (kg/L):

D_B = BW / body volume

$1/D_B = fm/D_{FM} + ffm/D_{FFM}$

fm/D_{FM} and ffm/D_{FFM} are the proportions of the fat mass (fm = FM (kg) / BW (kg)) and fat-free mass (ffm = FFM (kg) / BW (kg)) divided by their respective densities (57).

$$\% \text{ fat} = 100 \times [D_{FM} \times D_{FFM} / (D_B \times (D_{FFM} - D_{FM})) - D_{FM} / (D_{FFM} - D_{FM})] \quad (61)$$

TECHNICAL REPORTS ON AIR DISPLACEMENT PLETHYSMOGRAPHY

No significant difference was reported in measured and predicted thoracic gas volume (62, 63), and not in % fat by using these (63, 64). Reliability of ADP in almost a thousand women and men was assessed using duplicate measurements. There was no significant difference in mean of the first and second body density measurements, with a coefficient of variation (CV) reported as 0.15 % (65). Also, repeated measurements were reported with a technical error of 0.8 % fat (62).

VALIDITY OF BODY DENSITY BY AIR DISPLACEMENT PLETHYSMOGRAPHY

Body density by ADP has been validated against body density assessed by hydrodensitometry, and no significant difference was found in an elderly population (66) or in adult women (67). Also, body density was considered as accurately estimated by ADP in an overweight and obese population (68). However, biased body density by ADP has also been reported, with both significant and systematic underestimation (69), and significant overestimation (70).

FAT MASS BY AIR DISPLACEMENT PLETHYSMOGRAPHY

Mean FM by ADP was accurate compared with DXA in overweight and obese women (71), whereas ADP underestimated FM in adults compared with the four-component model, although with a mean bias of only 0.6 kg and not systematically, i.e. the difference did not depend on small or large FM (72). Also, ADP underestimated % fat compared with the four-component model in adult women (67) and in young adults (70), and underestimated mean FM compared with DXA both before and after a weight loss intervention (73). There was a significant overestimation of % fat by ADP compared with DXA in elderly (74, 75), but ADP was anyway considered by Bosy-Westphal *et al*. as a valid approach for body composition assessment in elderly (74).

For estimation of body composition *changes*, ADP was reported to correctly assess an exogenous addition of 1 kg FM (76). Also, changes in % fat during the above mentioned weight loss intervention were accurately assessed by ADP compared with DXA (73).

1.4.2 BIOELECTRICAL IMPEDANCE ANALYSIS

In the human body, water, which is part of the FFM (77), with electrolytes give rise to the lowest resistance, and is therefore the principal conductor of an electrical current (78). Capacitance from cell membranes together with resistance from ICF and ECF are combined into impedance (79). Impedance is also described as "the frequency-dependent resistance of a conductor to the flow of an alternating current" (78). Impedance methods use one single frequency or multiple frequencies (77). Single-frequency BIA is mainly performed at 50 kHz, which passes through both ICF and ECF (79), and can assess TBW and FFM (79). Multi-frequency BIA uses many frequencies to assess FFM, TBW, ICW and ECW from regression equations (79).

Also, there is the bioelectrical impedance spectroscopy (BIS) methodology which is based on mixture equations and mathematical modeling (79). BIS varies the frequency of the electrical current and then measures the reactance and resistance (78). Resistance at low frequency mirrors the ECW (78). At high frequencies, resistance mirrors both ECW and ICW (78). Currents at zero frequency or very high frequencies cannot be used, and therefore resistance at these frequencies must be predicted by mathematical modeling using the "Cole-Cole plot" (79). Furthermore, there is a mixing theory which states that resistance from a conductor increases if there are increasing quantities of suspended non-conducting particles (79). Both ICW and ECW can be assessed by BIS (80).

In addition to assessment of water compartments and FFM, impedance prediction equations have also been developed to estimate skeletal muscle mass by ourselves and others (81-83).

Impedance methods are considered as safe, easy to measure, and are not dependent on the operator (78). Electrodes are placed on the patient, and the potential drop is measured (78). Low skin temperature is a possible source of error during measurement (77), and standardized methodology is recommended to optimize BIA measurements, e.g. concerning previous food intake and exercise (84).

There are numerous BIA regression equations, developed from several equipment, and validated against varying reference methods in different populations. Validity of BIA body composition estimation is dependent on a regression equation that is adequate (78), and it should be validated against a reference method (79). Thus, it is challenging to get an overarching opinion on accuracy for impedance methods.

1.4.3 QUANTITATIVE MAGNETIC RESONANCE

QMR is noninvasive, safe (85), and highly precise (86, 87). The QMR methodology uses nuclear magnetic resonance (NMR) technique (88). There are different NMR signals from muscle mass, fat, and free water when different radio frequency pulses are applied at static magnetic fields (89). Static magnetic field puts hydrogen nuclei in a lower energy state (89). Application of an alternating radio frequency pulse flips hydrogen nuclei spins into a higher energy state (89). Thereafter, hydrogen nuclei return to the lower energy state and emit energy when the radio frequency pulse is turned off (89). NMR uses differences in hydrogen density and in relaxation time of the hydrogen spins to separate soft tissues (89).

Lean mass, fat, and free water are estimated by linear regression analysis, calibrated against lean animal tissues, canola oil, and tap water respectively (87). TBW is estimated as the difference between the total amount of protons and the fat found by regression analysis (87). Thus, the sum of free water and water in lean mass constitutes TBW by QMR (87). FM, free water, and lean mass by QMR from a measurement yields BW together (87). The output data from a QMR measurement yield total FM, total lean tissue mass, total water mass and free water mass.

1.4.4 OTHER BODY COMPOSITION METHODS

HYDRODENSITOMETRY

Hydrodensitometry, or underwater weighing, was the original densitometric body composition method, and is the gold standard for assessment of body volume (78). Nowadays, hydrodensitometry is less used in favour of ADP (48). During a hydrodensitometric procedure, BW is measured on a scale in air and in water, while breathing through a snorkel (60). Archimedes´ principle is used to assess body volume, and body volume = (BW in air – BW in water) / the density of water (57). Body density is calculated after adjusting for residual lung volume and air in the gastrointestinal tract (57).

DUAL-ENERGY X-RAY ABSORPTIOMETRY

Dual-energy X-ray Absorptiometry (DXA) was initially developed for measurement of bone mineral density, but can also yield precise measures of body composition (90). X-ray beams of different wavelengths are attenuated characteristically in different tissues (78). DXA measurements can identify and distinguish bone mineral content, lean soft tissue and FM (78, 90). Bone mineral content is one of the components used in the four-component gold standard model. Also, DXA scans can identify regional body composition, and e.g. differentiate between android and gynoid fat (77). The radiation from a whole-body DXA scan is approximately 5 µSv (91), which is comparable to the natural background radiation in Sweden, reported as approximately 0.04 µSv per hour (92) – reflecting 1 µSv per day.

HYDROMETRY

TBW can be estimated by dilution with isotopes that equilibrate with the TBW compartment; such as deuterium ($^{2}H2O$) (60), tritium ($^{3}H2O$) or H_2 ^{18}O (78). Subjects should be fasting and euvolemic at the time of measurement, and the isotope dose can be administered orally (93). A baseline sample of saliva, urine, or plasma is taken before administration of the isotope dose. After equilibration of 3 and 4 hours, plateau samples are taken (93). Alternatively, back extrapolation is used, with sample assembling up to two weeks after dose administration (56). The body water volume is calculated as the amount of tracer divided with the concentration in the body (78). However, there need to be a correction for exchange with nonaqueous molecules (78), and TBW is reported to be estimated with a precision of 1-2 % and an accuracy of 1 % (93). Estimations of ECW can be assessed by bromide dilution, which however slightly overestimates the ECW due to penetration of certain cells (93). Also, bromide concentrations in ECF distributes slightly unequally (93). Therefore, bromide dilution approximations of ECW needs correction, and an accuracy of

1 % was reported (93). ICW can be estimated as the difference from TBW and ECW by dilution (93).

MISCELLANEOUS

Whole body *magnetic resonance imaging*, and the four-component model (i.e. body density by densitometry, TBW by dilution technique, bone mineral content by DXA) are judged as body composition gold standard methods / models (48). Other important methods for body composition assessment that are not mentioned further here are *computed tomography* and *whole-body counting* by K^{40}, which are based on X-ray technique, and the natural occurrence of the radioactive K^{40}, respectively.

1.5 BODY COMPOSITION IN OBESITY

Obesity entails a large amount of adipose tissue. Additionally, obesity is associated with increased ECW and heightened ECW / ICW ratio (94). Increased water content lowers FFM density and may therefore introduce overestimation of FM in two-component models (95). Thus, the varying content in FFM is not adjusted for in the two-component model (96).

1.6 BODY COMPOSITION DURING PREGNANCY

1.6.1 BODY COMPOSITION CHANGES DURING PREGNANCY

During pregnancy, weight gain is composed by the foetus, placenta, mammary glands, uterus, plasma expansion, amniotic fluid, and also by adipose tissue gain. TBW expansion estimated by gold standard method during pregnancy was reported as 6-8 kg in all BMI classes (30, 97). Mean FM gain in normal weight women was 4-5 kg, whereas overweight and obese mean FM gain ranged between 0-8 kg (30, 97). Mean FFM gain was 9 kg in normal weight women and 10 kg in women with BMI > 26 kg/m^2 (30). Thus, differences between BMI groups in body composition changes during pregnancy was mainly reported for mean FM gain.

1.6.2 BODY COMPOSITION METHODS DURING PREGNANCY

During pregnancy, the gold standard method for body composition assessment is a four-component model (56, 98) using TBW by stable isotope dilution, bone

mineral content by DXA (before or after pregnancy), and densitometry (BW, body volume) (98). The expansion of body water, together with an only minor increase of bone mineral, during pregnancy (98) lowers the FFM density (99). Also, early pregnancy FFM gain is mainly maternal tissues, whereas later FFM gain is of lower density, mainly foetal (98). Use of the two-component model with an assumed fixed FFM density of 1.1000 kg/L will then overestimate the amount of FM (56, 60). Based on literature studies of FFM hydration and density during pregnancy, equations for estimation of FM from body density for any part of pregnancy were suggested by van Raaij *et al*. (100). The FM density during pregnancy is considered constant at 0.900 kg/L (100).

ACCURACY OF ADP, BIA, AND QMR DURING PREGNANCY

ADP has only been validated once during pregnancy, in the third trimester (101). However, the ADP results were statistically compared with post-partum DXA measurements (101), i.e. the timing of measurements give rise to uncertain interpretations of results.

Impedance accuracy studies are equation specific and equipment specific. Cross-sectional TBW compartments by BIS during pregnancy were both accurate (102) and biased (103). The BIA equipment used in this thesis has not been validated during pregnancy.

Accuracy of QMR has not been validated during pregnancy.

1.7 FAT

Fat is a common expression for a group of molecules. The main dietary fat is triglycerides, which consist of three fatty acids and one glycerol (104). Fatty acids have 4-22 carbon atoms, and are either saturated, monounsaturated, or polyunsaturated with hydrogen (104). Other important fats are phospholipids and sterols, such as cholesterol (105). Fats in the human body have several functions, e.g. being an energy source available for oxidation, precursors for synthesis of steroid hormones, precursors for eicosanoid production, and for incorporation into cell membranes (106). Free fatty acids in blood are transported together with albumin (105), and these contribute with about 2 % of lipids in plasma (104), corresponding to 300 mmol free fatty acids every 24 hours (106). Other fats, i.e. triglycerides, cholesterol, and phospholipids, are compiled with apoproteins into different sorts of lipoproteins, which circulate in the blood (105) from the intestines, to and from the liver, and to and from peripheral tissues (104). The main lipoprotein classes are chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins, low

density lipoproteins (LDL), and high density lipoproteins (HDL), of which chylomicrons and VLDL are rich in triglycerides, whereas LDL and HDL are rich in cholesterol (106). The phospholipid percentage in lipoproteins ranges between 6-35 % (106). Thus, as mentioned above, fatty acids are not only an energy source; they are important in cell membranes, but they also exert biological activity (107). Examples of biological activities are that fatty acids can give rise to eicosanoids, and affect transcription factors (107). Further description of function and dietary sources of important polyunsaturated fatty acids (PUFA) follow below.

1.8 HORMONAL AND PHYSIOLOGICAL ADAPTATIONS DURING PREGNANCY

The placenta is an endocrine organ that produces hormones such as progesterone, estrogens, placental growth hormone, human placenta lactogen, leptin (108), and pregnancy is a passing period of relative hypercortisolism (109). Thus, pregnancy entails large hormonal and physiological changes. It is noteworthy that the mean blood volume increases with approximately 50 % during pregnancy (110), corresponding to a plasma volume increase of 1200 (111)-1500 (112) mL (with data from Hytten and Chamberlain (113)), which thus may affect concentration but not necessarily amounts of circulating nutrients.

1.8.1 GLUCOSE AND FAT METABOLISM DURING PREGNANCY

Placental hormones affect, among other things, nutrient metabolism. In early pregnancy, peripheral insulin sensitivity is normal, with increased initial insulin response to oral glucose, increasing maternal fat storage (114). As pregnancy proceeds, maternal insulin resistance is heightened, with increasing insulin concentrations; which enhances the delivery of nutrients to the foetus / child (114). Insulin sensitivity decreases approximately 50-60 % during pregnancy (115). Insulin response increases in late pregnancy compared with before pregnancy (116). The mechanism for maternal insulin resistance during pregnancy is not fully understood (114), and has been connected with progesterone, estrogens, human placenta lactogen, glucocorticoids, and placental growth hormone (108).

Serum triglyceride levels are elevated already during trimester 1, and continue to rise throughout pregnancy (112), explained by increased estrogen and insulin resistance (117). Also, there is an increased circulation of cholesterol, fatty acids, phospholipids, and lipoproteins from the first trimester, and the

remaining pregnancy (114). In total, pregnancy goes from an anabolic condition to a catabolic condition which brings glucose and amino acids for the foetus / child, whereas lipids are used for maternal energy (114), characterized by accelerated lipogenesis and hyperphagia in early pregnancy and rising of maternal fat breakdown in late pregnancy (117). Maternal triglycerides first need to get hydrolyzed before placental uptake (117). Fatty acid transport across the placenta occur via simple diffusion, and also through fatty acid binding proteins, which can be bound in membranes or in cytoplasm (118). Composition of fatty acids that are transferred to the foetus is mainly decided by the maternal blood content, but there is also a selective transfer of PUFAs by the placenta (118).

1.9 POLYUNSATURATED FATTY ACIDS AND SEAFOOD INTAKE DURING PREGNANCY

1.9.1 ESSENTIAL POLYUNSATURATED FATTY ACIDS: DIETARY SOURCES AND FOETAL DEVELOPMENT

Some fatty acids are essential for health but cannot be synthesized by humans, and thus need to be a part of the dietary intake (119). These essential fatty acids are linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3), which can be further desaturated and elongated in humans (120). Linoleic acid and α-linolenic acid give rise to the two essential PUFA families ω-6 fatty acids and ω-3 fatty acids respectively (119, 121).

Docosahexaenoic acid (DHA, 22:6 n-3) and eicosapentaenoic acid (EPA, 20:5 n-3) are long chain PUFAs (LCPUFA) (122) formed from the essential fatty acid α-linolenic acid, which is an ω-3 PUFA (119), but only approximately 7 % of dietary α-linolenic acid is reported to be converted to EPA in plasma phospholipids, and even less into DHA (123). Arachidonic acid (ARA, 20:4 n-6) is derived from the essential ω-6 fatty acid linoleic acid (119). Linoleic acid and α-linolenic acid can be found in seed oils (119). EPA and DHA can be found in seafood, and are also produced in algae (124). Egg yolk and lean meat are dietary sources for ARA (119).

LCPUFAs are vital for optimal development (125, 126), and the essential PUFAs are critical for structure and function of membranes, therefore important when new tissues are developed during pregnancy (119). E.g., the Swedish National Food Agency recommends a daily intake of 200 mg DHA during pregnancy for non-fish eating women (127). DHA and ARA are essential contributors to the central nervous system (128), and are ample in the

infant brain (129), where DHA constitutes more than 90 % of the ω-3 PUFAs (130). Furthermore, DHA is accumulated in the retina (131). The foetus rely on maternal availability of LCPUFA (132). The transport from maternal to foetal circulation is reported as selective; DHA > α-linolenic acid > linoleic acid > oleic acid > ARA (133) – a so called biomagnification process suggested to depend on placental mechanisms (134).

Also, ARA is usually the major substrate for eicosanoid production (135). The ARA-derived eicosanoids are in most cases pro-inflammatory, and these may be counteracted by eicosanoids emanating from EPA, which however often have less biological activity (136).

1.9.2 BIOMARKERS OF POLYUNSATURATED FATTY ACIDS IN BLOOD: ASSOCIATION WITH TIME

Exogenously produced fatty acids may be used as dietary intake biomarkers (137). Immediate reflection of dietary intake after a meal can be estimated in fatty acids in chylomicrons (137), whereas blood triglycerides mirror the latest meals (138). Dietary intake of the last days can be estimated in plasma or serum individual fatty acids, phospholipid fatty acids, or cholesterol esters (137). Erythrocyte membranes likely mirror dietary intake of the past month (137) or months (139). However, changes of linoleic acid in erythrocyte phosphatidylcholine, plasma cholesterol ester and plasma phospholipid were associated and displayed a similar change rate over time; mirroring dietary intake of the past one-two weeks (140). Adipose tissue fatty acids can estimate the longstanding ω-3 fatty acid intake (141).

1.9.3 POLYUNSATURATED FATTY ACID LEVELS IN PLASMA AND ERYTHROCYTES DURING PREGNANCY

Concentration of PUFAs in blood can be assessed as either absolute or relative (i.e. percentage), and as previously described above, in different blood fractions such as plasma or serum phospholipids, or in the erythrocyte membrane. The absolute plasma phospholipid concentration of DHA increased during pregnancy (142), whereas the relative amount of ARA lessened (143). The relative concentration of ARA in erythrocyte phospholipids decreased during pregnancy (144). Furthermore, the erythrocyte membrane ARA concentration decreased during pregnancy, whereas no significant difference was detected in the corresponding EPA and DHA measurements (145). However, both the relative and absolute amount of DHA concentration in erythrocytes increased during gestation, whereas no significant differences were detected in EPA or ARA (139).

1.9.4 SEAFOOD INTAKE DURING PREGNANCY AND OFFSPRING HEALTH

A high periconceptional intake of lean fish was linked to increased risk for low birth weight (LBW, < 2500 g) (146), whereas intake of lean fish and shellfish, but not oily fish, in mid-pregnancy was positively connected with birth weight (147). Also, a high mid-pregnancy overall seafood intake was linked to a smaller risk for having a LBW neonate (147). Women that did not eat fish at all during late pregnancy were linked to a heightened risk for LBW (148). Also, no fish intake in trimester 3 was linked to intrauterine growth retardation (149). Furthermore, moderate maternal fish intake during pregnancy resulted in slightly heavier babies (150), and birth size was reported to rise with maternal fish intake (151). In conclusion, most, but not all, of these studies detected a positive connection between maternal seafood intake during pregnancy and the subsequent foetal growth measures. A review on ω-3 PUFA supplementation during pregnancy found poor / weak support for an association between supplementation and birth weight (132).

Also, a high fish intake during pregnancy is associated with elevated risk for obesity during childhood (152). A moderate maternal fish intake during pregnancy is connected with lower risk of preterm delivery (150), and a moderate fish intake during the first half of pregnancy was linked to a decreased risk for repeating preterm delivery (153).

2 AIMS

In papers I and III, the overarching common aim was to compare body composition measurements by QMR and BIA respectively with ADP in normal weight and obese non-pregnant (paper I) and pregnant (paper III) women.

In paper II, the aim was to evaluate the effect of a dietary intervention during pregnancy in normal weight women, with focus on fish intake and meat intake, serum phospholipid PUFAs, GWG, and body composition changes.

Specific aims paper I:

- To evaluate assessment of FM by QMR and by BIA against ADP in normal weight and obese women of reproductive age.
- To compare estimates of TBW by BIA and by QMR.

Specific aims paper II:

- To determine whether the longitudinal dietary intervention "*the Pregnancy Obesity Nutrition and Child Health (PONCH) study*" could increase fish intake, affect serum phospholipid PUFAs, GWG, and body composition changes during pregnancy in normal weight women.
- To study possible effects in early pregnancy of fish intake and meat intake, respectively, on serum phospholipid PUFAs, GWG, and body composition changes.

Specific aims paper III:

- To compare body composition during pregnancy by pregnancy-adjusted ADP, QMR, and BIA in normal weight and obese women respectively.
- To compare FM changes during pregnancy.

3 MATERIALS AND METHODS

3.1 STUDY POPULATIONS

This thesis was produced on data from two study populations of women of reproductive age (Table 2). The PONCH study is a longitudinal dietary intervention study in pregnant normal weight and obese women (paper II and III) (154, 155), and the Vitamin D study is a cross-sectional control study of non-pregnant normal weight and obese women (paper I) (156). Both studies were performed at the Department of Neuroscience and Physiology, Sahlgrenska Academy at the University of Gothenburg, and at Sahlgrenska University Hospital, Gothenburg, Sweden.

Table 2. Study populations and measurements in papers I-III.

	Paper I	Paper II	Paper III
Study population	Vitamin D	PONCH	PONCH
Study design	Cross-sectional	Longitudinal	Longitudinal
Study visits (n)	1	3	3
BMI class	Normal weight+Obese	Normal weight	Normal weight+Obese
n at inclusion	44 + 43	101	124 + 31
Non-/Pregnant	Non-pregnant	Pregnant	Pregnant
Measurements			
Body composition	ADP, BIA, QMR	ADP	ADP, BIA, QMR
Dietary assessment	–	Dietary questionnaire, Fish and meat FFQ	–
Blood analyses	–	s-insulin, p-glucose, s-phospholipid ARA, DHA, and EPA	–

ADP, air displacement plethysmography. BIA, bioelectrical impedance analysis. QMR, quantitative magnetic resonance. FFQ, food frequency questionnaire. ARA, arachidonic acid. DHA, docosahexaenoic acid. EPA, eicosapentaenoic acid. PONCH, Pregnancy Obesity Nutrition and Child Health study.

3.1.1 ETHICS

The Vitamin D and PONCH studies were approved by the local ethics committee at the University of Gothenburg, number 402-08 T600-09 and 402-08, respectively. Women received written and oral information, and signed an informed consent before entering the study. Recruitment processes are described below.

3.1.2 THE VITAMIN D STUDY

The Vitamin D study was initiated as a control study for the PONCH study, and focused on vitamin D intake and on body composition, explaining the

naming of the study population. Vitamin D intake was however not the scope for this thesis. The Vitamin D study is a cross-sectional study of normal weight and obese non-pregnant women of reproductive age (157), inclusion and exclusion criteria are presented below. Obese and normal weight women were recruited through advertisement in a newspaper and from public billboard posters, where 154 women initially were interested in participating, and 59 of these were included (158). Also, obese women were recruited from the Obesity unit at Sahlgrenska University Hospital, Gothenburg, Sweden, before they started weight loss treatment. Of 219 invited women at the Obesity unit, 39 were interested, and 27 of these were finally included (158). Women that were enrolled to the study were fasting at least 4 hours before study visit. All study visits were performed from September 2009 to October 2011. Measurements of ADP, QMR and BIA were done within 2 hours, see description of body composition measurement procedures below and in paper I. Recruitment and measurements of ADP and BIA were performed by Therese Karlsson, registered dietitian. Study flow chart is available in Figure 2.

Inclusion criteria Vitamin D study:

- Self-reported BMI 18.5-24.9 kg/m^2 or ≥ 30.0 kg/m^2
- Age 20-45 years

Exclusion criteria Vitamin D study:

- Smoking
- Pregnancy
- Non-European descent
- Severe psychiatric disorder
- Diseases / medications known to affect vitamin D status
- Vegan diet

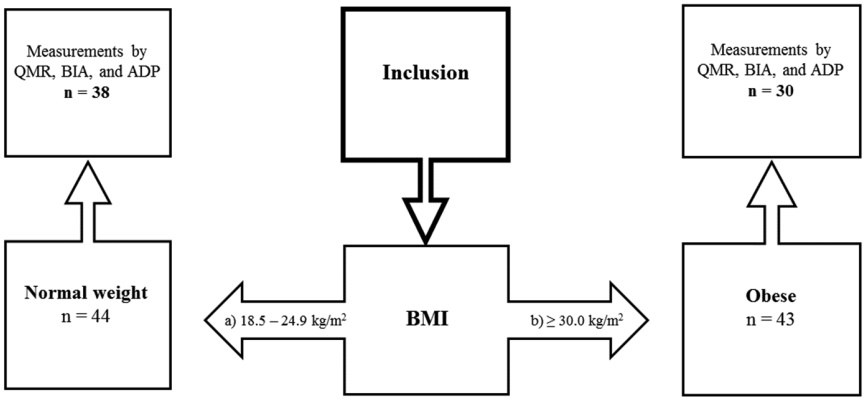

Figure 2. Flow chart of the Vitamin D study. ADP, air displacement plethysmography. BIA, bioelectrical impedance analysis. QMR, quantitative magnetic resonance.

3.1.3 THE PREGNANCY OBESITY NUTRITION AND CHILD HEALTH STUDY

The PONCH study started in April 2009 and is still an ongoing longitudinal randomized dietary intervention study in normal weight and obese pregnant women. Inclusion and exclusion criteria are presented below, with self-reported BW and body height used for inclusion BMI only. Women were recruited through maternity care centers in Gothenburg, by information given at websites addressed to pregnant women, and through postings at public billboards. Randomization into dietary counselling and control groups were matched for age, parity and BMI, using a computerized program developed at the unit. Included women visited the study at Sahlgrenska University Hospital in pregnancy weeks 8-12 (trimester 1), 24-26 (trimester 2), and 35-37 (trimester 3); aiming at performing study visits in all three trimesters of the pregnancy. In trimester 1, expected delivery date was counted from the first day of the last menstruation. In trimesters 2 and 3, the ultrasound done in the usual maternal health care dated the expected delivery. Women were instructed to be fasting overnight before study visits. Study flow chart of inclusion, randomization, and contents in study visits during pregnancy is available in Figure 3.

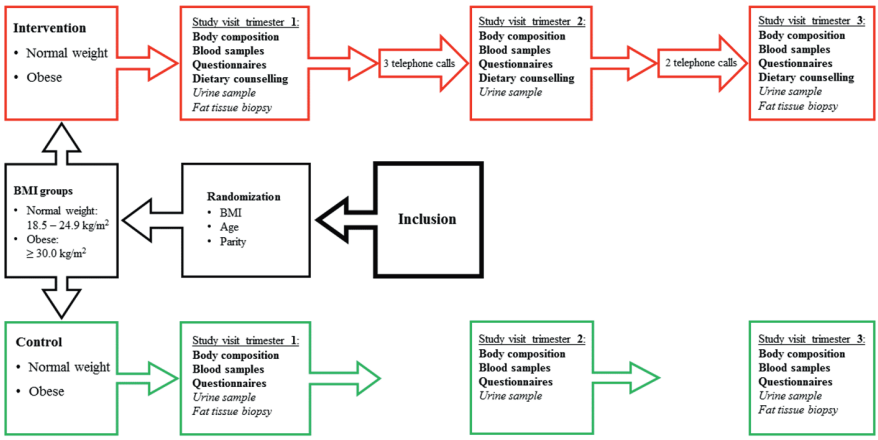

Figure 3. Flow chart of inclusion, randomization, and complete contents in study visits during pregnancy in the Pregnancy Obesity Nutrition and Child Health study. Measurements in italics were part of the study visits, but were not analyzed in the scope of this thesis.

For a comprehensive view, a complete study visit included filling in questionnaires on food intake (see below), psychiatric health, physical activity, and socioeconomic background before study visits, measurement of body composition by ADP, QMR, and BIA, waist- and hip circumference, blood samples, urine samples, and in the first and third trimester also a subcutaneous fat tissue biopsy. Results in this thesis used body composition measurements, blood sample analyses and data from questionnaires from women during pregnancy.

Although not the scope in this thesis, a brief compilation of additional partner and post-partum study parts follow below. Participants' partners were offered to participate; leaving one blood sample and filling in questionnaires on dietary intake and background information. Also, participants and partners were invited to take part of a second study part post-partum (Part II). The post-partum study part included continued study visits and proceeding with dietary intervention for the women 6, 12, and 18 months post-partum. Additionally, the second study part offered participation in following childrens' growth and health up to 6 years age, including measurement of body composition by ADP and questionnaires of dietary intake. A complete PONCH study visit protocol for women, partner, and children during pregnancy (Part I) and post-partum (Part II) is visible in Figure 4.

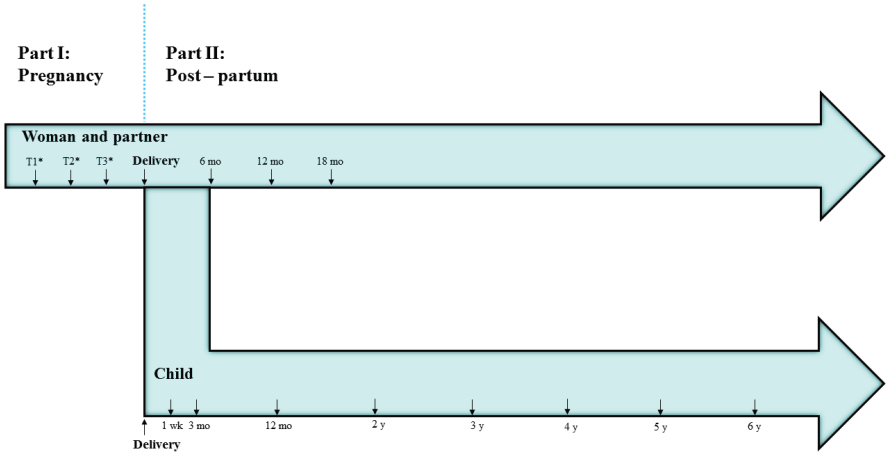

*Figure 4. Complete Pregnancy Obesity Nutrition and Child Health (PONCH) study visit protocol for women, partner, and children during pregnancy and post-partum. *Denotes time points included into this thesis. T1, trimester 1. T2, trimester 2. T3, trimester 3.*

Inclusion criteria PONCH:

- Self-reported BMI 18.5-24.9 kg/m^2 or ≥ 30.0 kg/m^2
- Age 20-45 years
- Singleton pregnancy

Exclusion criteria PONCH:

- Non-European descent
- Self-reported diabetes mellitus
- Use of neuroleptic drugs
- Vegetarianism or veganism
- Duplex pregnancy

3.2 ANTHROPOMETRY

Body height was measured to the nearest 0.5 cm, using a stadiometer. However, data input into the Tanita MC-180MA equipment only allows for whole cm. BW was measured by three different scales, one for each body composition method; i.e. ADP (Tanita BWB-627-A, modified by the ADP manufacturer), QMR (Tanita BWB-620, minimum weight graduation 0.05 kg),

and BIA (Tanita MC-180MA, minimum weight graduation 0.05 kg). Birth weight data was retrieved from hospital medical records after delivery.

3.3 BODY COMPOSITION MEASUREMENTS

ADP, BIA, and QMR were used for body composition measurements, and method characteristics are presented in Table 3. All methods are described in detail in each paper.

Table 3. Body composition method characteristics.

Method	ADP	Tanita MC-180MA	QMR
Examination time (~min)	2	1	4 × 3
CV %	2.4	3.0	0.3
Output body composition measures	FM, FFM	FM, FFM, muscle mass, TBW, ECW, ICW	FM, total lean tissue mass, total water mass, free water mass

ADP, air displacement plethysmography. QMR, quantitative magnetic resonance. FM, fat mass. FFM, fat-free mass. TBW, total body water. ECW, extracellular water. ICW, intracellular water. Coefficient of variation (CV) % from equipment used in this thesis. CV % for ADP and Tanita MC-180MA was calculated from duplicate FM measurements in 4 men, 2 obese pregnant women in trimester 1, and 16 non-pregnant women. CV % for QMR was calculated from triplicate measurements from data in paper I; i.e. non-pregnant women.

3.3.1 AIR DISPLACEMENT PLETHYSMOGRAPHY

Body volume measurement by ADP was conducted in a bathing cap in order to reduce a falsely large volume from the hair, and in light clothing. Bod Pod Gold Standard system (Bod Pod 2007 A, Life Measurement, Concord CA, software versions 4.2.1 and 5.2.0) was used to measure body volume twice, using predicted lung gas volume. The ADP software system requested a third volume measurement if the first two measurements were inconsistent. Body density was derived from data on BW and body volume. Body composition was either calculated with the Siri equation (50), and was output data from the ADP equipment; with assumed FM density of 0.9000 kg/L and FFM density of 1.1000 kg/L, or with pregnancy-adjusted equations, which takes into account that the FFM density decreases during the course of pregnancy. All pregnancy-adjusted equations were calculated with an assumed FM density of 0.9000 kg/L.

BW is body weight and D_B is body density.

The trimester 1 equation was published by van Raaij *et al*. (100), which did not state which FFM density that was assumed, but given from their figure 1, FFM density was approximately 1.099 kg/L. The FFM density in trimester 2 was

assumed to be 1.095 kg/L and the equation was developed by our laboratory, based on the FFM density data / figure 1 by van Raaij *et al*. (100). In trimester 3, FFM density was assumed to be 1.089 kg/L, and the equation by Hopkinson *et al*. (159) was based on the FFM data / figure by van Raaij *et al*. (100).

Siri equation: $\%\ \text{fat} = (4.95/D_B - 4.50) \times 100$

$\text{FM (kg)} = \%\ \text{fat} \times \text{BW} / 100$

Pregnancy-adjusted equations:

Trimester 1 (100): $\text{FM (kg)} = \text{BW} \times (4.964/D_B - 4.516)$

Trimester 2 (154): $\text{FM (kg)} = \text{BW} \times (5.0538/D_B - 4.6154)$

Trimester 3 (159): $\text{FM (kg)} = \text{BW} \times (5.19/D_B - 4.76)$

3.3.2 BIOELECTRICAL IMPEDANCE ANALYSIS

Body composition measurement by BIA was conducted standing in light clothing, barefoot on the Tanita MC-180MA multi-frequency eight-electrode equipment. The Tanita MC-180MA measures with a current ≤ 90 μA at 5 kHz, 50 kHz, 250 kHz, and 500 kHz. The manufacturer's proprietary software (160) used for body composition calculation are not public, i.e. cannot be presented here. The Tanita yielded both segmental and whole body composition data. In this thesis, output data of whole body TBW, FM, and FFM was used.

3.3.3 QUANTITATIVE MAGNETIC RESONANCE

QMR measurements were performed inside a chamber with a homogeneous low-intensity magnetic field of 6.5 mT (EchoMRI-AH, EchoMRI, Houston, Texas, US). Subjects were dressed in light clothing, and were halfway between sitting and lying down inside the chamber. The measurement chamber was shielded from external electric interference by using a copper net door. A single measurement lasted for three minutes, and each examination typically consisted of four contiguous measurements. Results were in most cases calculated as the mean of the last three measurements (papers I and III). QMR output FM and total water mass data was used. FFM was calculated as the difference between BW and FM. In paper I, TBW was also calculated as 0.73 × FFM.

3.4 QUESTIONNAIRES

Two questionnaires were used in paper II for assessment of dietary intake, and both questionnaires were used in connection with all study visits.

First, a self-administered dietary questionnaire, which was successfully validated in Swedish normal weight and obese men and non-pregnant women (161), assessed energy intake during the last three months. The questionnaire was described as a semiquantitative food frequency questionnaire (FFQ) (155).

Second, a FFQ with focus on fish and meat intake was designed at this unit. Weekly intake of cooked meals of fish, shellfish and meat was assessed:

"How many cooked meals per week do you eat of: a) Fish and shellfish b) Meat".

In paper II, the fish frequencies and meat frequencies were converted to grams, according to serving sizes recommended by the Norwegian Health Authorities (162); fish 150 g and meat 175 g.

Also, in paper II women were asked to fill in a questionnaire / were interviewed to ascertain any use of PUFA supplements containing fish oil / ω-3 fatty acids:

In trimester 1, women were asked;

a. "Have you taken any supplements (vitamins / minerals / other) the last 6 months before pregnancy?" Yes / No / Do not remember. If Yes, which supplement and frequency.
b. "Have you started taking any supplements since you became pregnant?" Yes / No. If Yes, which supplement and frequency.

In trimesters 2 and 3, women were interviewed during study visits: "Have you taken any supplements since the last study visit?" Yes / No / Do not remember. If Yes, which supplement and frequency.

3.5 DIETARY INTERVENTION

There was no dietary intervention in the Vitamin D study. A dietary intervention is a part of the PONCH study, but in this thesis the intervention was only analyzed in paper II. In paper III, women in the intervention group and control group were pooled into normal weight and obese BMI groups respectively, as independent samples *t*-test did not result in any significant difference in GWG or pregnancy-adjusted calculation of FM gain in intervention and control women.

Women assigned to the dietary intervention group received dietary counselling by registered dietitians at all study visits. Also, 3 telephone updates were done

between study visits 1 and 2, and 2 telephone updates between study visits 2 and 3. The intervention aimed at focusing on the advices for pregnant women published by the Nordic Nutrition Recommendations (NNR) 2004 (163):

1. Three meals of fish per week, including advices on types of fish.
2. Sugar intake < 10 E %.
3. A daily intake of 500 g fruits and vegetables, including advices on types and amounts of fruits and vegetables.
4. Increase daily energy intake with 350 kcal in trimester 2 and 500 kcal in trimester 3.
5. Obese women were advised to follow an energy restriction of 20 %. Energy requirement was calculated by the Harris-Benedict equation (164) for estimation of the basal metabolic rate, and a physical activity level of 1.4 was added. Obese women were not advised to increase energy intake in trimesters 2 and 3.

In addition to the recommendations above, individual advices were given by the dietitians when needed on nutrient density, fat quality, fibre intake, and food frequency, all according to the NNR (163).

3.6 BLOOD SAMPLES

Venous blood samples were drawn at all study visits after overnight fasting.

3.6.1 ANALYSES OF SERUM PHOSPHOLIPID LONG CHAIN POLYUNSATURATED FATTY ACIDS

Cecilia Svelander and Robert Jakubowicz performed gas chromatography-mass spectrometry analyses of LCPUFAs in serum phospholipids at the Division of Life Sciences / Food Science, Department of Chemical and Biological Engineering, at Chalmers University of Technology, Gothenburg, Sweden.

3.6.2 ANALYSES OF SERUM INSULIN AND PLASMA GLUCOSE

Clinical Chemistry at Sahlgrenska University Hospital, Gothenburg, Sweden (accredited laboratory, SWEDAC ISO 15189) analyzed serum insulin and plasma glucose. Serum insulin and plasma glucose were also used for calculation of Quantitative Insulin Sensitivity Check Index (QUICKI); 1 / (Ln(s-insulin)(mU/L) + Ln(p-glucose)(mmol/L)) (165).

3.7 RESULTING STUDY POPULATIONS

The analyzed study populations were selected differently in the three papers, with a reasonably different approach. The longitudinal design enabled drop-outs in the PONCH study (i.e. papers II and III), whereas the cross-sectional design in the Vitamin D study allowed for a less complex study flow and analysis process. Thus, the selection of data for analysis has been either focused on having maximum number of participants in each separate analysis, or focused on making subgroups with complete data;

1. In paper I (156), there were initially 44 normal weight and 43 obese women recruited. However, only women with complete ADP, BIA, and QMR measurements were selected for analysis, resulting in 38 normal weight and 30 obese women (Figure 2).
2. In paper II (155), the longitudinal design allowed for drop-outs and exclusion (Figure 5). Among the initially 101 recruited normal weight women, 73 women were analyzed as one group in trimester 1 ("*Early pregnancy"*, not divided into control and intervention groups). Also, analyses were done in a group of 35 women that participated in all three trimesters and had complete data on body composition, fish intake, and serum PUFA measurements; divided into control and intervention groups ("*Accomplished study visits in all trimesters*").
3. In paper III (154), maximum number of women were used in each separate analysis, from 124 / 88 / 76 normal weight participants in study visits trimesters 1 / 2 / 3. The corresponding obese participants in study visits were 31 / 25 / 17.

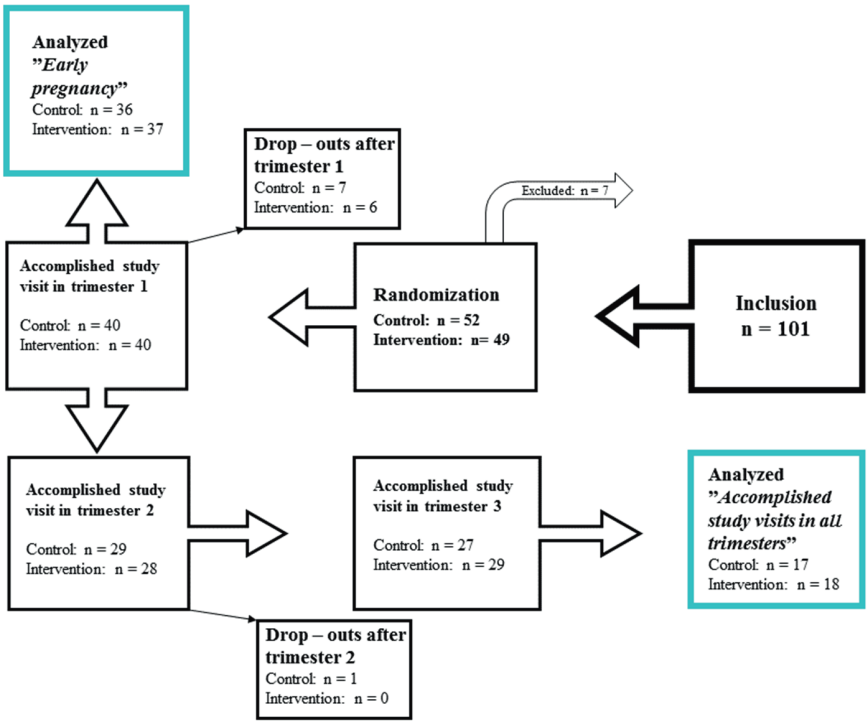

Figure 5. Flow chart of normal weight pregnant women in paper II. Illustration of included and excluded normal weight women, number of drop-outs, and the two normal weight populations analyzed in paper II, drawn from original figure (155). Control group and intervention group were pooled into one common group for "Early pregnancy" analysis in trimester 1. Also, analyses were conducted for women in the control group and intervention group that "Accomplished study visits in all trimesters".

3.8 STATISTICAL ANALYSES

Statistical analyses and methods used in the manuscripts in this thesis are described below.

Differences in number of participants (n) in baseline data and in different analyses were owing to missing data in some measurements, i.e. the number of women that participated in study visits were higher than the numbers for different measurements.

Statistical analyses were performed in PASW Statistics 18 (Release 18.0.2, IBM Software), and SPSS versions 21, 22, 23 (SPSS, Chicago, IL).

GWG and FM / FFM gain (Δ) were calculated as the difference between trimester 3 minus trimester 1. Differences in FM between trimesters 1 and 2, and trimesters 2 and 3 were calculated as the latest trimester minus the previous trimester.

Results of parametric analyses were presented as mean ± standard deviation (SD) or mean (SD). Results of non-parametric analyses were presented as median with interquartile range (IQR) (25^{th} percentile, 75^{th} percentile).

For parametric analyses, differences between groups were examined by using independent samples *t*-test, and differences within groups were analyzed by using paired samples *t*-test. For non-parametric cross-sectional analyses, the Mann-Whitney U test was used to compare data from two groups.

Correlations were examined with Pearson's correlation test or Spearman rank order correlation for parametric and non-parametric analyses respectively.

Friedman test followed by post-hoc Wilcoxon signed rank-test was used as non-parametric analysis for repeated measures.

Fisher's exact probability test was used to analyze if there was a relationship between two categorical variables, using crosstabs with at least one expected frequency < 5.

Box-plots were used to illustrate differences between methods, with first and third quartiles at box ends, median line inside box, whisker ends representing minimum and maximum values, and outliers denoted as rings outside whiskers.

Bland-Altman plots (166) were used for analyzing systematic bias in differences between methods. This figure illustrates and examines the relationship, using linear regression, between mean value of the two compared methods (x-axes), and the bias / difference between the two methods (y-axes). Limits of agreement were defined as mean ± 2 SD.

A p-value < 0.05 was considered significant, although Bonferroni correction for multiple testing was in some cases accounted for / discussed.

4 RESULTS

All results are described in detail in each paper. Below follows a description of baseline characteristics, a summary of key results, and subsequently an illustrative compilation of main findings.

4.1 BASELINE CHARACTERISTICS

Mean baseline age was consistently 31-32 years, except for a higher mean baseline age in the obese non-pregnant group (Table 4). Mean BMI was fairly equal in the non-pregnant and the pregnant normal weight groups. However, BMI in the obese non-pregnant group was 38.3 kg/m^2, but 34.6 kg/m^2 in the pregnant group.

Table 4. Baseline characteristics of participants.

	n	Age (years)	BMI (kg/m^2)
Paper I			
Normal weight	38	31.9 ± 6.7	21.4 ± 1.8
Obese	30	34.8 ± 6.2	38.3 ± 4.2
Paper II			
Normal weight pregnant control	35 – 36	31.2 ± 4.0	22.0 ± 1.3
Normal weight pregnant intervention	34 – 37	31.4 ± 3.9	22.0 ± 1.6
Paper III			
Normal weight pregnant	122	31.2 ± 3.8	22.1 ± 1.6
Obese pregnant	29	31.6 ± 3.3	34.6 ± 3.6

Mean ± SD.

4.2 ANALYSIS OF DROP-OUTS

In paper II, an analysis of study completers and drop-outs could not detect any differences in BMI, age or parity (Mann-Whitney U test).

4.3 SUMMARY OF KEY RESULTS

PAPER I:

- In normal weight non-pregnant women, mean FM was overestimated by QMR and underestimated by BIA, compared with ADP.
- In obese non-pregnant women, mean FM was underestimated by both QMR and grossly by BIA, compared with ADP.

- Mean estimates of TBW were considerably larger by BIA compared with TBW estimates by QMR in non-pregnant women.

PAPER II:

- Fish intake during pregnancy increased in the intervention group.
- In trimester 1, fish intake correlated with s-EPA and s-DHA.
- In trimester 1, meat intake correlated with s-ARA, and also with the subsequent FFM gain.

PAPER III:

- Mean FM gain during pregnancy, assessed by pregnancy-adjusted ADP, was 4 kg in normal weight women and 2 kg in obese women, whereas mean FFM gain was 7 kg in both BMI classes.
- In obese pregnant women, both mean FM and FM changes were similarly assessed by QMR and by pregnancy-adjusted ADP.
- In obese pregnant women, mean FM by BIA was grossly underestimated compared with FM by pregnancy-adjusted ADP.
- In normal weight pregnant women, QMR overestimated mean FM compared with FM by pregnancy-adjusted ADP.
- In normal weight pregnant women, mean FM changes were overestimated by BIA compared with FM changes by pregnancy-adjusted ADP.

4.4 PAPERS I AND III: COMPARISON OF BODY COMPOSITION METHODS IN WOMEN OF REPRODUCTIVE AGE AND DURING PREGNANCY

During pregnancy, GWG was 11.5 ± 2.9 kg in normal weight women (n = 71) and 8.8 ± 4.9 kg in obese women (n = 17). Δ FM by pregnancy-adjusted ADP was 4.1 ± 3.0 kg in normal weight women (n = 71) and 2.2 ± 4.1 kg in obese women (n = 17). Δ FFM by pregnancy-adjusted ADP was 7.4 ± 2.2 kg in normal weight women (n = 71) and 6.6 ± 3.5 kg in obese women (n = 17). Body composition during pregnancy is illustrated in Figure 6.

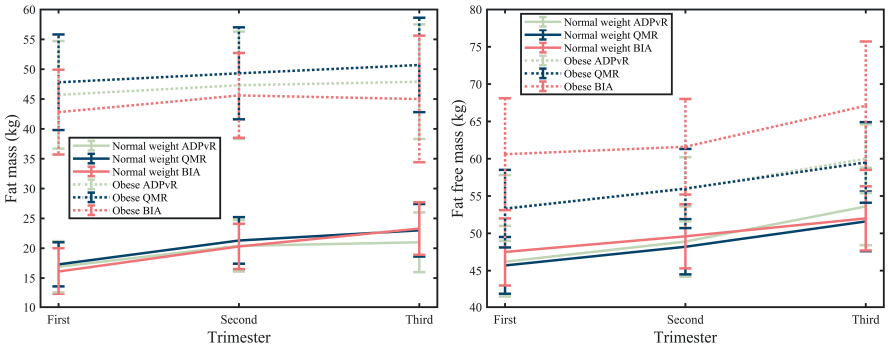

Figure 6. Body composition by three methods during pregnancy. BIA, bioelectrical impedance analysis. QMR, quantitative magnetic resonance. ADPvR, air displacement plethysmography calculated with pregnancy-adjusted fat-free mass densities. Mean with ± SD. Normal weight women n: ADPvR = 64, QMR = 50, BIA = 42. Obese women n: ADPvR = 17, QMR = 14, BIA = 10.

Body composition results for each method in each trimester, and data of all cross-sectional FM comparisons and comparisons of FM changes during pregnancy is available in paper III. In both papers I and III, FM by QMR and by BIA were compared with ADP in non-pregnant and pregnant women respectively.

Below follows first a description of FM results in trimesters 1 and 2, and of FM changes between trimesters 1 and 2, and between trimesters 2 and 3; all data details are available in paper III.

In trimester 1, mean FM by BIA was lower than by pregnancy-adjusted ADP in both normal weight and obese women, whereas mean FM by QMR was higher than by pregnancy-adjusted ADP in normal weight women, but no difference was detected in obese women.

In trimester 2, mean FM by QMR was higher than by pregnancy-adjusted ADP in normal weight women, but no difference was detected in obese women. Again, BIA yielded lower mean FM estimates compared with pregnancy-adjusted ADP in obese women also in trimester 2, but no difference was detected in normal weight women.

Between trimesters 1 and 2, mean FM change by QMR was similar to pregnancy-adjusted ADP in normal weight and obese women, whereas estimates by BIA were higher.

Between trimesters 2 and 3, both QMR and BIA yielded mean FM changes that were similar to pregnancy-adjusted ADP in obese women. However, both QMR and BIA yielded higher mean FM changes between trimesters 2 and 3 compared with pregnancy-adjusted ADP in normal weight women.

Data of cross-sectional FM comparisons in trimester 3 and in non-pregnant women, and of FM changes from trimesters 1 to 3 follow in Table 5 below. Mean FM by QMR was higher than FM by ADP in normal weight women both in the non-pregnant state (Figure 7) and in trimester 3 (Figure 8). However, in non-pregnant obese women, mean FM by QMR was lower than FM by ADP. Mean FM by BIA was much lower than FM by ADP in obese women both in the non-pregnant state and in trimester 3. On the contrary, in pregnant normal weight women, mean FM by BIA was higher than ADP in trimester 3.

During pregnancy, mean FM change from trimester 1 to trimester 3 (Δ FM) was estimated as 3 kg higher by BIA compared with pregnancy-adjusted ADP in normal weight women ($p < 0.001$).

Bland-Altman plots are illustrated within papers I and III, with ADP as reference method in both non-pregnant and pregnant (pregnancy-adjusted ADP) women;

Table 5. Comparison of body composition methods in women of reproductive age.

	Pregnant normal weight		Normal weight	
	Mean ± SD	p	Mean ± SD	p
FM: ADP – QMR (kg)	-2.5 ± 2.4 # [a]	<0.001	-1.1 ± 1.6 [d]	<0.001
FM: ADP – BIA (kg)	-2.1 ± 3.5 # [b]	<0.001	1.2 ± 3.4 [d]	0.032
ΔFM: ADP – QMR (kg)*	-1.6 ± 2.0 [c]	<0.001	-	-
ΔFM: ADP – BIA (kg)*	-3.0 ± 3.2 [c]	<0.001	-	-
	Pregnant obese		Obese	
	Mean ± SD	p	Mean ± SD	p
FM: ADP – QMR (kg)	-1.6 ± 3.3 # [e]	0.057	1.7 ± 1.9 [h]	<0.001
FM: ADP – BIA (kg)	4.7 ± 7.7 # [f]	0.035	9.3 ± 3.4 [h]	<0.001
ΔFM: ADP – QMR (kg)*	-0.5 ± 2.9 [f]	0.495	-	-
ΔFM: ADP – BIA (kg)*	-1.3 ± 7.6 [g]	0.630	-	-

#Denotes trimester 3. *Denotes difference between trimesters 3 and 1. FM, fat mass. ADP, air displacement plethysmography. QMR, quantitative magnetic resonance. BIA, bioelectrical impedance analysis. During pregnancy, ADP was calculated with pregnancy-adjusted fat-free mass densities (100, 159). Paired samples *t*-test. [a] n = 56, [b] n = 53, [c] n = 49, [d] n = 38, [e] n = 17, [f] n = 15, [g] n = 9, [h] n = 30

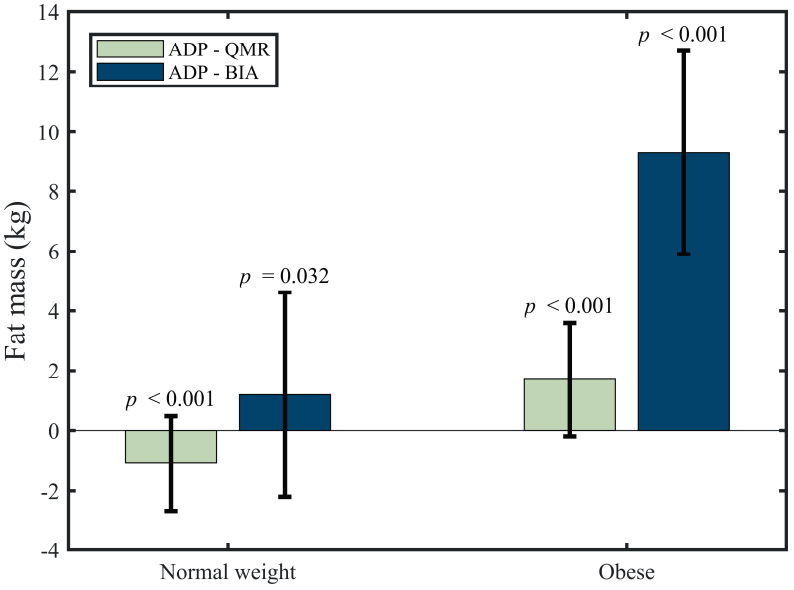

Figure 7. Comparison of body composition methods in non-pregnant women of reproductive age. ADP, air displacement plethysmography. QMR, quantitative magnetic resonance. BIA, bioelectrical impedance analysis. Mean with ± SD. Paired samples t-test. Error bars denote 1 SD in each direction from mean value. n: normal weight = 38, obese = 30.

Systematic significant biases between methods were not detected when non-pregnant normal weight and obese BMI groups were analyzed separately. However, when normal weight and obese non-pregnant women were analyzed as a combined group, it was shown that FM underestimation bias by BIA increased at higher FM values ($R^2 = 0.58$, $p < 0.001$). Also, the combined BMI groups analysis of non-pregnant women indicated that QMR overestimated FM systematically at lower FM values, and underestimated FM at higher FM values ($R^2 = 0.27$, $p < 0.001$).

Systematic significant FM differences between methods were also analyzed in pregnant women, with a slight systematic FM overestimation by QMR at low FM values in normal weight women in both the first ($R^2 = 0.06$, $p = 0.013$) and last ($R^2 = 0.08$, $p = 0.037$) trimester, and with systematically aggravated FM underestimation by BIA at high FM values in obese women during the middle trimester ($R^2 = 0.31$, $p = 0.006$).

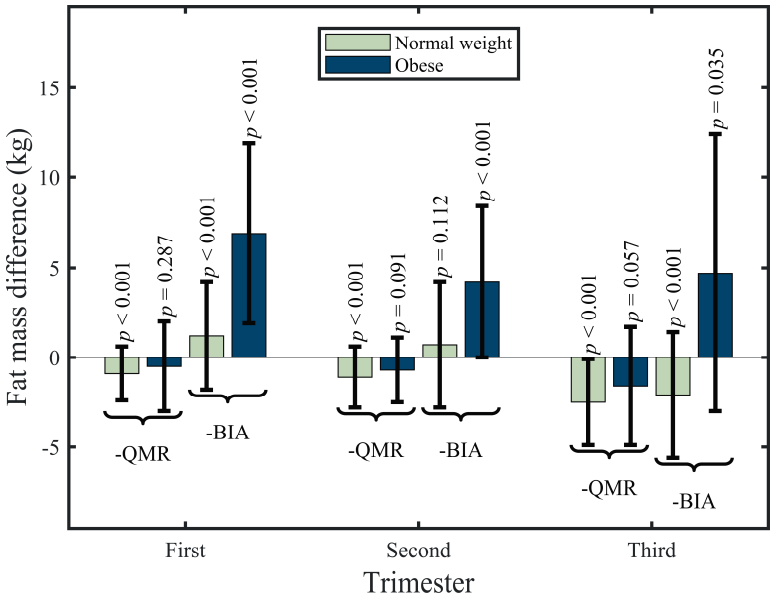

Figure 8. Comparison of body composition methods during pregnancy. Mean with ± SD. Paired samples t-test. Quantitative magnetic resonance (QMR) and bioelectrical impedance analysis (BIA) were compared with pregnancy-adjusted air displacement plethysmography. n normal weight women (trimester 1, 2, 3): QMR (98, 75, 56); BIA (95, 66, 53). n obese women (trimester 1, 2, 3): QMR (27, 21, 17); BIA (22, 23, 15)

As an additional comparison, which was not included in any paper, pregnancy-adjusted FM by ADP during pregnancy was compiled with the standard Siri output data from the ADP equipment (Table 6). In both normal weight and obese women respectively, the mean FM difference was approximately 1 kg in trimester 2, and 2 kg in trimester 3, with higher FM values from the Siri calculations.

Table 6. Body composition measured by air displacement plethysmography during pregnancy in normal weight and obese women.

	Normal weight (n= 64)			Obese (n=17)		
Trimester	1	2	3	1	2	3
$FM_{ADPSiri}$ (kg)	17.0 ± 4.3	21.4 ± 4.2	23.0 ± 5.0	45.9 ± 9.0	48.4 ± 9.0	50.0 ± 9.5
FM_{ADPvR} (kg)	16.9 ± 4.3	20.4 ± 4.3	21.0 ± 5.0	45.7 ± 9.0	47.3 ± 9.0	47.9 ± 9.6

ADP, air displacement plethysmography. FM, fat mass. Siri, calculated with the Siri equation (50). vR, calculated with pregnancy-adjusted fat-free mass densities (100, 154, 159). Mean ± SD.

During pregnancy, mean TBW estimates by BIA were consistently higher than QMR estimates in both normal weight and obese women (Table 7), data was

not included in any paper. Mean TBW in non-pregnant normal weight women was 3.8 ± 2.8 kg ($p < 0.001$) higher by BIA than by QMR, and the corresponding difference for obese non-pregnant women was 9.8 ± 3.6 kg ($p < 0.001$).

Table 7. Total body water during pregnancy in normal weight and obese women.

		Pregnant normal weight			Pregnant obese		
		n	Mean ± SD	p	n	Mean ± SD	p
TBW: BIA – QMR (kg)	Trimester 1	75	3.4 ± 2.0	<0.001	22	9.1 ± 4.3	<0.001
TBW: BIA – QMR (kg)	Trimester 2	60	3.3 ± 2.5	<0.001	20	6.7 ± 3.0	<0.001
TBW: BIA – QMR (kg)	Trimester 3	43	2.0 ± 2.2	<0.001	14	8.5 ± 5.5	<0.001

Comparison of total body water (TBW) by bioelectrical impedance analysis (BIA) and quantitative magnetic resonance (QMR). Paired samples *t*-test.

4.5 PAPER II: DIETARY INTERVENTION, FISH INTAKE, MEAT INTAKE, GESTATIONAL WEIGHT GAIN, BODY COMPOSITION, AND SERUM PHOSPHOLIPID POLYUNSATURATED FATTY ACIDS DURING PREGNANCY

In paper II, numerous data analysis were performed. All data and results are thoroughly presented in the paper, and below follows an overview of the most important and / or significant results. Analyses were initially performed for an *early pregnancy group* with all women in trimester 1 – before the dietary intervention had influenced the intervention group. Subsequently, *the dietary intervention group* and *the control group* were analyzed within each group separately, and the groups were also compared.

4.5.1 EARLY PREGNANCY GROUP

In the pooled early pregnancy analysis group in trimester 1, fish intake, meat intake, energy intake, and significant correlations are presented in Table 8. In early pregnancy, both s-EPA and s-DHA correlated with fish intake in normal weight women. Meat intake correlated with s-ARA. Also, early meat intake correlated with the subsequent Δ FFM.

Table 8. Early pregnancy / baseline group in trimester 1.

	n	Mean ± SD	r	p
Fish intake / week (g)	69	384 ± 210		
Meat intake / week (g)	69	1112 ± 525		
Energy intake / day (kcal)	69	2234 ± 543		
s-EPA × fish intake	69		0.36	0.002
s-DHA × fish intake	69		0.34	0.005
s-ARA × meat intake	69		0.28	0.02
Δ FFM × meat intake	45		0.39	0.009

Δ FFM denotes the change in fat-free mass assessed by air displacement plethysmography between trimesters 1 and 3. EPA, eicosapentaenoic acid. DHA, docosahexaenoic acid. ARA, arachidonic acid. Pearson's correlation.

4.5.2 ACCOMPLISHED STUDY VISITS IN ALL TRIMESTERS: DIETARY INTERVENTION GROUP AND CONTROL GROUP

FISH INTAKE

The intervention group increased their fish intake significantly from both trimester 1 to trimester 2, and from trimester 1 to trimester 3.

MEAT INTAKE

No statistically significant differences were detected in meat intake between or within the intervention group and the control group.

ENERGY INTAKE

No statistically significant differences were detected in energy intake between or within the intervention group and the control group.

SERUM PHOSPHOLIPID PUFA

Within both groups, median s-ARA and s-DHA increased during pregnancy (Figure 9). No significant longitudinal differences were detected for s-EPA, and neither any significant differences in s-ARA, s-DHA, and s-EPA between groups.

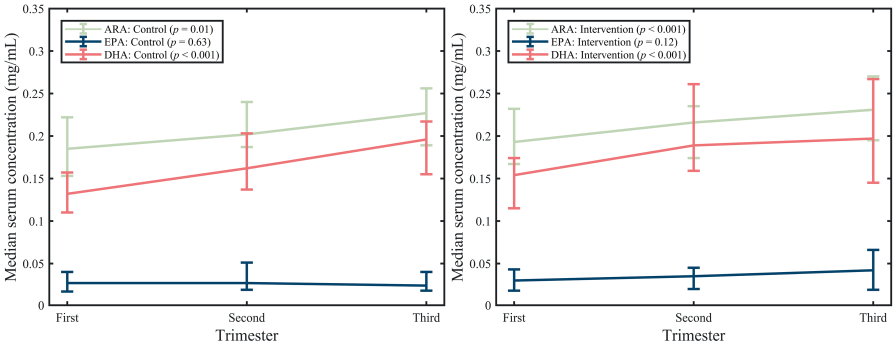

Figure 9. Serum levels of arachidonic acid (ARA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) during pregnancy. Median, interquartile range. p-values denote Friedman test within each group. n: control group (left) = 17, intervention group (right) = 18.

SERUM PHOSPHOLIPID PUFA, FISH INTAKE, AND MEAT INTAKE

In trimester 1, s-EPA correlated with fish intake in both groups, whereas s-DHA correlated with fish intake in the intervention group in trimester 1 and in the control group in trimester 2. For meat intake, only a negative correlation was found; for s-EPA in the control group in trimester 2.

GWG, BODY COMPOSITION CHANGES BY ADP, BIRTH WEIGHT, AND BIRTH LENGTH

No differences were found between the intervention group and the control group in body composition changes, and median GWG was 11.6 kg in both groups (ns). Also, there were no differences between the intervention group and the control group in babies' birth weight or birth length.

PUFA SUPPLEMENTS

Three women used PUFA supplements in the pooled early pregnancy group in trimester 1. Thereafter, women were analyzed in subgroups; no women in the intervention group used PUFA supplements, whereas 1 / 2 / 4 control women were PUFA supplement users in trimesters 1 / 2 / 3.

5 DISCUSSION

5.1 STUDY POPULATION

5.1.1 POSSIBLE SELECTION BIAS AND ASSESSMENT OF FISH INTAKE, MEAT INTAKE, AND ENERGY INTAKE

The study population in the PONCH study was highly educated, which could have introduced a source of selection bias, i.e. women that were already health conscious accepted to participate. Among the normal weight participants in paper II, 72 % and 76 % had at least three years of higher education in the control group and intervention group respectively. In the Swedish population among women aged 25-34 years, the corresponding education level was 36 % (167). During pregnancy, a high education level and a "Health conscious" dietary pattern were linked (168), whereas a low education level was linked to a low intake of ω-3 fatty acids in trimester 1 (169). A possible selection bias could be reflected in the number of normal weight women, 77 %, that already at baseline had three meals of fish every week, also expressed as a mean weekly intake of 384 g. As a comparison, women aged 31-44 years reported a 150 g less mean weekly intake of fish and shellfish in the large Swedish national survey Riksmaten (170). Mean meat intake was now estimated as 1112 g per week in trimester 1, compared with 644 g per week in the Riksmaten survey in women aged 31-44 years (170), i.e. almost the double amount. The Riksmaten survey collected data from a four-day food register, but also from a complementary FFQ, and it is not completely clear which fish and shellfish data that was analyzed; but the presentation indicates that the four-day food register including portion sizes was used. Thus, then a possible source of difference could be our standardized portion size of 150 g. Obviously, standardized portion sizes of 150 g for fish, and 175 g for meat, brings uncertain estimates of intake sizes. Also, we assessed energy intake, and estimation of energy intake has been reported as a challenge during pregnancy. As much as 45 % of pregnant women were detected as potential energy intake underreporters (171). Overweight and obese pregnant women were more likely to underreport energy intake, and a lower education level was also linked to energy intake underreporting (171).

If there was a selection bias with highly educated fish-eating women, this could influence the intervention in two ways. First, they can have been more prone to follow dietary advises, i.e. effects of the intervention would be too large and

not representative for the general population. However, it might also have been a lesser space for increasing fish intake, as they already had a high intake.

The Vitamin D study population was however rather different, as 2/3 of the normal weight women had at least three years of higher education, but only 21 % of the obese participants (158). That is, a rather low education level in the obese non-pregnant women.

5.1.2 OBESE PREGNANT SAMPLE SIZE

A large population was excluded from the PONCH study, namely overweight pregnant women. Inclusion of overweight women would have increased the number of participants. However, in order to have a clear distinction between BMI class differences, only normal weight and obese women were included. The rather small obese population sample size has been pointed out throughout the publication processes, and might have reduced the power to detect significant differences. Although hard work and enthusiasm from the PONCH study co-workers and midwifes at maternity care centers, it has been difficult to recruit obese women, and obese women are still being included, whereas the normal weight population inclusion was finished in September 2015. Difficulty to recruit obese women for intervention studies during pregnancy is a well known problem, has been reported by others (172-174), and was partly explained by a fairly low invitation rate of 65 %, and also that only about 6/10 accepted participance (173). However, for a truly fair comparison; there are much less obese than normal weight pregnant women in Sweden, i.e. there are simply fewer obese women than normal weight women to recruit. Roughly, there are approximately almost 5 times more normal weight than obese pregnant women in Sweden (3), and in paper III the start numbers in trimester 1 were 4 times more normal weight women than obese women, i.e. a rather representative number.

5.2 BODY COMPOSITION BY BIA AND BY QMR IN NON-PREGNANT WOMEN OF REPRODUCTIVE AGE

Mean FM was underestimated by Tanita MC-180MA and overestimated by QMR compared with ADP in normal weight non-pregnant women of reproductive age. Furthermore, mean FM was underestimated by both Tanita MC-180MA and by QMR compared with ADP in obese non-pregnant women.

The specific BIA equipment that was used in this thesis, Tanita MC-180MA, underestimated % fat compared with DXA in adults (175), and in obese adolescents (176), but could estimate mean % fat loss (176). Also, the Tanita MC-180MA underestimated FM compared with DXA in an elderly population (177). Furthermore, Tanita MC-180MA underestimated mean FM compared with DXA in overweight and obese women two- three months post-partum, but could assess mean FM changes one year later, although with wide limits of agreement (178). Thus, all studies, including the present one, conclude that Tanita MC-180MA underestimates mean FM / % fat in different types of populations, with different BMIs included, but could possibly assess mean changes.

Previous human cross-sectional QMR validation measurements both underestimated (87, 179) and overestimated (86) mean FM, with larger bias at higher BW (179). Mean FM decrease in obese subjects was underestimated approximately 1 kg by QMR compared with the four-component model (180). Thus, the previous cross-sectional QMR studies were inconclusive with mean FM biases in different directions, and our results in non-pregnant women adds to these conflicting differences. Interestingly, mean FM was 2 kg more by ADP compared with QMR in women (87), which is a difference in a similar direction as in our obese non-pregnant women.

5.3 FOOD FREQUENCY QUESTIONNAIRE: METHODOLOGICAL CONSIDERATIONS

Use of FFQ for dietary assessment is practical for assessment of intake of specific foods, and can be both qualitative, semiqualitative, and quantitative, with reference to portion sizes (181). A qualitative FFQ was used for assessment of fish intake and meat intake in this thesis. However, a FFQ should preferably be validated against a more exact reference method (181), which was not accomplished for the present FFQ. As we analyzed fish intake and meat intake using a non-validated FFQ, there could be methodological problems which we have not observed. The FFQ used in this thesis has additional data on types of fish (lean and oily), and of types of meat, such as pork, red meat, and poultry. Future PONCH analyses could therefore study effects of different types of fish and meat.

5.4 FISH INTAKE AND MEAT INTAKE IN NORWEGIAN AND DANISH BIRTH COHORTS: A COMPARISON

The two largest pregnancy cohorts in the world are Norwegian and Danish; the Norwegian Mother and Child cohort study (MoBa) and the Danish National Birth cohort (182). Both cohorts used FFQs as dietary assessment methods. The Danish National Birth cohort included 101 042 pregnancies from 1996-2002, and the MoBa cohort included 95 200 women from 1999-2008. Pregnant women filled in a FFQ during pregnancy week 25 in the Danish cohort, and during pregnancy weeks 17-22 in the MoBa cohort. The Danish pregnant women had a mean daily fish intake of 27 g, and the Norwegian pregnant women 37 g. The corresponding mean meat intakes were 108 g and 86 g, respectively (182). Thus, the Danish mean weekly fish intake and meat intake was 189 g and 756 g, and the Norwegian mean weekly fish intake and meat intake 259 g and 602 g, respectively. Compared with the MoBa cohort, weekly mean fish intake and meat intake in trimester 1 were now higher, especially the present meat intake. Furthermore, compared with the Danish National Birth cohort, weekly median fish intake and meat intake in trimester 2 were considerably higher in both the intervention group and the control group (paper II).

5.5 ADHERENCE TO DIETARY GUIDELINES DURING PREGNANCY

In early pregnancy, 77 % of the normal weight women already reached the goal of three weekly fish servings. We could however not detect that the energy intake was increased as suggested by the advices given in the intervention counselling, however with a hidden reservation for possible energy intake underreporting. Thus, the normal weight pregnant women did not adhere to energy guidelines, whereas already at baseline a large majority consumed fish according to the recommendation.

The first step to be able to adhere to dietary guidelines is, naturally, to have knowledge about the recommendations. In Australia, almost 2/3 of pregnant study participants were actually not familiar with the Australian dietary guidelines during pregnancy (183), and another Australian study of dietary intake during pregnancy concluded that none of the participants followed the dietary guidelines completely, although 6/10 still thought that their diet was healthy (184). Additionally, Australian adherence to dietary guidelines was

investigated in a nationally representative rather large group of pregnant women; there was a low adherence for “meat and alternatives”, “dairy”, and “cereals”, and half of the women reached the fruit intake recommendations (185). Furthermore, weekly seafood intake recommendations for American women during pregnancy is 8-12 oz. (186), corresponding to an amount of 227 g to 340 g. However, in a quite small observational study in Louisiana, United States, only 22 % of the pregnant women reported eating fish at all (186). Adherence to dietary guidelines during pregnancy in New Zealand was studied by an interview and a semiquantitative FFQ in a cohort study (187). Seafood and meat recommendations were however included and analyzed in a common food group, “Lean meat, meat alternatives and eggs”; and pregnant women were advised to consume at least two daily servings. Only 21 % had at least two daily servings of this food group (187), and further sub-group analyses on fish and meat were not presented.

The aim with dietary guidelines is of course to promote health, and adherence is intended to be beneficial. This was shown in the Norwegian MoBa cohort, where pregnant women who followed the Norwegian food guidelines seemed to be linked to lower weight retention after pregnancy (188).

5.6 DIETARY INTERVENTION IN NORMAL WEIGHT WOMEN DURING PREGNANCY

In the PONCH study, the dietary intervention focused mainly on diet quality, but also on energy intake according to the NNR (163). The dietary counselling aimed at reaching three meals of fish per week, and also advised the women to daily eat 500 g vegetables and fruits, to lower sugar intake, and in normal weight women also to increase energy intake in trimesters 2 and 3. However, in the scope of this thesis only fish intake, meat intake, and energy intake was analyzed. Fish intake during pregnancy increased within the intervention group. Few previous studies have aimed to improve diet quality in women of normal weight during pregnancy. In a Finnish study, women with mean normal weight BMI were given dietary counselling and foods in order to follow advises from the NNR 1996 (189). Women in the intervention group were advised to consume fish twice weekly, and in trimester 3 women in the intervention group had a 9 g higher daily mean fish intake compared with control women, with a mean daily intake of 29 g (189). The mean difference in fish intake was however not significant (189). The Finnish mean fish intake corresponds to approximately 200 g fish per week – i.e. considerably lower than women in the PONCH normal weight intervention group, but on the other hand women in the PONCH study were advised to consume fish three times

per week, whereas the Finnish advise was only two times per week. Again, a possible source of difference could also be the different food recording. We used a FFQ and standardized portion sizes, whereas the Finnish study used a three-days food record. In a Norwegian population-based study, all pregnant women in Trondheim were invited to a non-randomized intervention study; the control group was an earlier birth cohort. Thus, women of all BMI classes were invited. Women were advised to eat oily fish twice per week, and also to daily consume cod liver oil. During pregnancy, the intervention group had a higher fish intake frequency compared with the control group (190).

5.7 MATERNAL FISH- AND MEAT INTAKE AND BIRTH WEIGHT

5.7.1 BIRTH WEIGHT IN RELATION TO MATERNAL FISH INTAKE DURING PREGNANCY

No association was found between reported maternal fish intake during pregnancy and birth weight. As described in the Introduction, several studies have found positive associations between maternal fish intake during pregnancy and foetal growth measures. The lack of association here could however naturally be our true result. As previously described, women in the PONCH study had a high fish intake, with about ¾ already at baseline having three weekly fish meals.

5.7.2 BIRTH WEIGHT IN RELATION TO MATERNAL MEAT INTAKE DURING PREGNANCY

No association could be found between maternal meat intake and birth weight. Few studies have investigated possible connections between solely maternal meat intake during pregnancy and birth weight as outcome. Most studies reported dietary patterns, which we did not analyze in this thesis. In late gestation, poor maternal meat intake was linked to lighter birth weight (191), whereas maternal meat intake during pregnancy appeared to rise the risk for having a SGA neonate, based on data from a case-control study (192). Also, pregnant women eating a “Western” dietary pattern, including meat, were more likely to have a SGA neonate, compared with women that consumed a “Health conscious” dietary pattern with vegetables, fruits, fish, and poultry (193). Furthermore, there was a heightened risk for having SGA neonates connected with a dietary pattern rich in bread, soft drinks, and confectioneries compared with a diet rich in fish, vegetables, and rice (194), and poor

adherence to a “Mediterranean” dietary pattern in early gestation was linked to a lighter birth weight (195).

5.8 GESTATIONAL WEIGHT GAIN

5.8.1 GESTATIONAL WEIGHT GAIN IN RELATION TO RECOMMENDATIONS AND TO OTHERS

The mean GWG in normal weight women was in the lower range of the IOM GWG recommendations (25), whereas mean GWG in obese women was in the upper range of the recommendations, and also with a large variation. Furthermore, the mean GWG in normal weight women was lower than the 13.8 kg model (24). Additionally, mean GWG in both BMI groups was lower than in the previously described large Swedish compilation (26), which now however is getting rather out of date. A more recent compilation of data from the Swedish Maternal Health Care Register used data from 178 716 pregnancies in 2011 and 2012. Mean GWG for all BMI classes was 12.6 kg, but BMI class specific GWG was unfortunately not reported (29). Also, recently Swedish BMI specific GWG-for-gestational age z-score charts were developed, based on data from Stockholm and Gotland (196).

5.8.2 GESTATIONAL WEIGHT GAIN IN RELATION TO FISH INTAKE AND MEAT INTAKE

We did not detect any significant correlations between fish intake or meat intake during pregnancy and GWG. Associations between single foods and GWG are rather complex to study, as there are possible other determinants of GWG that are not always controlled for, and often dietary patterns are analyzed instead of single foods. However, there are some studies of the possible relation between fish intake and GWG, whereas few studied meat intake. Fulfilling the fish guidelines of two weekly meals was not connected with reaching appropriate GWG, and neither was intake of fruits or vegetables (197). On the other hand, in the Danish National Birth Cohort, there was a positive link between oily fish intake and GWG (198). Bärebring *et al.* (199) detected a positive connection between fish intake, bread intake, snacks intake, caloric beverages and excessive GWG in another sample of pregnant women in Gothenburg, Sweden. Red meat was not associated with excessive GWG (199). Also, a dietary pattern with “margarine, sugar, and snacks” was linked to a higher risk for excessive GWG (200).

5.9 BODY COMPOSITION DURING PREGNANCY

5.9.1 BODY COMPOSITION CHANGES DURING PREGNANCY IN RELATION TO OTHERS

Although a slightly lower mean GWG in normal weight women compared with the 13.8 kg model (24), mean FM gain was more similar to the model; 4.1 kg (154) and 4.3 kg (24) respectively. The normal weight mean FM gain was rather similar to the FM gains in normal weight women reported by gold standard measurements by Butte *et al*. (30) and Lederman *et al*. (97). Mean FM gain in strictly obese women was reported as only 0.2 kg (97), i.e. lower than our results. A much larger mean FM gain was detected in a combined overweight and obese group, 8 kg (30) – a substantially higher value compared with the present 2 kg in obese women (154).

5.9.2 BODY COMPOSITION CHANGES DURING PREGNANCY IN RELATION TO FISH INTAKE AND MEAT INTAKE

In trimester 1, reported meat intake correlated with the subsequent FFM gain in normal weight women. No other studies could be found that investigated the possible relation between maternal body composition changes during pregnancy and maternal fish intake or meat intake. Possible mechanisms for the connection between meat intake and FFM gain are beyond the scope for this thesis, but diets rich in protein have been associated with positive results on FM, and proteins of milk origin were linked to muscle protein synthesis (201). However, pregnant women have a heightened protein requirement, but approximately 12 E % is enough, and this is in most cases covered by the usual diet (202). As a comparison, in the Riksmaten survey women aged 31-44 years had a mean protein intake of 17 E % (170). Future PONCH study analyses of maternal meat intake and subsequent FFM gain would benefit of controlling for other available parameters, such as physical activity.

5.9.3 BODY COMPOSITION DURING PREGNANCY: METHODOLOGICAL CONSIDERATIONS

QMR was similar to pregnancy-adjusted ADP for assessment of mean FM and FM changes in obese women during pregnancy, whereas QMR overestimated mean FM in normal weight pregnant women. BIA by Tanita MC-180MA

underestimated mean FM grossly in pregnant obese women, and overestimated mean FM changes in normal weight pregnant women.

Whole body composition of woman and foetus as one unit was measured by three safe methods; ADP, BIA, and QMR. The body composition measurements in pregnant women were not compared with the gold standard four-component model, including hydrometry with deuterium or tritium dilution for TBW assessment. Use of tritium during pregnancy is not safe, but deuterium would however had been an excellent gold standard method, and has been used by others during pregnancy (101, 203). The PONCH study, with a longitudinal design, includes many measurements. Thus, including deuterium measurements was unfortunately not practicable.

ADP, which is a densitometric two-component model, has only been scarcely validated during pregnancy. Reported mean FM in late pregnancy by pregnancy-adjusted ADP was about 6 kg more than by a four-component model, but no statistical analyses were presented on these two methods (101). However, the use of a densitometric two-component model using hydrodensitometry during pregnancy has been evaluated. Compared with a four-component model, van Raaij pregnancy-adjusted two-component densitometry by hydrodensitometry estimated mean FM gain accurately during pregnancy in a small sample, whereas the standard Siri equation overestimated mean FM gain (204). In trimester 3, there was no significant difference in mean FM by the four-component model and by van Raaij pregnancy-adjusted hydrodensitometry, whereas the standard Siri equation overestimated mean FM (159). Compared with a three-component model, using body density, deuterium, and BW, pregnancy-adjusted two-component hydrodensitometry could assess mean % fat accurately in pregnancy weeks 14 and 32 (203). Also, FFM density was reported as 1.093 kg/L in pregnancy week 32 (203), and as 1.087 kg/L in pregnancy week 36 (159), which can be compared with 1.089 kg/L used in trimester 3 in this thesis. Additionally, a higher biological variability in FFM density was reported in pregnancy week 14 compared with pregnancy week 32 (203). Our ADP equipment had a CV % of 2.4 % in duplicate FM measurements. A high CV % could derange a methods' possibility to follow longitudinal changes accurately. In conclusion, although not evaluated in mid-pregnancy, and the biological variability in FFM density, there is support for use of pregnancy-adjusted densitometric two-component models for assessment of mean FM on a group level in early and late pregnancy, and for FM gain estimation.

As mentioned in the Introduction, QMR accuracy has not been validated during pregnancy. The highly precise QMR method has several possible

advantages during pregnancy; as changes in FFM hydration should not affect FM results (88), which otherwise influence accuracy of two-component models, and the NMR measurements are probably not affected by body shape (86). Also, the measurement procedure was well tolerated by the study participants. However, free water could possibly influence FM results, with a minimal FM overestimation in the presence of free water (88). In conclusion, QMR measurement during pregnancy would gain of more studies for assuring accuracy, preferably against gold standard methods.

Only few studies have validated accuracy of impedance during pregnancy. Cross-sectional TBW by BIS in each trimester was similar to TBW by deuterium in a small sample of pregnant women (102). Cross-sectional ECW and TBW by BIS, when using software by the manufacturer in pregnancy weeks 14 and 32 respectively, were lower compared with the corresponding bromide / isotope dilution values (103). Also, increases of ECW and TBW from pre-pregnancy to trimester 3 by BIS were biased compared with reference dilution methods (103). Although the present study does not validate BIA against a gold standard methodology, our results indicate that the Tanita MC-180MA BIA equipment in general is unsuitable for FM assessment and FM change assessment during pregnancy. Furthermore, the present BIA CV % of 3.0 % for FM could be compared with our previously reported TBW CV % of 2.8 % for another BIS equipment (205), i.e. an acceptable CV % in the same magnitude. In conclusion, impedance measurement accuracy during pregnancy have shown rather discouraging results, but would gain of more validation studies from more different equipment, and also in larger populations.

5.10 DHA, EPA, AND ARA IN BLOOD: BIOMARKERS FOR NUTRITIONAL INTAKE

In early pregnancy, fish intake correlated with s-EPA and s-DHA, whereas reported meat intake correlated with s-ARA in normal weight women. In trimesters 2 and 3, the control group and intervention group were separated, and a positive correlation could only be detected in the control group for fish intake and s-DHA in trimester 2.

Pregnancy is a complex state with increased blood volume, and the addition of the placenta and the foetal circulation. When fish intake in trimester 2 was assessed by both a weighed food diary, and with a FFQ, a positive association was detected for DHA dietary intake (including supplements) and DHA proportion in erythrocytes, but not for EPA (206). Furthermore, nutrient intake

in Canadian pregnant women in trimester 3 was assessed by a FFQ, and erythrocyte fatty acids were analyzed. Fish was the main contributor of DHA and EPA, and the main sources of ARA were meat, eggs, and poultry. The ratio of ARA to DHA plus EPA in erythrocyte membranes was lower with higher reported fish intake (207).

In the non-pregnant state, blood levels of EPA and DHA are used as biomarkers for fish intake, e.g. were DHA and EPA plasma phospholipid levels positively associated with nonfried fish intake, assessed by a FFQ (208). Also, serum EPA and DHA were used as biomarkers for fish intake in European adolescents (209). Reported red meat intake was positively connected with plasma ARA, whereas a reported high fish intake was linked to high plasma EPA and DHA in adults, dietary intake was assessed by a semiquantitative FFQ (210). Furthermore, intake of ARA correlated with consumption of foods with animal origin, and intake of EPA and DHA were associated with consumption of fish and seafood in adults; dietary intakes were assessed by 24-hours recalls (211).

There are studies that investigated the feasibility of, and found support for, EPA, DHA, and ARA in blood as biomarkers for dietary / supplementation intake. Postprandial plasma DHA mirrored herring consumption (212), and supplementation with fish oil yielded higher plasma phospholipid EPA, compared with controls (141). Furthermore, plasma phospholipid and erythrocyte DHA and EPA were linear biomarkers for intake in a rather small but thoroughly controlled sample (213). In a carefully controlled cross-over study, men received two different diets; 2 × 3 weeks with one high saturated fat diet, and one high unsaturated fat diet. Erythrocyte fatty acids were analyzed, and the high unsaturated fat diet resulted in higher erythrocyte ARA, and correlations were significant between dietary DHA and erythrocyte DHA (214). Also, although a very small sample, plasma phospholipid ARA indicated an increase after one week with a high ARA content diet (215).

Use of supplements must be considered when fatty acids in blood and dietary intake are analyzed. A low percentage of the normal weight women used PUFA supplements during pregnancy in this thesis; none in the intervention group and only 6-24 % in the control group. As a comparison from the Norwegian MoBa cohort, 62 % of normal weight women in pregnancy weeks 17-24 used fish oil / cod liver oil (216), i.e. a considerably higher Norwegian percentage.

6 SUMMARY AND CONCLUSIONS

In these studies, body composition measures by three methods were compared in normal weight and obese non-pregnant women of reproductive age and during pregnancy. Also, a dietary intervention in normal weight women during pregnancy was evaluated, with focus on fish intake. Additionally, serum phospholipid levels of DHA, EPA, and ARA were measured during each trimester of pregnancy.

In non-pregnant normal weight women of reproductive age, mean FM estimates by QMR and by BIA were biased 1 kg compared with ADP. In non-pregnant obese women of reproductive age, mean FM estimates by QMR and by BIA were underestimated 2 kg and 9 kg respectively, compared with ADP. Mean TBW estimates by BIA were larger compared with QMR estimates in both normal weight and obese non-pregnant women.

Reported fish intake increased from the first trimester to the second and third, respectively, in a group of normal weight women that took part in a dietary intervention. In trimester 1, reported fish intake correlated with serum phospholipid DHA and EPA, and reported meat intake correlated with serum phospholipid ARA in normal weight women. Also, reported meat intake in trimester 1 correlated with the subsequent maternal FFM gain.

Mean GWG during pregnancy was 12 kg in normal weight women, and 9 kg in obese women. Mean FM gain during pregnancy, assessed by pregnancy-adjusted ADP, was 4 kg in normal weight women and 2 kg in obese women. Mean cross-sectional FM and FM changes measured by QMR and by pregnancy-adjusted ADP were similar in obese women during pregnancy. Mean FM was underestimated by BIA in obese pregnant women, compared with pregnancy-adjusted ADP. Mean FM measured by QMR and FM changes measured by BIA yielded higher values than pregnancy-adjusted ADP in normal weight women during pregnancy.

For a practical perspective, normal weight women receiving dietary counselling during pregnancy could increase fish intake, and this finding shows the importance of proper dietary information to pregnant women. FM estimates by QMR were biased in normal weight and obese non-pregnant women, and QMR is therefore probably not accurate enough for individual body composition assessment. FM measurements by BIA as Tanita MC-180MA were grossly underestimated in non-pregnant obese women and hence not suitable for use in obese women. In pregnant women, QMR and BIA by

Tanita MC-180MA would need validation against a gold standard method, and therefore no certain conclusions could be made. However, the present results indicate that the software specific Tanita MC-180MA is unsuitable in pregnant women for cross-sectional and longitudinal FM assessment.

7 FUTURE PERSPECTIVE

A future perspective includes both upcoming analyses in the PONCH study, and also possible research questions in thinkable future studies.

First, the PONCH study is still an ongoing study, with inclusion of more obese women. A natural step will be the evaluation of the dietary intervention in the full sample of all normal weight and all obese women. The evaluation can focus on fish intake, but also on energy intake, intake of fruits and vegetables, and sugar E % intake. Dietary intake analyses can include both macronutrients, micronutrients, whole single foods, and also dietary patterns. Also, other intervention outcomes to analyze are GWG, maternal body composition, birth weight, neonatal body composition at birth, and possible connections between variables such as maternal fish intake or meat intake during pregnancy and birth weight or neonatal body composition at birth. Additionally, it can be interesting to check whether the correlation between meat intake in early pregnancy and the following FFM gain detected in normal weight women also exists in obese pregnant women. The FFQ used in this thesis also gives data on different types of fish and meat, and these data could be analyzed in relation to serum phospholipid fatty acids, birth weight, and neonatal body composition. Also, future analyses can be post-partum comparisons of women in the dietary intervention group and control group, with focus on post-partum weight retention, body composition, and also of body composition development in their children, assessed by ADP. Additionally, future analyses will investigate the association between maternal body composition during pregnancy, or body composition changes during pregnancy, in normal weight and obese women, and neonatal body composition.

Second, a possible study could be to evaluate the fish and meat FFQ used in this thesis.

Third, another conceivable study would be to validate the three body composition methods used here during pregnancy, against gold standard methodology including hydrometry. This is of considerable value especially for the promising and comfortable QMR methodology, and for the user friendly ADP method. Although the QMR equipment is rare, the value of validation against gold standard would be of great scientific interest, and a useful method in research. ADP equipment are more commonly used, and validation also during pregnancy could naturally be of scientific interest, but also possibly for clinical use, and for use in laypersons that are interested in body composition.

ACKNOWLEDGEMENT

Det finns ett antal personer som jag skulle vilja tacka:

Agneta Holmäng, stort tack för att jag har fått möjlighet att arbeta i PONCH-studien. Det har varit fantastiskt att få förmånen att studera dessa intressanta frågeställningar!

Lars Ellegård, ett stort tack för att du, redan när jag var student, introducerade nutrition/näringslära. Du har alltid kunnigt och noggrant besvarat frågor, och lärt mig att försöka skriva vetenskaplig text så okomplicerat som möjligt. Tack för all konstruktiv hjälp under alla år som student, anställd och slutligen doktorand!

Holger Nilsson, tack för att du alltid har varit hjälpsam, framför allt för goda och otroligt kunniga råd i fysiologiundervisningen. Jag har verkligen uppskattat att få undervisa i fysiologi!

PONCH-gruppen – vilken tur jag har som har fått arbeta med just er, **Nina Jansson, Therese Karlsson, Aysha Hussain, Louise Andersson, Robert Jakubowicz, Jeanette Pettersson, Carolina Gustavsson, Hanna de Maré, Ulrika Andersson Hall** och **Evelina Järvinen**. Ni har alla varit otroligt ambitiösa och trevliga kollegor. Det har varit ett nöje att få samarbeta med er, och tillsammans har vi vridit och vänt på utmaningar för att kunna arbeta på bästa möjliga sätt med studien. Ett särskilt tack till Evelina och Ulrika som har förgyllt det sista året!

Fettvävsgruppen, **Malin Lönn, Henrik Svensson, Birgitta Odén**. Tack för gott samarbete genom åren, både vad gäller allt praktiskt runt fettvävsbiopsier och för trevliga PONCH-möten. Det är alltid inspirerande att höra er berätta om fettväv!

Dessutom, tack till alla trevliga medarbetare i fysiologihuset – ingen nämnd ingen glömd – som alltid är tillmötesgående och har gett upphov till många skratt i lunchrummet.

Tack också till alla medarbetare i undervisningen. Ni har alltid varit hjälpsamma och gett fina introduktioner inför kommande nya undervisningsmoment.

Dessutom vill jag tacka kusin-familjen, **Sara, Mattias, Stella** och **Thea**, för allt kul genom åren. Speciellt tack till Sara för goda råd på vägen från en tidigare fysiologen-doktorand.

Ingvar, tack för att jag, innan vi blev släkt, fick börja arbeta på Klinisk nutrition. Med din expertis inom nutrition inklusive kroppssammansättning fick jag en väldigt god start innan forskarutbildningen. Numera är du framför allt en väldigt snäll och uppskattad farfar!

Ann – världens bästa farmor! Din hjälp på vardagarna har underlättat otroligt. Tack för all tid som du har hand om barnen.

Stort tack till mina kära systrar med familjer som alltid finns där! **Sara, Anders, Edvin, Valter, Anna-Pia, John, Mika** och **Olle** – vilket gäng!

Mamma **Pia** och pappa **Pentti,** tack för allt, exempelvis målning av lägenhet, härliga Långasandsvistelser, omsorg av barnen och ständig uppmuntran!

Ingrid och **Ivar,** våra älskade roliga ungar. Ni betyder allt! Jag förundras dagligen över er fantasi, glädje, förmåga och vilja att lära er nya saker. Jag är så stolt över er!

Niklas, mitt stora stöd i livet och min bästa vän! Tack för ALLT. Jag hade inte kunnat genomföra detta utan dig, du är bäst!

Slutligen ett stort tack till alla medverkande mödravårdscentraler och deltagande kvinnor i studien – utan er hade denna studie inte varit möjlig.

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