Effects of micronutrients on infant birth weight

The importance of iron during pregnancy

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Photo on front page from: Langman’s Medical Embryology, by Thomas W. Sadler

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Abstract

OBJECTIVE: The link between nutrition, fetal development and growth, and future health, is gaining increasing attention. Non-optimal infant birth weight (<2500 g, > 4000 g), micronutrient intake and an inadequate maternal and fetal iron status have been linked to adverse health risks in both short- and long term perspective of the mother and child. The purpose of this thesis was to examine possible associations between micronutrients, iron status and infant birth weight.

METHODS: The study was a prospective cohort study including 1031 Norwegian pregnant women and their infants, during gestational week 14-16 and 30-32 in the period 2002-2008. The dietary intake was ascertained by using a food frequency questionnaire (NORKOST 1997). Plasma was analyzed for iron status biomarkers: iron, transferrin, soluble transferrin-receptor, transferrin-saturation and ferritin.

RESULTS: The dietary intake of vitamin D, iron and folate did not reach the New Nordic Recommendations for pregnant women in either early or late pregnancy. In addition, the vitamin A intake exceeded both the daily- and upper limit recommendations in many of the women. Iron and calcium were positively associated with infant birth weight in univariate analyses in gestation week 14-16, whereas magnesium was positively associated with infant birth weight in gestational week 14-16 and 30-32, respectively. No other significant correlations were found. The plasma concentrations of iron, ferritin and transferrin-saturation decreased during the period, whereas transferrin and soluble transferrin-receptor increased. All iron status biomarkers indicated profound iron deficiency in several of the women, with exception of soluble transferrin-receptor. Four percent of the women went into the pregnancy with empty iron stores (defined as P-ferritin < 12 µg/l).

CONCLUSION: We found that micronutrient deficiency and low iron stores is common in healthy, Norwegian pregnant women. Significant correlations between iron, calcium and magnesium and infant birth weight were observed in univariate analyses. Further trials are needed to evaluate whether changes in micronutrient intake and iron status may improve clinical outcome for the mother and child.
Acknowledgements

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Last, but not least, I want to thank Ingvild Torset Voldhaug. You had the most beautiful and pure soul, whom still guides me today. I am really looking forward to live by your principles the rest of my life: enjoy each day as it was your last. “Du har satt dype spor. Mer enn du aner, mer enn du tror”.

Oslo, June 2012

Karianne Nordahl
# Table of contents

1 INTRODUCTION ........................................................................................................ 1

2 BACKGROUND ........................................................................................................... 3
  2.1 Micronutrients: A brief summary .............................................................................. 3
  2.2 Placental transfer- and metabolism ......................................................................... 3
  2.3 Developmental origins of health and disease .......................................................... 5
  2.4 Epigenetics ............................................................................................................ 5
  2.5 Vitamin A .............................................................................................................. 6
    2.5.1 Metabolism .................................................................................................... 6
    2.5.2 Sources and recommendations ....................................................................... 6
    2.5.3 Functions ..................................................................................................... 7
  2.6 Vitamin D ............................................................................................................. 7
    2.6.1 Metabolism .................................................................................................... 7
    2.6.2 Sources and recommendations ....................................................................... 8
    2.6.3 Functions ..................................................................................................... 9
  2.7 Vitamin E ............................................................................................................. 9
    2.7.1 Metabolism .................................................................................................... 9
    2.7.2 Sources and recommendations ....................................................................... 10
    2.7.3 Functions ..................................................................................................... 10
  2.8 Vitamin K ............................................................................................................ 11
    2.8.1 Metabolism .................................................................................................... 11
    2.8.2 Sources and recommendations ....................................................................... 11
    2.8.3 Functions ..................................................................................................... 11
  2.9 Vitamin C ............................................................................................................ 12
    2.9.1 Metabolism .................................................................................................... 12
    2.9.2 Sources and recommendations ....................................................................... 12
    2.9.3 Functions ..................................................................................................... 12
  2.10 Thiamine (B₁) .................................................................................................... 13
    2.10.1 Metabolism .................................................................................................. 13
    2.10.2 Sources and recommendations ..................................................................... 13
    2.10.3 Functions ..................................................................................................... 13
  2.11 Riboflavin (B₂) .................................................................................................. 13
    2.11.1 Metabolism .................................................................................................. 13
    2.11.2 Sources and recommendations ..................................................................... 14
    2.11.3 Functions ..................................................................................................... 14
  2.12 Niacin (B₃) ........................................................................................................ 14
    2.12.1 Metabolism .................................................................................................. 14
    2.12.2 Sources and recommendations ..................................................................... 15
    2.12.3 Functions ..................................................................................................... 15
  2.13 Pantothenic acid (B₅) ........................................................................................ 15
    2.13.1 Metabolism .................................................................................................. 15

VII
3 STUDY AIMS ............................................................................................................. 38

4 MATERIALS AND METHODS .................................................................................. 39

4.1 SUBJECTS AND STUDY DESIGN ........................................................................ 39

4.2 METHOD .................................................................................................................. 40

4.2.1 DATA ASSESSMENT ......................................................................................... 40

4.2.2 COST REGISTRATION ....................................................................................... 41

4.2.3 CALCULATION OF THE SAMPLE SIZE ......................................................... 41

4.2.4 BLOOD SAMPLES AND ANALYZES ............................................................... 42

4.2.5 ANTHROPOMETRIC MEASURES ..................................................................... 43

4.2.6 MICRONUTRIENTS AVAILABLE FOR ANALYSES ....................................... 44

4.2.7 CALCULATION OF BASAL METABOLIC RATE (BMR), AND UNDER- AND OVERREPORTING ................................................................. 44

4.2.8 STATISTICAL ANALYSIS ................................................................................. 44

4.3 ETHICS .................................................................................................................... 45

5 RESULTS .................................................................................................................... 46

5.1 CHARACTERISTICS OF THE WOMEN AND NEWBORNS ................................ 46

5.2 CHARACTERISTICS OF THE NUTRIENTS ....................................................... 47

5.3 CHARACTERISTICS IN THE IRON STUDY GROUP ........................................ 49

5.4 CORRELATION BETWEEN INTAKE OF MICRONUTRIENTS AND BIRTH WEIGHT ........................................................................................................ 53

5.5 CORRELATION BETWEEN IRON STATUS IN BLOOD AND BIRTH WEIGHT .... 55

5.6 REGRESSION ANALYSIS ....................................................................................... 55

5.7 SUMMARY OF THE RESULTS .............................................................................. 56

6 DISCUSSION ................................................................................................................. 57
1 Introduction

Factors that influence health outcomes for both mother and child are of interest in preventive and clinical medicine and nutrition. It is desirable to identify modifiable factors that may contribute to the prevention of both short and long term complications and other diseases in mother and child (1). Low birth weight is a great problem in developing countries, whereas high birth weight is an increasing global problem in developed countries (2). Birth weight is influenced by various factors such as genetics, gestational age, maternal and paternal anthropometric measures, maternal weight gain during pregnancy, and child's gender and intrauterine environment. The intrauterine environment is influenced by several factors such as pregestational weight, weight gain during pregnancy, maternal physical fitness, maternal metabolism and maternal nutritional status (2-6).

Maternal nutrition during pregnancy may influence genes and intrauterine environment through epigenetics (7). Epigenetics is modifications of DNA and proteins associated with DNA, and plays a central role in eukaryotic biology and cellular differentiation. The modifications are changes in gene expression that are not due to changes in the nucleotide sequence, for example DNA methylation, and may be stable for several generations (6, 8, 9). Metabolism and absorption of micronutrients may also be influenced by factors other than the mother's diet, such as infection, genetics and smoking (9-12). Although the conclusions remain unclear about micronutrients contribution to fetal growth and development, several studies have found associations between micronutrients and infant birth weight (13-19).

The haemostatic mechanisms show profound alterations compared with non-pregnant women (20), and is a diverged subject among Norwegian physicians. The disagreement on how and when to treat iron deficiency and anemia (21), is one example. The former Norwegian guidelines yield that both the hemoglobin (Hb) concentration and S-ferritin level should be measured during pregnancy, while the new guidelines from 2005 yield that only the hemoglobin concentration should be measured and followed through pregnancy. Several studies imply that hemoglobin alone will not detect an iron deficiency or anemia, and that hemoglobin in context with ferritin and soluble transferrin-receptor (sTfR) presents the best pictures of iron deficiency and anemia (22, 23).
It is still a controversy whether all women should take iron prophylactically during pregnancy. Our greatest experience in prevention derives from countries where iron deficiency is least common. In Norway, about 12-16% of fertile women have depleted iron stores, and some studies have found that the dietary iron intake among Scandinavian pregnant women is too low (21, 23, 24). This may lead to deficiency in the mothers and low iron stores in the newborns (25, 26).

This master thesis is part of a Norwegian cohort: 'STORK (Store barn og komplikasjoner)', a study that aimed to identify better methods of recognizing pregnant women in the risk zone for giving birth to macrosomic infants, so that early intervention/treatment can be provided. In English (U.S.) the study is called "The increasing prevalence of fetal macrosomia: A prospective study including endocrinological, metabolic, placental and nutritional characteristics of pregnant women given birth to macrosomic infants defined by the World Health Organizations (WHO) definition\textsuperscript{1}" (2, 6, 27).

STORK was conducted in two rounds, STORK 1 and 2, where a total of 1031 pregnant women were followed longitudinally through their pregnancies from gestational week 14-16 to three-four days after birth. One of the main goals was to investigate various factors affecting intrauterine growth, with focus on children who were born macrosomic (6).

The purpose of this masterthesis was to examine the intake of various micronutrients and the iron status during pregnancy, and possible associations with infant birth weight.

\textsuperscript{1} Infant birth weight > 4000 g
2 Background

2.1 Micronutrients: A brief summary

Nutrition supply to the fetus is regulated by a number of ways, for instance by maternal diet intake, metabolic adaptation and absorption, and fetal uptake and placental transfer. Nutritional imbalance is most likely to affect the fetal development before conception or in the 1st trimester (when rapid cell differentiation and formation of organs are taking place) and the fetal birth weight in the 3rd trimester (when rapid growth is taking place) (28).

There are few randomized, epidemiological studies on micronutrients and development of the fetus in humans, but some have confirmed that pregestational- and gestational nutrition may contribute to conditions like low birth weight and various birth defects (29). The vast majority of the studies have looked at multivitamin- or iron/folic acid supplements in relation to infants born small for gestational age (SGA) in developing countries: few studies have regarded specific micronutrients in relation to birth outcomes in developed countries.

2.2 Placental transfer- and metabolism

The placenta is an active endocrine organ that is of great importance for intrauterine fetal development and growth, and acts in concert with the fetus (the feto-placental unit) and the maternal metabolism. Some hormones are transferred between the placenta and the fetus independently of the mother (e.g. protein hormones), while other hormones (e.g. steroids) remains separated from the fetus (20).

It is difficult to achieve direct measurement of placental transfer to the fetus because of the ethical and technical constraints. Different patterns of transfer are simple diffusion, flow limitation\(^1\), passive diffusion, facilitated diffusion and active transport (20).

\(^1\) Occurs when the rate of supply or the removal of the substance from the membrane surface becomes a rate-limiting factor

\(^1\) A gene containing a short DNA sequence of about 180 base pairs referred to as a homeobox (which code for a
Table 1: Overview of placental transfer of macro-and micronutrients. The facts are based on the book “Clinical physiology in Obstetrics”, by F. Hytten and G. Chamerlain 1995 (20)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Transfer method</th>
<th>Control mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>Facilitated and passive diffusion driven by a concentration gradient</td>
<td>Unknown whether insulin affects the absorption or not. Oestrogen and progesterone might inhibit the uptake</td>
</tr>
<tr>
<td>Lipids</td>
<td>Passive transfer of free fatty acids. Cholesterol is transferred by unknown mechanisms. No evidence of triglyceride transfer</td>
<td>Might be dependent on a concentration gradient</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Active transport systems</td>
<td>Unknown</td>
</tr>
<tr>
<td>Lipid-soluble vitamins</td>
<td>Passive diffusion down a materno-fetal concentration gradient</td>
<td>Unknown</td>
</tr>
<tr>
<td>Water-soluble vitamins</td>
<td>Active transport against a concentration gradient</td>
<td>Unknown</td>
</tr>
<tr>
<td>Sodium (Na⁺)</td>
<td>Paracellular pathways or co-transported carriers</td>
<td>Unknown</td>
</tr>
<tr>
<td>Potassium (K⁺)</td>
<td>Carrier-mediated</td>
<td>Possible modulation by Ca²⁺</td>
</tr>
<tr>
<td>Calcium (Ca²⁺)</td>
<td>Active transport up a concentration gradient through transcellular routes</td>
<td>Unlike adults, the Ca²⁺ uptake in the fetus is not under control of calcium binding proteins or vitamin D</td>
</tr>
<tr>
<td>Phosphate (P⁺)</td>
<td>Active transport</td>
<td>Na⁺ and energy dependent transport</td>
</tr>
<tr>
<td>Chloride (Cl⁻)</td>
<td>Passive diffusion</td>
<td>Unknown</td>
</tr>
<tr>
<td>Iron</td>
<td>Transport systems that involve transferrin (Tf)</td>
<td>Unknown</td>
</tr>
<tr>
<td>Zinc (Zn⁺)</td>
<td>Passive and active transfer systems</td>
<td>Unknown</td>
</tr>
<tr>
<td>Iodide (I⁻)</td>
<td>Possibly carrier-mediated</td>
<td>Unknown</td>
</tr>
<tr>
<td>Other minerals and trace elements</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
Disturbed placental epigenetics has been demonstrated in cases of intrauterine growth retardation and small for gestational age (SGA) (30).

2.3 Developmental Origins of Health and Disease

In the last 10-15 years there has been increasing evidence that nutrition during early life can alter future disease risk, so-called fetal programming. Fetal programming means that the nutrients may affect an individual's genetic expression permanently, so that individuals with the same genotype have different phenotypes. An important mechanism for fetal programming seems to be epigenetic regulation (31) (32) (32) (33).

The hypothesis that impaired fetal growth might be a cause of development of chronic and degenerative diseases later in life (28, 31, 34), is often called the Forsdahl hypothesis, the Barker hypothesis or the hypothesis of fetal origin. Internationally, it is often called Developmental Origins of Health and Disease (DOHaD).

2.4 Epigenetics

Maternal imbalanced diet- and/or macro-and micronutrient intake either throughout pregnancy, or at defined stages periconceptionally, might have pronounced effects on fetal development and organogenesis (35). Adverse short- and long-term consequences reflect a mismatch between different early fetal/neonatal conditions and the conditions that the individual will confront later in life (e.g. environmental, nutritional). The mechanisms underlying this risk remain unclear, but epidemiological and experimental observations suggest that epigenetic changes (in regulatory genes and growth-related genes) might play a major role in fetal programming and set disease susceptibility later in life (cardiovascular disease, hypertension, obesity, type 2 diabetes mellitus and some cancers) (36, 37).

Epigenetics is modifications of DNA that changes the gene expression, which are not due to changes in the nucleotide sequence. Epigenetic marks like post-translational modifications of histones, deoxyribonucleic acid (DNA) methylation and non-coding ribonucleic acids (RNAs)
are some of the epigenetic modifications being investigated (9, 30). The imprinting of insulin-like growth factor (IGF2) expression, which modulates fetal growth among other things, is one example of maternal and paternal epigenetic marking (38).

2.5 Vitamin A

2.5.1 Metabolism

Retinoids are a collective term for the metabolites of vitamin A (retinol, its endogenous metabolites and synthetic analogues), and are closely chemical related to carotenoids. The different retinoids are converted to retinol (ROH) after digestion and packed into micelles, which are further absorbed by enterocytes. There, ROH is re-esterified to retinyl ester (RE) and incorporated into chylomicrons, which is transported through the lymphatic system to the liver and different target organs. The bioavailability of retinoids and carotenoids are 70-90% and 9-22% respectively, where fiber, fat and bile affect the accessibility. Because of the low regulation of the uptake, overdose and toxic levels of the vitamin may easily occur (29, 39).

Chylomicron-remnants are taken up by the liver, where RE is hydrolyzed to ROH, and further bound to retinol binding protein (RBP) and transthyretin/prealbumin/thyroxine binding globulin (TTR). The complex is subsequently secreted into the bloodstream or stored in stellate cells (approximately 80% of the store is located in the liver). ROH is generally recycled between the liver, plasma and extrahepatic tissues. In the latter, ROH are converted to retinal (RAH), which is further converted to retinoic acid (RA) (29, 39).

2.5.2 Sources and recommendations

Vitamin A is in the form of RE or provitamin A carotenoids in the diet, and are found in foods like liver, fish liver oil, yellow, red and green vegetables and fruit, eggs and dairy products. The boundary of retinol toxicity is controversial, but the upper limit intake is set to 3000 µg/day. It is desirable that vitamin A intake is kept within the recommended target of 800 µg/day (40), as both deficiency and excessive of ROH can cause fetal damage. The increased need during pregnancy is based on accumulation of the vitamin in fetal liver (29).
2.5.3 Functions

Vitamin A is important for the regulation of gene expression for embryonic development of the vertebrae, spinal cord, limbs, heart, eyes and ears, the visual process, reproduction, growth, cell differentiation and proliferating, and for immune responses. This involves regulation of retinoid receptors and their subsequent effects on homeobox genes\(^1\). Disruption of these processes through retinol deficiency or excess may lead to under or over-expression of homeobox genes during susceptible stages of development and may eventually result in adverse effects like teratogenesis (41).

Numerous studies have documented deleterious effects of retinol deficiency on fetal development, with target organs like heart, ocular tissues, respiratory-, urogenital- and circulatory systems. Hypovitaminose A is shown to have teratogen effects such as the development of embryopathy\(^2\), anophthalmia\(^3\) and hydrocephalus\(^4\). Excessive exposure to vitamin A is more or less the same as for deficiency: congenital-, heart-, skull and craniofacial-, skeleton-, limbs-, brain-, eyes- and central nervous system (CNS) malformations (41). Hypervitaminosis has also showed teratogenic effects, especially in 1\(^{st}\) trimester (the period of fetal organogenesis) where neural tube defects (NTDs) have highest occurrence. The risk of teratogenicity can persist for many months after a high vitamin A intake (29).

2.6 Vitamin D

2.6.1 Metabolism

There are two main types of calciferol (vitamin D) depending on whether they derive from the diet or skin: ergocalciferol (vitamin D\(_3\)) and cholecalciferol (vitamin D\(_2\)). Ergocalciferol is absorbed in the intestine through chylomicrons and transported via chylomicron remnants

---

\(^1\) A gene containing a short DNA sequence of about 180 base pairs referred to as a homeobox (which code for a homeodomain). Homeobox genes encode proteins that bind and regulate DNA transcription. Involved in bodily segmentation and cell differentiation during embryonic development.

\(^2\) Fetal malformations like small/malformed ears, mandibular hypoplasia, heart defects and cheiloschisis

\(^3\) Congenital disorder where the eye is severely underdeveloped or not developed at all

\(^4\) A buildup of fluid inside the ventricles that leads to brain swelling
(CMR) to the liver (roughly 80%). The rest is transported to extra hepatic tissues for storage as calcitriol. Cholecalciferol formed in the skin is bound to vitamin D binding protein (DBP) in plasma and transported primarily to the liver. Calciferol is inactive and has to go through two hydroxylation steps to be an active hormone (see Figure 1). The first hydroxylation step is in the liver, where calciferol is converted to calcidiol (25(OH)2D3). This conversion is poorly regulated, which makes vitamin D toxic when the intakes from the diet are too high. Calcidiol is further transported by DBP to the kidneys for the last hydroxylation step, which yields the active metabolite calcitriol (1,25(OH)2D3). In contrast to the calcidiol level, the calcitriol level is carefully regulated by parathyroid hormone (PTH), phosphate deficiency, various feedback loops and calcitriol itself (29, 39).

![Figure 1: Vitamin D production in the skin (cholecalciferol) and absorption in the lumen (ergocalciferol), and further transportation to the liver and kidneys. Source: Silje Sæby Dybvik, Clinical nutritionist.](image)

### 2.6.2 Sources and recommendations

Sunlight is the main source of vitamin D, while important dietary sources are cod liver oil, fatty fish, fortified milk, margarine and eggs. Recommendations for pregnant women are 10 µg/d (40). The upper limit is set at 50 µg/d in healthy adults, although there is evidence that up to 2.5 mg/d do not affect fetal development negatively (29).
2.6.3 Functions

Vitamin D exerts its effects through gene regulation. Various functions are maintenance of calcium- and phosphate homeostasis, stimulation of optimal bone development- and mineralization, cellular differentiation, differentiation of promyeloid leukemia cells (the macrophages) and an antiproliferative agent against tumor cells (e.g. in breast, colon, lung and prostate cells) (29). There are few data that elucidate the effect of vitamin D on pregnancy and fetus, and specific functions in fetal development are unclear (42).

Deficiency may lead to adverse, permanent changes in the fetus. Studies have found low maternal vitamin D status to be associated with reduced intrauterine long bone growth, shorter gestation, low birth weight, SGA, neonatal hypocalcemia, rickets, osteomalacia, osteoporosis, reduced bone mass at 9 years of age, cataracts and abnormal brain development perinatally (43, 44). Vitamin D excess has been proposed as part of the pathogenesis of several diseases and syndromes including increased risk of having a child born SGA, supravalvular aortic stenosis\(^1\) and Williams syndrome\(^2\) (41, 45). Recent evidence links not only low but also high maternal vitamin D status with increased risk of SGA. In addition, due to vitamin D’s role in the immune system, it has been hypothesized that vitamin D status is negatively associated with preeclampsia (46).

2.7 Vitamin E

2.7.1 Metabolism

Vitamin E is a collective term for different stereoisomers, where alpha-tocopherol is the most potent of the eight (four tocopherols and four tocotrienols). Vitamin E is cleaved from triglycerides or tocopheryl esters by pancreatic lipase, mainly in the duodenum and jejunum. The bioavailability is less than 50 %, depending on the fat content in the lumen (since the absorption is dependent on micelles). Vitamin E is incorporated in chylomicrons after passive

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1 A congenital disorder with a left ventricular outflow tract (LVOT) obstruction that occurs as a localized or diffuse narrowing of the ascending aorta
2 A rare genetic disorder that affects a child's growth, physical appearance, and cognitive development. Genes on chromosome 7 are missing, including elastin, which leads to disorders of the circulatory system and heart defects because of the lack of elastin
diffusion (via micelles) into the epithelial cells, and transported with lymph to the liver, other tissues or to high-density lipoproteins (HDLs). Vitamin E can be transferred amongst the lipoproteins in the circulation. Different uptake mechanisms in tissues are via lipoprotein lipase (LPL) activity, low-density lipoprotein receptor (LDL-receptor) or scavenger receptor. Over 90% of the vitamin is stored in adipose tissue, but the usage of this storage is still undefined. Erythrocytes, liver and spleen have highest vitamin E turnover. Generally, an overload of the vitamin will rapidly be oxidized, conjugated and excreted fecally or in the urine (29, 39).

**2.7.2 Sources and recommendations**

Vitamin E is solitary synthesized in plants, where the richest sources are vegetable oils (especially sunflower oil), sunflower seeds, almonds, peanuts, margarine, oatmeal and avocado. The daily needs during pregnancy are 10 mg (29).

**2.7.3 Functions**

Vitamin E’s main function lies in its antioxidant properties, where it prevents oxidation of polyunsaturated fatty acids (PUFAs) in phospholipids, cell membranes and lipoproteins. Other functions are inhibitory effects of protein kinase activity (during cell proliferation and differentiation (29)) and arachidonic acid metabolism, and regulation of smooth vascular muscle cells.

Vitamin E deficiency may lead to hydrocephalus\(^1\) in the human fetus. In mice, high doses of the vitamin led to growth inhibition and palatoschisis\(^2\) (41).

---

1. A buildup of fluid inside the ventricles that leads to brain swelling
2. Cleft palate
2.8 Vitamin K

2.8.1 Metabolism

Vitamin K is a group of chemically related vitamins. The most common forms are vitamin K₁ (phylloquinone), vitamin K₂ (menaquinone) and vitamin K₃ (menadione). Most of the vitamin is absorbed in jejunum and ileum as part of chylomicrons. The bioavailability is 40–70 % depending on the fat amount in the meal. The vitamin is either transported in plasma or absorbed in the liver, where there is a tiny store (1-10 µ/kg) that only lasts for 24–36 hours. Excretion of the vitamin occurs through unconjugated bile, or via the bowel and bladder when it is conjugated (29, 39).

2.8.2 Sources and recommendations

Vitamin K₂ are found in the diet or produced from intestinal bacteria, whereas vitamin K₁ is found in oils, cauliflower, broccoli, brussel sprouts, mayonnaise, cabbage, lettuce and soy margins. The Norwegian recommendation of 75-90 µ/d for pregnant and lactating women is exclusively related to vitamin K₁(40). Doses up to 500 times RDI should not cause toxic effects, but an intake of 100 mg has shown to trigger thromboembolic complications (29).

2.8.3 Functions

The vitamin is a coenzyme in the synthesis of proteins involved in bone metabolism and coagulation. There is no data for the vitamin K content in fetal tissue (29).

Lack of vitamin K might cause various birth defects (in the nose, mouth and the middle part of the face, shortened fingers, ossification¹ and cupped ears²). The risk of malformations of the central nerve system (CNS) is increased in fetuses carried by mothers who use vitamin K antagonists (41).

¹ Ossification (or osteogenesis) is the process of laying down new bone material by osteoblasts
² Congenital disorder where the rim of the ear is tightened or constricted
2.9 Vitamin C

2.9.1 Metabolism
Vitamin C is actively absorbed throughout the intestines and transported via vena cava to the liver and other organs, and excreted through the kidneys. Almost all cell types accumulate vitamin C, where our highest store is in skeletal muscles (which lasts for 2-3 months). Ascorbic acid is the dominate plasma form of the vitamin (29).

2.9.2 Sources and recommendations
Fruit, vegetables and berries are rich vitamin C sources. The recommended daily intake (RDI) during pregnancy is 85 mg/d (40).

2.9.3 Functions
Vitamin C’s main functions are through actions as a coenzyme (in monooxygenases or dioxigenases) or as an antioxidant (in lipid peroxidation, low density lipoprotein-oxidation (LDL-ox) and DNA-oxidation). Examples of affected pathways are the transformation of dopamine to noradrenalin, the biosynthesis of carnitine, hydroxylation of prolin and lysine (in procollagen), facilitation of iron absorption and oxidation of lipids, LDL, DNA and nitrite. In addition, vitamin C acts as a regulator of gene transcription of collagen 1 and 3, elastin, acetylcholine receptor, cytochrome P450, tyrosine hydroxylase, collagen integrin and ubiquitin.

Vitamin C deficiency during pregnancy is associated with increased risk of infection, premature birth and eclampsia (29). Several studies on maternal vitamin C deficiency in fetal development have found no adverse effects, while some animal studies have found birth defects like retarded skin- and muscle development, fetal haemorrhages, edema and irregular ossification (41).
2.10 Thiamine \((B_1)\)

2.10.1 Metabolism

Thiamine is absorbed in jejunum and ileum, either actively (using adenosine triphosphate (ATP)) or passively, and transported freely in the portal vein to the liver, and excreted by the kidneys. We have minor stores of vitamin B\(_1\) (25-35 mg), mainly in skeletal muscle, followed by liver, kidney and brain tissue (29).

2.10.2 Sources and recommendations

Foods rich in thiamine are grains, legumes, meat, milk and milk products. Daily requirement is 1.4 mg during pregnancy (40).

2.10.3 Functions

Thiamine is a coenzyme in carbohydrate-, amino acid- and protein metabolism. The fetus is especially vulnerable in the 3\(^{\text{rd}}\) trimester: the deficiency may contribute to fetal alcohol syndrome\(^1\) or cause intrauterine growth retardation\(^2\) (29). Animal studies have shown increased death rate, hemorrhages, edema of the head and trunk and exencephaly\(^3\) in rat fetuses with thiamine deficiency (41).

2.11 Riboflavin \((B_2)\)

2.11.1 Metabolism

Riboflavin is found in three different forms in the diet: free, phosphorylated and bound to protein. The vitamin is actively transported into the enterocytes of the upper small intestine, and phosphorylated into flavin mononucleotide (FMN), which can be phosphorylated into

---

\(^1\) Fetal alcohol syndrome is growth, mental, and physical problems that may occur in an infant when a mother drinks alcohol during pregnancy

\(^2\) Intrauterine growth restriction (IUGR) refers to the poor growth of the fetus perinatally

\(^3\) A rare malformation of the neural tube, which leads to absence of the bones of the cranial vault, with protrusion of brain tissue into the amniotic cavity
flavin adenine dinucleotide (FAD). Little riboflavin is stored, so any excess is rapidly excreted through the kidneys (29).

### 2.11.2 Sources and recommendations

The main sources are milk, cheese, fish, green vegetables, eggs and lean meat. The recommended intake is 1.3 mg/d (40).

### 2.11.3 Functions

Vitamin B₂ is a coenzyme (in the form of FMN and FAD) in a variety of redox reactions: the electron transport chain, formation of superoxide in macrophages, the formation of pyridoxal-5-phosphate and reduction of 5.10-methyltetrahydrofolat (MTHFR). In addition, riboflavin is essential for the conversion of pyridoxine to a coenzyme, and the conversion of tryptophan to niacin (29).

There is no convincing evidence that riboflavin deficiency or overload might lead to adverse effects in the human fetus. However, deficiency in maternal rats has led to short jaws, palatoschisis¹, syndactyly², short extremities and hydronephrosis³ (41).

### 2.12 Niacin (B₃)

#### 2.12.1 Metabolism

Niacin appears in two forms in food (nicotinic acid and nicotinamide) and is absorbed in the ventricle and small intestine, either actively or passively. The vitamin drifts freely or bound to protein in the blood, and is found in the form of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) in tissues (29).

---

¹ Cleft palate
² Congenital malformation with webbing or fusion of two or more digits, a result of failure of early interdigital tissue to degenerate
³ Swelling of a kidney due to a backup of urine
2.12.2 **Sources and recommendations**

Vitamin B₃ is found in fish, meat, nuts, milk and eggs. The needs depend on energy balance, but it is generally set at 16 NE/d for pregnant women. The upper limit is 500 mg/d (40).

2.12.3 **Functions**

Niacin is necessary for the formation of NAD and NADP, and is involved in energy metabolism (NAD in catabolic reactions, and NADP in anabolic reactions), and synthesis of fatty acids and steroids (29).

Few studies on niacin, teratogenicity and birth outcomes exist (41).

2.13 **Pantothenic acid (B₅)**

2.13.1 **Metabolism**

Pantothenate is hydrolyzed from CoA in the intestines, absorbed and further transported to the liver where the vitamin is resynthesized. Pantothenate is found freely in plasma, or transported through the body via red blood cells (RBC). We have an abundant storage in muscle, liver and intestines. Excretion of the vitamin occurs through the kidneys (29).

2.13.2 **Sources and recommendations**

Our riches sources are meat, chicken, liver, egg, tomato products, broccoli, potatoes and whole grain. Little is known about the increased needs or status during pregnancy (29), but the RDI are set at 5 mg/day (40).

2.13.3 **Functions**

As part of CoA, pantothenic acid participates in the tricarboxylic acid cycle (TCA-cycle), β-oxidation of fatty acids, oxidative degradation of amino acids, elongation of long-chain fatty acids, biosynthesis of cholesterol and steroids, biosynthesis of vitamin A, vitamin D and heme
A\(^1\), production of ketone bodies, biosynthesis of porphyrines and acetylation. Panthothenate itself participates in the biosynthesis of leucine, arginine and methionine (29).

### 2.14 Pyridoxine (B\(_6\))

#### 2.14.1 Metabolism

Vitamin B\(_6\) is a common name of pyridoxine (PN), pyridoxamin (PM) and pyridoxal (PL). The vitamins are absorbed passively in jejunum and ileum. Further transportation to the rest of the body occurs through albumin in plasma, or via hemoglobin in erythrocytes. The greatest storage of the vitamin is in muscle, mitochondria and cytosol, and lasts for approximately 75 days (29).

#### 2.14.2 Sources and recommendations

Different food sources are meat, liver, fish, egg, corn and milk products. RDI for pregnant women are 1.2 mg (40).

#### 2.14.3 Functions

Vitamin B\(_6\) is involved in the metabolism of amino acids, lipids and one-carbon units (including the conversion of homocysteine to cysteine), gluconeogenesis and in pathways that include heme- and neurotransmitter synthesis\(^2\).

Few studies in human fetuses exist, but animal studies have found that deficiency during pregnancy lead to a smaller spleen and thymus, palatoshisis\(^2\), feet-and finger defects, omphalacele\(^3\) and exencephaly\(^4\) (29).

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\(^1\) A derivative of heme found in cytochrome aa3 (cytochrome c oxidase)
\(^2\) Cleft palate
\(^3\) A birth defect in which the infant's intestine or other abdominal organs stick out of the belly button
\(^4\) A rare malformation of the neural tube with a large amount of protruding brain tissue and absence of calvarium
2.15 Biotin (B₈)

2.15.1 Metabolism
Absorption of biotin is most active in the small intestines, and occurs through sodium-dependent transporters. Afterwards, biotin is either transported freely or together with biotinidase or albumin to organs like liver, kidneys, muscles or lymphocytes. The vitamin is stored as biotinyl-5-adenylate in the liver, muscles and kidneys. When biotin is not incorporated in carboxylases, the vitamin is transformed to an inactive metabolite and excreted in the urine.

2.15.2 Sources and recommendations
Different biotin sources are meat, egg yolk, yeast and intestinal bacteria. RDI during pregnancy is 30-35 µg (40). The increased requirements during pregnancy are based on fetal growth, increased energy needs and increased excretion in the urine (29).

2.15.3 Functions
Vitamin B₈ is important for the energy metabolism (carbohydrate-, protein- and fatty acid metabolism), and acts as a coenzyme in bicarbonate dependent carboxylation. Animal studies suggest that biotin deficiency is teratogenic (may lead to cheiloshisis¹, micrognathia², exencephaly³ and micromelia⁴) (41, 47).

---
¹ Cleft lip
² Underdeveloped and small jaws
³ A rare malformation of the neural tube, which leads to absent of the bones of the cranial vault, with protrusion of brain tissue into the amniotic cavity
⁴ Unusual small or short arms and legs
2.16 Folic acid (B₉)

2.16.1 Metabolism

Folic acid is absorbed in the intestines as 5-methyltetrahydrofolate, which can be inhibited by alcohol. One third of the vitamin is transported freely in the plasma, whereas the rest of the vitamin is bound to albumin and other proteins. Our main storage is in the liver (about 4-15 mg). About 200 μg is excreted in the feces every day, and only small amounts are lost in the urine (29).

2.16.2 Sources and recommendations

Folic acid is found in a range of different foods like vegetables (spinach, broccoli and kidney beans), nuts, whole grain, fruit, egg, liver, milk- and dairy products, chicken and meat. RDI for pregnant women are 400 μg (40). A folic acid intake at 400 μg/d is especially recommended one month before conception and during 1st trimester. However, an adequate intake of 200 μg/d is important through the rest of the pregnancy and during lactation.

2.16.3 Functions

The vitamin plays an important role in the transfer of one-carbon units in nucleotide- and amino acid metabolism, which means that folic acid is essential for DNA synthesis and normal cell division (29).

Inadequate intake can lead to megaloblastic cells and birth defects such as NTD’s (29). Thomas et. al did a meta-analysis and found that the risk of giving birth to an infant with NTD’s, decreased with increased folic acid intake (41).
2.17 Cobalamin (B<sub>12</sub>)

2.17.1 Metabolism
Cobalamin is bound to haptocorrin (TC l) after food digestion in the ventricle. The cobalamin-TC l complex is released in the intestines, whereas TC-l is replaced by intrinsic factor (IF). The cobalamin-IF-complex is principally absorbed in ileum by IF-receptor. Cobalamin is released in the enterocytes and further bound to haptocorrin ll, and mainly transported to the liver. The body storage is approximately 2500 µg (29).

2.17.2 Sources and recommendations
Bacteria synthesize cobalamin, which means that our primary sources are animal products. RDI for pregnant women are 2.8 µg (40).

2.17.3 Functions
The vitamin acts as a coenzyme in the metabolism of fatty acids, carbohydrate and protein, in reactions involving methylations, and is essential for normal blood formation and neurological function. An increased need during pregnancy comes from hemodilation, fetal development and accumulation in fetal liver (29).

Maternal cobalamin deficiency may lead to hydrocephalus<sup>1</sup>, bone defects, eye defects, hydronephrose<sup>2</sup>, cheilosis<sup>3</sup> and NTD’s (41).

2.18 Iron

2.18.1 Metabolism
Iron is primarily absorbed in duodenum as inorganic ferrous iron (Fe<sup>2+</sup>) by divalent metal transport 1 (DMT1) or as heme-iron through heme-binding protein 1 (HCP1) (see Figure 2).

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<sup>1</sup> A buildup of fluid inside the ventricles that leads to brain swelling
<sup>2</sup> Swelling of a kidney due to backup of urine
<sup>3</sup> Cleft lip
The bioavailability of these to compounds is 2-20% and 15-35%, respectively. Iron uptake depends on both physiological and dietary factors (the latter affects solitary the absorption of non-heme iron). Iron status (e.g. anemia, deficiency, hemochromatosis), fasting, pregnancy, hypoxia, meat factor and ascorbic acid will promote absorption, whereas hemochromatosis, low levels of stomach acid, copper deficiency, polyphenols (tannin acid in coffee, tea), phytic acid (corn), calcium and high copper and zinc concentrations may inhibit the uptake. In other words, the iron absorption depends on the rate of erythropoiesis, iron stores, the content of the diet and whether supplements are being given (20, 48).

\[ \text{Duodenum} \quad \text{Enterocyte} \quad \text{Blood plasma} \quad \text{Hepatocyte} \]

\begin{align*}
\text{Heme} & \rightarrow 1 \quad \text{Enterocyte} \\
\text{Fe}^{2+} & \rightarrow 2 \quad \text{Enterocyte} \\
\text{Fe}^{3+} & \rightarrow 3 \quad \text{Storage} \\
\text{Porphyrin} & \rightarrow 4 \quad \text{Enterocyte} \\
\text{Fe}^{2+} & \rightarrow 5 \quad \text{Enterocyte} \\
\text{Fe}^{3+} & \rightarrow 6 \quad \text{Blood plasma} \\
\text{Fe}^{3+} & \rightarrow 7 \quad \text{Hepatocyte} \\
\text{Fe}^{3+} & \rightarrow 8 \quad \text{Blood plasma} \\
\text{Fe}^{3+} & \rightarrow 9 \quad \text{Hepatocyte} \\
\end{align*}

Figure 2. Absorption of nonheme and heme iron in the duodenum, and further transportation to tissues. Heme enters the enterocyte by HCP1 (1). The absorption of nonheme iron occurs almost exclusively as Fe\(^{2+}\) via DMT1 (2). An apical ferrireductase reduces any free Fe\(^{3+}\) to Fe\(^{2+}\)(3). Inside the cell, heme oxygenase releases Fe\(^{3+}\) which is then reduced to Fe\(^{2+}\)(4). Fe\(^{2+}\) is then transported through the basolateral membrane by ferroportin (5), and further oxidized to Fe\(^{3+}\) by hephaestin (7). Once in the blood plasma, the Fe\(^{3+}\) binds to transferrin (8), which carries the iron to the hepatocyte, where it binds to transferrin receptor (9). The Fe\(^{3+}\)-Tf-TfR-complex is then taken up by endocytosis. Hepcidin (6) can bind to ferroportin (5) and inhibit iron absorption.

\(^1\) Low copper status → low levels of ceruloplasmin and hephaestin
Following the absorption, Fe\(^{2+}\) will either be used in enterocyte synthesis of iron-containing compounds or transported to the bloodstream via ferroportin (regulated by hepcidin). Fe\(^{2+}\) is oxidized to ferric iron (Fe\(^{3+}\)) by hephaestin in the blood stream, and further bound to transferrin (Tf) for transport to other tissues that use iron in synthesis, or for storage in the liver, spleen or bone marrow. The Tf/iron complex is bound to transferrin receptor (TfR) in tissues, and taken up by endocytosis. Iron is then released and incorporated mainly into heme iron or stored in ferritin. Hepatocytes can also take up iron in the form of free hemoglobin (Hb) (29, 49).

Iron has important functions in all metabolically active cells, and the total body content is 3-4 g. Most of the iron is bound to hemoglobin, but also to ferritin or hemosiderin intracellularly, in the reticuloendothelial system (RES), as a part of myoglobin, cytochromes and various enzymes, and in plasma. The basal iron loss is approximately 1 mg/d, but varies individually. The only known mechanism for control of iron loss is through the regulation of absorption, which should prevent both iron deficiency and excess under normal conditions (29, 49).

The daily requirement of iron during pregnancy is 0.8 mg in 1\(^{st}\) trimester and increases to 12.0 mg in 3\(^{rd}\) trimester. This can only be met by mobilizing iron stores, in addition to maximum absorption of dietary iron (20, 50). The total requirement of iron during pregnancy is in the order of 700-1400 mg. The different demands consist of expansion of red-cell mass (~ 570 mg), losses in skin, feces and urine (~ 270 mg), iron transferred to the fetus (~ 200-370 mg), content in placenta and cord (~35-100 mg) and blood loss at delivery (~ 100-250 mg). 1 \(\mu\)g/L S-ferritin corresponds to approximately 7-8 mg stored iron, which means that pregnant women should have a ferritin concentration of at least 60 \(\mu\)g/L (~ 500 mg) to meet the iron demands during pregnancy. Since some of the iron is recycled, it is believed that 500 mg would be sufficient to meet the iron demands. Full-term infants are normally born with high Hb level and adequate iron stores in the liver and haematopoietic tissue, which usually lasts for six months (20).

### 2.18.2 Hematology and iron status biomarkers

The haemostatic mechanisms show profound alterations compared with the non-pregnant state. The blood volume increases (both plasma- and red-cell mass), and the greatest changes are seen in women who have the lowest blood volumes pre-conceptionally. The amount of increase in blood volume is correlated with infant birth weight, and is approximately 1500 mL.
(1250 mL plasma and 250 mL RBC’s) in healthy women in a normal first pregnancy. Multiple pregnancy and multigravidae have a proportionately higher increment of plasma volume (20).

Iron status can be considered as normal iron status with varying amounts of stored iron, iron overload, and iron deficiency with or without anemia. Definitions of iron status during pregnancy vary; normal ranges are illustrated in Table 2.

Table 2: Biomarkers of iron status. The knowledge and facts are based on the book "Clinical physiology in Obstetrics", by F. Hytten and G. Chamberlain 1995 (20)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Normal ranges in women</th>
<th>Ranges during pregnancy</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Abnormal ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Hb (g/dL)</td>
<td>11.7-15.3</td>
<td>&gt; 11</td>
<td>When viewed in relation to the women’s normal S-Hb value, it might give an indication of deficiency.(^{[51]})</td>
<td>Declines with increasing blood volume(^{[20]})</td>
<td>Low in iron deficiency, and high in hemocromatosis.</td>
</tr>
<tr>
<td>S-iron (μmol/L)</td>
<td>8-35 (NOR) 13-27 (US)</td>
<td>12-35</td>
<td>When viewed in relation to the women’s normal S-iron value, it might give an indication of deficiency.(^{[51]})</td>
<td>Significant individual variability and daily fluctuations. Hard to interpret(^{[51]}).</td>
<td>Low in iron deficiency, active processes and chronic diseases, and high in hemocromatosis.</td>
</tr>
<tr>
<td>P-transferrin (g/L)</td>
<td>2.2-3.9 (NOR) 1.2-2.0 (US)</td>
<td>Increases slightly</td>
<td>Useful measure of total body iron stores in non-pregnant women.(^{[51]})</td>
<td>Altered by pregnancy independently of iron status(^{[52]}).</td>
<td>High in iron deficiency, and low in hemocromatosis, active processes and chronic diseases.</td>
</tr>
<tr>
<td>P-Tf-saturation (%)</td>
<td>15-45</td>
<td>15-45</td>
<td>Great screening step in haemochromatosis(^{[51]}).</td>
<td>Significant individual variability and daily fluctuations(^{[51]}).</td>
<td>Low in iron deficiency and high in hemocromatosis (&gt; 75 %).</td>
</tr>
<tr>
<td>P-sTf-receptor (mg/L)</td>
<td>1.9-4.4</td>
<td>1.9-4.4</td>
<td>Useful measure of iron needs of cells. Sensitive. Unaffected by infection or inflammation. Does not vary with age, gender or pregnancy.(^{[52, 53]})</td>
<td>May be elevated with increased red cell production or turnover.(^{[53]})</td>
<td>High in iron deficiency, and low in hemocromatosis.</td>
</tr>
<tr>
<td>P-ferritin (μg/L)</td>
<td>10-200 (NOR) 15-300 (US)</td>
<td>15-150</td>
<td>Stable, unaffected by recent iron consumption, accurate and sensitive. Great measure of total body iron stores.(^{[51, 54]})</td>
<td>Affected by infection or inflammation. Limit usefulness in late pregnancy.(^{[51]})</td>
<td>Low in iron deficiency, and high in active processes, chronic diseases, hemocromatosis, pregnancy, liver failure and leukemia.</td>
</tr>
</tbody>
</table>
2.18.3 Iron deficiency and iron overload

Iron deficiency is the result of long-term negative iron balance, with diminished iron stores that no longer meet the needs of normal iron turnover. This results in an increase in transferrin, a decrease in transferrin-saturation and an increase in transferrin-receptor, along with depleted stores. This is opposite of what we see in iron overload: decrease in transferrin, increase in transferrin-saturation and a decrease in transferrin-receptor, along with overflowed iron stores. Iron deficiency, defined by WHO, occurs when there are no mobilizable iron stores, and when signs of compromised supply of iron to tissues are noted. Iron deficiency anemia is the result of more severe stages of iron deficiency (51).

Mild to moderate iron deficiency has adverse functional consequences that affects cognitive performance (55), behavior (56), growth of infants and children (51), immune status (57), morbidity from infections (57-59), and physical capacity and work performance (60). During pregnancy, iron deficiency may increase perinatal risks for mothers and neonates, and increase overall infant mortality (51).

Iron overload in the population is often a reflection of a hereditary disease, rather than an excess of dietary iron (61). The preventative doses for iron deficiency given to some sub-populations or food fortification, is too low to cause the harmful effects that iron excess might impose (e.g. acceleration of infection, promotion of chronic diseases) in most people, with exception of those with iron overload diseases (57, 61, 62).

Almost half of the pregnant women in the world are estimated to be anemic (52 and 23 % in developing- and developed countries, respectively). When multiple indices are available, iron deficiency is best defined by ferritin concentrations in absence of infection. Transferrin-saturation is less reliable as an indicator because of intra- and inter-day variability in serum iron. As a consequence of the limitations of each test, sensitivity might be low even though the specificity increases, when they are considered jointly. However, the best combination would be hemoglobin, transferrin-receptor and ferritin, which would reflect functional impairment, tissue avidity for iron and iron storage, respectively (51).

When iron-, transferrin-saturation- or ferritin levels are less than 12 µmol/L, 15 % and 15 µg/L, respectively, iron deficiency occurs (20). Depleted iron stores follows when ferritin concentrations are less than 12 µg/L, whereas anemia is defined when the hemoglobin
concentration is under 11 g/L in pregnant women. Severe risk of iron overload occurs when the ferritin levels are >150 µg/L (51).

2.18.4 Iron prophylaxis

A low dose of ferrous iron (30-40 mg) during pregnancy seems to improve women’s iron status and protect the infants from iron deficiency (63). When possible, individual iron prophylaxis tailored by the ferritin concentration should be preferred to general prophylaxis (64). However, the full benefits and adverse effects of iron supplementation remain unclear. Some studies have found adverse effects like hypotension, constipation-related hemorrhoids and decreased mineral absorption (65-67). It is important to notice that some of the studies that have regarded iron supplementation have used high iron doses (~100 mg ferrous iron) in women with close to normal iron status. Such high doses are only recommended to women with depleted iron stores. In addition, most of the studies that have regarded effects of iron supplementation have compared the effect of supplementation with iron/folic acid versus multiple vitamin-mineral supplementations, mostly in developing countries.

There may be several possible explanations of the effects seen in the studies with deleterious effects. Firstly, supplementation for a longer period may increase the risk of delivery complications during labour because of increased birth size. Secondly, early supplementation may have altered the metabolic regulation and led to complications during pregnancy and consequently to perinatal death. Thirdly, supplementation may prevent early spontaneous abortion and allow mothers to carry frail fetuses to much later stages of pregnancy. In addition, iron may interact with DNA and disrupt ligand binding or protein function, or induce oxidative damage on embryotic tissue. Few studies have examined the effects of micronutrient supplementation on long-term child health outcomes, such as child mortality, morbidity, growth and cognitive development. This is important because greater infant survival or other beneficial effects most likely are not just reflected in birth weight alone.

2.18.5 Sources and recommendations

Iron exists in the diet as heme iron and non-heme iron. The main iron sources in Norway are cereals, meat and fish, vegetables, fruits and berries, and potatoes. The recommended intake for pregnant women in Norway without signs of iron deficiency or iron deficiency anemia (IDA) is 15 mg/d (40).
Based on the knowledge that iron excess may be harmful (as iron participates in the formation of free oxygen radicals that may contribute to cell damage and stress), iron supplements are only recommended for pregnant women with anemia (Hb <11 g/L and s-ferritin <20 µg/L) and for those that do not meet the iron needs through diet (e.g. vegan). The concentration of hemoglobin is measured in the first control and in gestation week 28, with an eventual adjustment of iron deficiency with supplements in week 28. The supplementation doses depend on the degree of iron deficiency. The former guidelines yielded that the Hb-concentration should be followed independently of question about iron supplements or not, and that the concentration of ferritin should be measured before gestation week 15 (22) (64):

- S-ferritin < 20 µg/L: iron supplements from start or from gestation week 12-14
- S-ferritin 20-60 µg/L: iron supplements from gestation week 20
- S-ferritin > 60 µg/L: No iron supplements
- Supplements in the order of 30-50 mg Fe²⁺ per day.

The extra requirements during pregnancy derive from blood volume expansion, placental and fetus demands (20, 21).

### 2.18.6 Functions

Important component of O₂-transport (as part of hemoglobin), O₂-store (as part of myoglobin), electron transport in various enzyme systems (cytochromes, dehydrogenases, oxidases e.t.c.), iron transport (as part of transferrin) and iron storage (as ferritin or hemosiderin). In addition, iron participates in antibacterial activity (as lactoferrin).

Severe iron deficiency during pregnancy is associated with premature birth, low birth weight, palatoschisis¹, limb defects and perinatal mortality (29, 41, 49).

---

¹ Cleft palate
2.19 Calcium

2.19.1 Metabolism

Most of the calcium absorbed in ileum is in the form of ion, but some might be absorbed as calcium oxalate and calcium carbonate. The bioavailability decreases with increasing age, and remains between 5-30%. Calcium is absorbed and released from enterocytes down a concentration gradient (transcellular or paracellular), which can be influenced positively by 1.25(OH)\(_2\)D\(_3\) levels (not in infants), hypophosphatemia and pregnancy. Menopause, decreased gastric acid content in the intestinal lumen, oxalate and phytic acid may inhibit calcium absorption. Most of the excretion of the mineral occurs through faeces and urine, but some calcium is also lost through sweat, skin, hair, nails, fetal growth and lactation. Daily Ca\(^{2+}\)-turnover is 700 mg between the bones and rest of the Ca\(^{2+}\)-pool. Women’s Ca\(^{2+}\) content is 1000 g, where 99% is present in bones and teeth. The remainder is found in plasma, extracellular fluid (ECF) and intracellularly. Calcium homeostasis is maintained by registering the ECF concentration of the mineral (via Ca\(^{2+}\)-surface receptors in parathyroid gland, thyroid, kidney, intestines, bone marrow and other tissues) and by regulation of parathyroid hormone (PTH), calcitonin (CT) and calcitriol (see Figure 3) (29, 39).

Figure 3: Calcium homeostasis. Source: Silje Sæby Dybvik, Clinical nutritionist
2.19.2 Sources and recommendations

The richest calcium sources are cheese, milk, cereals, fruits, berries and vegetables. RDI is 900 mg/d during pregnancy. Generally, the increased need for calcium in the fetus is covered by the mother's increased absorption in the intestine (in response to increased vitamin D concentration in the mother). The upper daily limit is set at 2.5 g/d (40).

2.19.3 Functions

Calcium is essential for dental and skeletal development, vascular and muscle contraction, vasodilation, nerve transmission and glandular secretion. Optimal calcium intake during pregnancy may lead to improved fetal skeletal development and bone mineralization (68), reduced risk of preeclampsia, hypertension and preterm birth (69). Meta-analyses have shown no overall effect on the risk of stillbirth or reduced infant mortality, but a reduction in maternal death and serious morbidity (14). Animal models suggest that Ca$^{2+}$ deficiency can be teratogen during fetal development, and increase the risk of NTD’s (29).

2.20 Phosphorus

2.20.1 Metabolism

Phosphorus absorption occurs in the small intestine through specific transporters, and is inhibited by phosphate-binding antacids and phytic acid. The process is under control of vitamin D. Our body contains roughly 700 g phosphorus, of which 80 % is contained in the bone and 9 % in skeletal muscle. The mineral$^1$ is primarily excreted by the kidneys (70).

2.20.2 Sources and recommendations

Great sources of phosphorus are foods that are rich in protein and whole grains. The same mechanisms that contribute to increased intestinal calcium absorption during pregnancy, provides increased phosphorus absorption as well. RDI in pregnant women are 700 mg (40).

$^1$ Essential minerals are defined by their RDI > 100 mg/d, and that its contents in the body is in the order of grams.
Because of the increased bioavailability in pregnancy, the upper limit is set at 3500 mg/d (4000 mg/d in non-pregnant women) (40).

### 2.20.3 Functions

Phosphorus is a crucial component of all tissues with regulatory- and structural functions (in phospholipids, nucleotides and nucleic acids) (29).

### 2.21 Magnesium

#### 2.21.1 Metabolism

The absorption of magnesium is satiable and occurs throughout the whole gut, but primary sites of intestinal absorption are in the jejunum and the ileum (bioavailability of 25-75%). Some studies have found that calcium, phosphate, zinc and dietary fiber might inhibit the bioavailability. Around 25 g exist in the body, mainly in bones, muscles and soft tissues. Excretion routes of the mineral are through the feces, urine and sweat (29).

#### 2.21.2 Sources and recommendations

Magnesium is abundant in fruits, vegetables, grains, animal products, dairy products and drinking water. RDI for pregnant women are 280 mg (40).

#### 2.21.3 Functions

The mineral has both catalytic and structural roles. Magnesium is, among other functions, a cofactor for over 300 different enzymes, including the enzymes in the ATP/ADP reactions, DNA polymerases, DNA topoisomerases, DNA repair enzymes, adenylate cyclase, protein kinases and as part of the ubiquitin-proteasome. Mg$^{2+}$ stabilizes the DNA helix (binds to RNA and DNA via phosphate groups) and is important in tubulin polymerizing (and thus chromosome segregation during mitosis) (29).

Few studies have looked at different intakes of magnesium during pregnancy and consequences related to fetal development. However, some studies have explored magnesium
deficiency during pregnancy and maternal and fetal nutritional problems, where decreased risk of preterm birth, decreased risk of preeclampsia and induction of metabolic syndrome later in life have been discussed (72, 73). In addition, some animal studies have found association between magnesium deficiency and birth defects (skeletal, cheilosis\textsuperscript{1}, hydrocephalus\textsuperscript{2}, heart-, lung- and urogenital anomalies). There are absent data on human pregnancy and birth defects (41).

### 2.22 Iodine

#### 2.22.1 Metabolism

Iodine is efficiently absorbed in jejunum and ileum as iodide, and further transported through the circulation. Iodine homeostasis is basically controlled by thyroid gland, where our major iodine store relays (70-80 \% of the total body iodine content (15-20 mg)). The kidneys excrete the trace element\textsuperscript{3} through urine (29).

#### 2.22.2 Sources and recommendations

Fish, shellfish, seaweed, milk and dairy products, iodide salt and drinking water might be rich sources in iodine. However, the content varies dramatically. RDI is 200 µg to compensate for the loss of iodine that is not recycled back to the thyroid gland (40).

#### 2.22.3 Functions

Iodine is essential for normal thyroid function, and production of triiodothyronine (T3) and thyroxine (T4), and is thus part of gene transcription, protein synthesis and other enzymatic activity. Thyroid hormones are important in myelination of nerves in CNS and are most active in the prenatal period (shills). Deficiency might lead to brain damage, mental retardation, hypothyroidism, goiter\textsuperscript{4} and endemic cretinism\textsuperscript{1} (as a result of maternal hypothyroidism).

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\textsuperscript{1} Cleft lip

\textsuperscript{2} A buildup of fluid inside the ventricles that leads to brain swelling

\textsuperscript{3} A trace element is defined by a RDI < 100 mg/d, where the content in the body is in the order of mg or µg

\textsuperscript{4} Abnormal enlargement of the thyroid gland
Excessive intake of iodine prenatally may cause goiter and hypothyroidism in newborns (29, 41).

2.23 Selenium

2.23.1 Metabolism

Selenium enters the body in several forms, where selenomethionine (from plants) and selenocysteine (from animals) are the main forms. Absorption rate is in the range of 50-100%, and is unaffected by selenium nutritional status. The selenium metabolic pool is responsible for the trace elements homeostasis in the body, by regulating its incorporation in different proteins, tissues and organs. Excretion of selenium occurs through the liver, the kidneys and by breath (70).

2.23.2 Sources and recommendations

Seafood, fish, egg, cereals and meat are good selenium sources. Some claims that the selenium from fish and shellfish are not available for the human gut. We have no specific recommendations during pregnancy since data on the subject is incomplete. The US recommendation is 60-70 µg/day (40). The increased need during pregnancy reflects the fetal accumulation of selenium for the formation of selenoproteins. Selenium can be toxic at high intakes over a longer period (2-3 mg/ d) (40).

2.23.3 Functions

Selenium has enzymatic and structural roles as part of glutathione peroxidase, iodothyronine deiodinase, thioredoxin reductase, selenoproteins and sperm. This means that selenium protects cells from oxidative stress, regulates thyroid hormones (converts T4 to T3, in addition to removing iodine from T3) and reduces many other proteins, including insulin. Furthermore, selenium protects us against toxic metals (e.g. mercury (Hg), arsenic (As), cadmium (Cd)) (29).

1 Disease that affects the fetus if the mother has extreme hypothyroidism. Characterized by mental impairment, deafness and dumbness, spasms, and growth retardation.
2.24 Zinc

2.24.1 Metabolism

The absorption of zinc is transporter mediated, with highest absorption rate in jejunum. However, the full absorption mechanisms are unclear. Zinc is present in all body tissues and fluids (approximately 2-2.5 g), where just 0.1% of the total is in the plasma. The main excretion route is fecally, but we also have significant loss of the mineral through skin, hair, sweat, sperm, menses and urine (29).

2.24.2 Sources and recommendations

Different zinc sources are egg, milk and dairy products, poultry, fish, seafood, grains, nuts and legumes. RDI is 9 mg for pregnant women, with a general upper limit of 0.3 mg/kg/d (40).

2.24.3 Functions

Zinc function as a cofactor in catalytic reactions (about 200 enzymes depend on zinc), as a structural part (stabilizing the cell membranes, in ribosomes and in hormone receptor complexes) and has regulatory functions (via zinc finger proteins). Furthermore, the mineral is important for cellular differentiation and cell survival during early embryonic development (41, 70). Zinc deficiency can increase the risk of complications, lead to premature birth, prolonged labor, low birth weight, teratogenicity (NTD’s, CNS defects, anencephalus\(^1\), intrauterine growth retardation, spina bifida\(^2\), cheilosis\(^3\) and eye defects) and embryonic death/fetal death (29).

2.25 Various minerals and trace elements

\(^1\) Severe neural tube defect where the foetus is born without a head
\(^2\) Congenital disorder caused by the incomplete closing of the embryonic neural tube
\(^3\) Cleft lip
## Table 3: Overview of some minerals and trace elements, based on facts from the book "Modern nutrition in health and disease", by Shils (29).

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Metabolism</th>
<th>Sources</th>
<th>Recommendations</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fluor (F)</strong></td>
<td>Absorbed in the gut. Deposited in teeth and bones. Excreted mainly by the kidneys.</td>
<td>Water, milk and toothpaste.</td>
<td>No RDI have been set.</td>
<td>Stimulates regeneration of bone tissue. Protects against pathological demineralization of calcified tissue.</td>
</tr>
<tr>
<td><strong>Potassium (K)</strong></td>
<td>About 90 % of ingested K⁺ is absorbed by passive diffusion. 95 % is found within cells. Excretion through feces, urine and sweat.</td>
<td>Fruit, vegetables, coffee, meat, fish, eggs and dairy products.</td>
<td>3.1 g/dag</td>
<td>Fundamental role in acid-base regulation, fluid balance, muscle contraction and nerve conduction. Main kation in ECF(29). Deficiency in mice caused abnormal branching of tubules and cystic dilatation in kidneys (41).</td>
</tr>
<tr>
<td><strong>Copper (Cu)</strong></td>
<td>Absorbed across the enterocyte and transported to the liver by albumin, transcuprein and amino acids (AAs). Incorporated in ceruloplasmin in the liver, for further transported into the circulation. 70-150 mg in the body.</td>
<td>Whole grains, nuts, seeds, legumes, liver and shellfish.</td>
<td>1 mg/d. Upper limit is 10 mg/d.</td>
<td>Energy metabolism, production of connective tissue, glucose-, cholesterol- and neuropeptid metabolism, production of Hb and cofactor for several enzymes. Low levels are associated with low birth weight (29, 74) and teratogenicity in brain, heart, blood vessels, lungs, skin, skeletal, immun and blood system (41, 74).</td>
</tr>
<tr>
<td><strong>Chrome (Cr)</strong></td>
<td>Low bioavailability from the gut (&lt;2%). Tf transports Cr to organs like bone, spleen, liver and kidney. Fecal excretion.</td>
<td>Cereals, fruits and vegetables.</td>
<td>25 µg/d. Cr (III) has low toxicity, Cr (IV) is an irritant, while Cr (VI) is carcinogenic when inhaled.</td>
<td>Potentiates insulin’s effect(29), and has beneficial effects on the blood lipid profil.</td>
</tr>
<tr>
<td><strong>Manganese (Mn)</strong></td>
<td>Absorbed by diffusion and active transport. Bioavailability is 6-16 %. Bound to Tf, macroglobulin and albumin in the circulation. Excretion occurs primarily by bile. 10-20 mg in the body.</td>
<td>Whole grains, legumes, nuts and tea.</td>
<td>2 mg/d. Upper limit is 9 mg/day.</td>
<td>Essential for bone development, and part of the metabolism of AAs, cholesterol and carbohydrate(29). Part of several enzymes. Teratogenic, and associated with b.w(75).</td>
</tr>
<tr>
<td><strong>Molybdenum (Mo)</strong></td>
<td>Rapidly absorbed with a bioavailability of 55-95 %. Accumulation in the liver, kidney, adrenal gland and in the bone. Excreted by the kidneys.</td>
<td>Legumes, grains and nuts.</td>
<td>50 µg/d. Upper limit is 2000 µg/d.</td>
<td>Cofactor for enzymes involved in the catabolism of sulphur containing AAs and heterocyclic substances(29). Electrontransferer in redox reactions.</td>
</tr>
<tr>
<td><strong>Sodium (Na)</strong></td>
<td>Effective absorption of 90 % of the dietary intake. The body pool is ~ 100 g, where half is found in ECF and 10 % in the cells. Main excretion route is through the kidneys, some losses in feces and sweat.</td>
<td>Processed foods, bread, cheese, meat, fish, added salt, e.t.c.</td>
<td>1500 mg/d. Upper limit is 2.4 g/d.</td>
<td>Vital role in the regulation of fluid balance, blood pressure and transmembrane gradients.</td>
</tr>
</tbody>
</table>
### 2.26 Macronutrients

#### 2.26.1 Previously findings in STORK regarding nutrients

Pregestational physical inactivity, glucose and high BMI are independent decisive factors of fetal macrosomia (infant birth weight > 4200 g). Voldner et al showed a tendency, but no statistical significance between macronutrient intake and fetal growth were found (2). However, they looked at the mean intake of macronutrients during gestation weeks 14-32, and not the mean intakes as separately analyses (gestational weeks 14-16 and 30-32, respectively) (76).

High maternal protein intake may decrease the risk of giving birth to a macrosomic infant. In addition, many of the women that participated in this cohort had an unbalanced diet that did not correspond with the Norwegian guidelines. A large proportion of the women ate to little fiber (66 %), 30 % had too high sugar intake, 85 % ate saturated fat excessively and 42 % had too low intake of n-3 fatty acids (6).

A relatively high proportion of the women in STORK entered the pregnancy overweight or obese, 30.8 % were overweight (BMI 25-29.9 kg/m²) and 11.2 % were obese (BMI ≥ 30 kg/m²). Furthermore, a high energy intake was associated with a higher weight gain and skinfold thickness, compared to the women with a lower energy intake. Smaller total skinfold thickness was reported among women with a fiber intake > 25 g/d (27).

#### 2.26.2 Energy

Energy requirements during pregnancy vary greatly individually, and are estimated to be 20, 85 and 310 kcal extra per day in 1st, 2nd and 3rd trimester, respectively (70), due to fetal- and maternal endogenous tissue growth (blood volume, extracellular water, uterus, breasts, adipose tissue and placenta). It is thought that many women compensate for their increasing energy needs by reduced physical activity. Recommendations for weight gain (77) are given in relation to pre-pregnancy weight, with regards to best health outcomes for both mother and child (70, 77):
<table>
<thead>
<tr>
<th>Pre-pregnancy BMI (kg/m²)</th>
<th>Weight gain during pregnancy (kg)</th>
<th>Argumentations against excessive weight gain</th>
<th>Argumentations against a too low weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI &lt; 18.9</strong> (under weight)</td>
<td>13.5-18</td>
<td>Increased risks for complications</td>
<td>Increased risk for low birth weight, SGA, spontaneous preterm delivery, maternal delivery complications and adult diseases (coronary artery disease, hypertension and type 2 diabetes) in fetus.</td>
</tr>
<tr>
<td><strong>BMI 18.5-24.9</strong> (normal weight)</td>
<td>11.5-16</td>
<td>Increased long-term risks of obesity for the mother, increased risk for high birth weight (LGA or macrosomia) and delivery complications.</td>
<td>Increased risk for low birth weight</td>
</tr>
<tr>
<td><strong>BMI &gt; 25.0-29.9</strong> (over weight)</td>
<td>6.8-11.5</td>
<td>Increased risk for stillbirth, neonatal death, preeclampsia, gestational diabetes mellitus, hypertension, cardiovascular disease, miscarriage, caesarean deliveries, shoulder dystocia, post-op complications, neural tube defects, macrosomia and maternal breast cancer.</td>
<td>Maintenance of adequate nutrition status for both mother and child.</td>
</tr>
<tr>
<td><strong>BMI &gt; 30</strong> (obese)</td>
<td>5-9</td>
<td>Same risks as for the overweight pregnant women</td>
<td>Maintenance of adequate nutrition status for both mother and child.</td>
</tr>
</tbody>
</table>

### 2.26.3 Protein

The optimal protein intake during pregnancy is unknown, but the suggestion of 1.1 g/kg/day (10-20 E %) (77) is based on extra tissue and organ growth in both mother and child (70).

### 2.26.4 Carbohydrate

The carbohydrate intake should be 50-60 E % (77) since the fetus burns carbohydrate exclusively, but no official recommendations during pregnancy exist (70).

### 2.26.5 Fat

The extra fat demands (for structural lipids, energy and possibly the absorption of fat-soluble vitamins and some other substances) ought to be covered by the extra energy needs (20), and should be approximately 25-35 E % (77). No official recommendations, with exception for an adequate long-chain n-3 polyunsaturated fatty acids intake (PUFA’s) to ensure optimal neural
growth and development in the fetus. PUFA’s should contribute with 5 E %, where at least 1 E % is n-3.

2.27 Nutrition recommendations: Summary

There are only a few exceptions in the recommendations for nutritional demands in pregnant women, compared to non-pregnant women (40):

- Increased energy requirements for fetal development and growth of endogenous tissues, which is greatest during 3rd trimester.

- Essential fatty acids should contribute with at least 5 % of the total energy intake.

- Taking vitamin D supplements in the winter, folic acid one month preconceptionally and one month into pregnancy, and iron supplements if needed. In addition, women who take vitamin A supplements preconceptionally, are advised to discontinue this supplement three months before a planned pregnancy

- Increased need for almost all micronutrients:

<table>
<thead>
<tr>
<th>Table 5: Overview of the recommendations based on the NNR 2004 (40):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women (18-30 y)</td>
</tr>
<tr>
<td>Pregnant women</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Ca2+ (mg)</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Women (18-30 y)</td>
</tr>
<tr>
<td>Pregnant women</td>
</tr>
</tbody>
</table>

35
2.28 Birth weight

2.28.1 Maternal impact on birth weight

Fetal growth rate is assumed to be an independent predictor for short- and long term health of the newborn. There are several maternal factors assumed to affect infant birth weight: age, anthropometry, weight gain during pregnancy, smoking habits, physical activity, maternal genetics, nutritional and endocrinological factors (12, 79-86).

2.28.2 Small for gestational age (SGA) and low birth weight

There is no universal definition of SGA. The most used cut-off points for infants born SGA are a birth weight or a crown-heel length below the 10th percentile and 2 SDs below the mean for gestational age. Cut-offs like 2.5th percentile, 5th percentile or 3 SDs below the mean are also frequently used (87, 88). In addition, different levels of low birth weight are defined by low birth weight (LBW < 2500 g), very low birth weight (VLBW < 1500 g) and extreme low birth weight (ELBW < 1000 g).

The incidence in Norway (2008) for LBW, VLBW and ELBW was 5.4, 1.2 and 0.7 % (89), respectively.

Being born SGA or with a low birth weight is related to both short- and long term problems. Short-term complications are conditions related to prematurity such as cerebral palsy, respiratory complications (e.g. bronchopulmonary dysplasia), necrotizing enterocolitis, severe retinopathy\(^1\), hypoglycemia and hypotension. Long-term complications may be impaired cognitive development and increased risk of insulin resistance, dyslipidemia, cardiovascular diseases, cancer, type 2 diabetes and obesity (76, 87, 88, 90, 91).

Several studies have found that specific micronutrients may affect the risk of giving birth to an infant with low birth weight, but the link between high or low micronutrient intake during pregnancy and its relation to low birth weight is not obvious. Most of the studies find that multivitamin-mineral supplementation increase birth weight and decrease the risk of having a child with low birth weight (18, 65, 92-97).

\(^1\) A general term that refers to some form of persistent (i.e. inflammation) or acute (i.e. photon beam radiation) damage to the retina of the eye
2.28.3 Optimal birth weight

The relationship between birth weight and best possible outcome when it comes to morbidity and mortality may be described by an U-formed- or reversed J-curve, but there is no consensus of what the optimal infant birth weight is. The minimal perinatal mortality risk varies considerably between different populations (98-101). No final conclusions about exact optimal birth weight can be set because of the lack of firm evidence. Yet, it is likely that the birth weight should be somewhere between all extreme values, when looking at total mortality and morbidity.

2.28.4 Large for gestational age (LGA) and high birth weight

Like SGA, diverse definitions of LGA and macrosomia exists: above the 90\textsuperscript{th} percentile or 2 SDs above the mean for gestational age, and $\geq 4000$ g, $\geq 4200$ g, $\geq 4500$ g or $\geq 5000$ g (84, 102, 103).

The incidence in Norway (2008) for infants born with a birth weight of 4000-4499, 4500-4999 and 5000-5499 g, was 14.4, 2.7 and 0.3 %, respectively (89).

Being born LGA or with a high birth weight is associated with both short- and long term consequences. The former includes delivery- and neonatal complications such as prolonged labor time, increased risk of caesarean section/operative deliveries, artificial labor induction, maternal post partum hemorrhages and soft tissue trauma, and neonatal shoulder dystocia, clavicular fracture, hypoglycemia and hyperbilirubinemia. Long-term complications for the mother may be post partum traumatic stress symptoms, poorer reproductive health, persistence perineal-, urinary- and anal dysfunction defects. Fetal long-term consequences may be increased susceptibility of chronic diseases like type 2 diabetes, cancer, cardiovascular diseases and obesity in adult life (76, 102, 104-107).

The knowledge and conclusions about micronutrients and infants born with high birth weight are inconclusive. Some studies find that micronutrient intake and supplementation might increase infant birth weight, whereas others have found that it might decrease the risk of having a macrosomic child (92, 108-110).
3 Study aims

1. To describe the micronutrients intake in the STORK population in gestation week 14-16 and 30-32, and a possible association with birth weight.

2. To investigate iron status during pregnancy in a subgroup of the STORK population, and whether the different iron status biomarkers (iron, transferrin, transferrin saturation, soluble transferrin-receptor and ferritin) are associated with infant birth weight.
4 Materials and methods

4.1 Subjects and study design

STORK is a voluntary, prospective cohort study of healthy Norwegian women of Scandinavian heritage registered for obstetric care at Oslo University hospital (OUS, Rikshospitalet) during the period 2002-2008 (76, 111). A total of 4122 women were invited to participate, which is roughly one-third of the qualified women that were scheduled for giving birth at the hospital. 1241 women accepted the invitation, and 110 of these were excluded before inclusion. This means that 1131 women were included in the final study. 97 of these participants were lost to follow-up or excluded due to various causes (stillbirth, fetal malformations, twin births, the mothers moved or gave birth at other hospitals, and dropouts). 553 women participated in STORK 1 (2002-2005), and 479 women participated in STORK 2 (2005-2008). Thus, the entire study population consisted of 1031 pregnant women.

Figure 4: Flow-chart of STORK 2002-2008
**Inclusions criteria:**

- Scandinavian, healthy pregnant women who gave birth at OUS, Rikshospitalet
- Singleton pregnancy

**Exclusions criteria:**

- Multiple pregnancies (twins)
- Pregnant women with serious diseases including diabetes mellitus type 1 (T1DM), lung-, kidney-, gastrointestinal- or heart disease
- Pregnancy with miscarriage, or fetuses with malformations

The participants were followed up at four visits during pregnancy (from gestation week 14-38). Data and blood samples used in this thesis are from visit 1 (gestation week 14-16) and 3 (gestation weeks 30-32), where a food frequency questionnaire (see Appendix 10.3) was filled out, and a standard oral glucose tolerance test (OGTT) was performed. The participants were also weighed at both visits.

### 4.2 Method

#### 4.2.1 Data assessment

Analyzed data and blood samples that were already collected in STORK study were used in this master thesis, with exception for the analyses of the women’s iron status, which were analyzed in 2012. The blood samples were taken and immediately frozen down in 2002-2008, whereas the actual analysis of the iron status was done in the start of 2012.
4.2.2 Cost registration

A Norwegian self-administered Food Frequency Questionnaire (FFQ), NORKOST 1997 (see Appendix 10.3), was used to record the participants' dietary habits during pregnancy.

The FFQ is designed to measure the average intake over the last 6-12 months in a typical Norwegian meal. The questions consisted of both quantitative amounts and consumption patterns depending on the foods and beverages, and dietary habits were to rely on the current period (what they consumed during gestation week 14-16 and 30-32). Approximately 180 food groups and supplements were listed in the questionnaire, and if something was missing, the participants were able to fill this in under "other". Intakes of vitamin and mineral supplements (cod liver oil, multivitamin-mineral, vitamin B-, C-, D- and E, iron, calcium and fluoride) are included in the nutrition calculations, and do not exist as separate variables in the data set, which means that the calculated micronutrient intake is a mean of both dietary and supplementation intakes.

Written instruction was given on the first page of the FFQ, so unless the participants asked, no further instruction was given.

The completed FFQ forms were scanned digitally (Teleform 6.0), and error-checked manually by the same person. The database IE96 (KBS software) for nutritional calculations (developed by the Department of Food Science, University of Oslo) was used to calculate food intake and nutrition content of the questionnaires, and is based on the Norwegian Food Composition Table from 1997 (112).

NORKOST 1997 is well validated for the Norwegian population, but has not been validated for pregnant women (113). At the start of the cohort, no other FFQ forms for Norwegian, pregnant women were available (114).

4.2.3 Calculation of the sample size

The number of blood samples required to get a strength of 90 % in the iron study group, was based on discovering a clinically relevant difference in birth weight of 10-50 g, and on clinical experience. The formulae for sample size determination taken from Medical Statistics by Kirkwood and Sterne were used, where the aim is to demonstrate a significant difference in the dependent variable (in this case: the infant birth weight):
\[
\frac{(u + v)^2 \sigma^2}{(\mu - \mu_0)^2}
\]

Where,
\( \mu - \mu_0 \) = Difference between mean, \( \mu \), and null hypothesis value, \( \mu_0 \),
\( \sigma \) = Standard deviation
\( u \) = One-sided percentage point of the normal distribution corresponding to 100 % - the power
\( v \) = Percentage point of the normal distribution corresponding to the two sided significance level

Different approaches were tried with varying values of the significance level (1-5 %), minimal detection in difference in birth weight (clinical relevance between 10-250 g) and power (80-95 %) to see how much the N changed with the different approaches. The most reasonable number of participants was 100.

### 4.2.4 Blood samples and analyzes

**Blood sampling for the Iron study**

A randomized sample size of 100 women was extracted in SPSS from 706 women (of the 1031 participants) where dietary iron data were available. A standard oral glucose tolerance-test with 75 g glucose was performed after 10-12 h fasting between 07:30 and 8:30 am (115) during STORK. The blood samples used for analyses were obtained from venipuncture and collected in 7-ml Vacutainer tubes. Vacutainer® tubes on ice containing EDTA blood were centrifuged at 3000 G for 25 minutes at 4ºC, and plasma was aliquoted and stored at –80ºC within 1 h at the Central Laboratory at Oslo University Hospital (OUS). To our knowledge, glucose does not affect the iron status in the serum. Therefore, we decided to use the 90 minutes serum from both gestation week 14-16 and 30-32.

Due to an unexpected increasing trend in fasting blood glucose concentrations over the 7 years of recruitment, all glucose measurements were de-trended prior to the analyses (111). To detrend data means that you subtract the mean or a best-fit line from your data. Removing a trend from the data enables you to focus your analysis on the fluctuations in the data about the trend, which in this case was a systematic trend. Whereas some trends can be meaningful, systematic trends yield better insight once you remove the trends.
Analysis

The samples were thawed overnight in the refrigerator the day before the analyses. All preparation was done in cooperation with Endocrinology Laboratory at OUS prior to the analyses. Of the 200 blood samples (two from each woman: one from gestation week 14-16, and one from gestation week 30-32), Central Laboratory analyzed 100 blood samples at a time (400-500 µL in each sample).

The analyses in blood plasma comprised of iron, transferrin (Tf), soluble transferrin-receptor (s-TfR), transferrin-saturation (Tf-saturation) and ferritin. The plasma levels of Tf, s-TfR and iron were measured by Modular P, while ferritin was auto analyzed by Modular E 170, both at an accredited laboratory at OUS, Rikshospitalet. Transferrin saturation is a calculated by dividing iron by transferrin (s-jern/2 × s-transferrin). Intra- and inter assay coefficients of variation (CVs) were < 10 % for all assays.

It would have been very desirable to measure hemoglobin, but since we only had serum (and not whole blood) this was not possible. Because of the transition from paper to e-journals in Norwegian hospitals, we were not able to find the Hb value in the medical records.

4.2.5 Anthropometric measures

All women were weighed on a digital weight (normal digital weight in STORK 1, and Tanita weight in STORK 2 (116)) to the nearest 100 g, with light clothes and shoes. The height is self-reported to nearest cm, but a comparison of measured height and self-reported height of 50 women during STORK 1 showed little discrepancy.

The same person made all measurements in STORK 1, while in the STORK 2, there were two different persons responsible for the collection.

Newborns were measured to the nearest 10 grams on a digital weight (Data Baby Scales 930 from SOLOTOP OY, Finland) within two hours after birth. Data from the first 150 births were collected from paper records, whereas the rest are from new digital records.
4.2.6 Micronutrients available for analyses

The micronutrients that were available in the dataset from STORK for analyses were: vitamin A, C, D and E, β-carotene, tiamin (B₁), riboflavin (B₂), folic acid (B₉), cobalamin (B₁₂), iron, calcium and magnesium. All were considered as continuously, independent variables.

4.2.7 Calculation of basal metabolic rate (BMR), and under- and overreporting

Reported body weight is related to BMR based on age and sex. Since the mean age in STORK was 30 years, it was decided to use the BMR formula for women in the age range 18-29 years (70), which means that the women get a slightly higher BMR than if the formula for women 30-59 years had been used.

\[
BMR = (14.8 \times kg \text{ body weight}) + 487
\]

Energy intake (EI, kcal) divided by BMR will give an estimate on whether participants are having an energy intake consistent with life over time. An EI/BMR < 1.35 is considered to represent underreporting (where EI/BMR < 1.14 is inconsistent with life), while an EI/BMR ≥ 2.4 is considered as overreporting (117).

4.2.8 Statistical analysis

Birth weight (dependent variable), micronutrients and iron status biomarkers (independent variables) were considered as continuously data in all analyses.

Characteristics of the subjects, the micronutrients and the different iron variables were examined by descriptive statistics. Most of the micronutrients and some of the iron biomarkers were highly skewed, and log transformation was done prior to analysis to identify the most extreme values and to see whether the data would become more normally distributed or not. Mild outliers (2 SD from the median) and extreme outliers (3 SD from the median) were thoroughly examined in the original dataset and in the women’s individual FFQ’s.

Possible differences and similarities in the participants, the diet and iron status biomarkers were examined by paired t-test (2-tailed) for normal distributed data, and Wilcoxon Signed Rank test for variables that were nonparametric. The log transformed values are only used in the paired t-test and the correlations tests.
The correlation between infant birth weight and the independent continuously variables were examined with Pearson’s $r (r_p)$ and Spearman’s rho ($r_{sp}$), depending on whether the variables were normally distributed or not. When the correlation coefficients $r_p$ and $r_{sp}$ gave the same conclusions of associations, $r_p$ was chosen for presentation.

Possible predictors of infant birth weight were analyzed with univariate- and multiple logistic regression analyses. The different explanatory variables (that were significant at the 0.10 level in the correlation analysis), were first examined separately in univariate analyses. The crude effects of micronutrients on infant birth weight that were significant at the 0.05 levels in the univariate analyses, were further analyzed in a multiple linear regression model (forward analysis). Possible confounders and colliders were taken into account in the final model. Standardized regression coefficients ($b$) are denoting the increase in the dependent variable for a standard deviation increase in the explanatory variable, and are presented with accompanying 95 % CI. Since the $R^2$ values of the individually explanatory variables were very low, these values are not shown.

All analyses were done by ´intention to treat´ when possible, and were systematically checked for possible violations of assumptions connected to the different analysis methods and models. The Statistical Package of Social Sciences (SPSS version 19.0, Inc., Chicago, IL, USA) was used for all statistical processing and analyses of the data. Statistical significance was considered when $p < 0.05$.

4.3 Ethics

The regional committees for medical and health research approved STORK. The study followed the Declaration of Helsinki. Using data from STORK to external analysis is approved by the IEC (The Regional Ethical Committee), see Appendix 10.2. All participants gave a written consent (Appendix 10.1), and all participated voluntarily and had the opportunity to withdraw at any time without any consequences for them.
5 Results

5.1 Characteristics of the women and newborns

Evaluations of the cohort (n=1031) and the iron study group (n=100) showed no significant discrepancies in maternal age, gestational age, gestational weight gain, BMI, smoking habits, plasma glucose values, infant birth weight or the child’s gender (data not shown). Demographic-, anthropometric-, plasma glucose- and newborn data are shown in Table 6. The data is presented as the mean (standard deviation), median (25-75 percentile) and min-max.

Table 6: Characteristics of the women in both the cohort (n=1031) and the iron study group (n=100).

<table>
<thead>
<tr>
<th>Demography and anthropometry (n = 1031)</th>
<th>n (%)</th>
<th>Mean (SD)</th>
<th>Min - Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (year)</td>
<td>1031 (100 %)</td>
<td>31.2 (3.9)</td>
<td>19.0 - 42.0</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>1029 (99.8 %)</td>
<td>39.9 (1.8)</td>
<td>26.0 - 43.1</td>
</tr>
<tr>
<td>BMI visit 1 (kg/m²)</td>
<td>1001 (97.1 %)</td>
<td>24.5 (3.9)</td>
<td>17.2 - 43.9</td>
</tr>
<tr>
<td>BMI visit 3 (kg/m²)</td>
<td>981 (95.2 %)</td>
<td>27.2 (3.9)</td>
<td>19.4 - 46.8</td>
</tr>
<tr>
<td>Daily smoking (yes)</td>
<td>31 (3 %)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Demography and anthropometry (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (year)</td>
</tr>
<tr>
<td>Gestational age (year)</td>
</tr>
<tr>
<td>BMI visit 1 (kg/m²)</td>
</tr>
<tr>
<td>BMI visit 3 (kg/m²)</td>
</tr>
<tr>
<td>Daily smoking (yes)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plasma glucose values (n = 1031)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-h plasma glucose visit 1 (mmol/L)</td>
</tr>
<tr>
<td>2-h plasma glucose visit 3 (mmol/L)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plasma glucose values (n = 100)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-h plasma glucose visit 1 (mmol/L)</td>
</tr>
<tr>
<td>2-h plasma glucose visit 3 (mmol/L)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Newborn data (n = 1031)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
</tr>
<tr>
<td>Gender (boy)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Newborn data (n = 100)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
</tr>
<tr>
<td>Gender (boy)</td>
</tr>
</tbody>
</table>

a Data from the iron study group
The percentage of infant birth weight being less than 2500 g, < 1500 g and < 1000 g was 3.8 %, 0.3 % and 0.1 %, respectively. The percentage of infant birth weight being between 4000-4499 g, 4500-4999 g and 5000-5499 g, was 15.6 %, 4.6 % and 0.4 %, respectively. Over 13 % of the infants weighed more than 4200 g.

5.2 Characteristics of the nutrients

Table 7 summarizes the dietary intake of macro- and micronutrients during gestation week 14-16 and 30-32. Results are presented as mean (SD) for normal distributed data, as median (25-75 percentile) for non-normal distributed data, and as minimum and maximum values.

Table 7: Intake of macro-and micronutrients during gestation week 14-16 and 30-32.

<table>
<thead>
<tr>
<th></th>
<th>Visit 1 (week 14-16)</th>
<th>Visit 3 (week 30-32)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SD)</td>
<td>Min - Max</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>1026</td>
<td>8497.7 (2093.4)</td>
<td>3336.0 - 18284.0</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>1026</td>
<td>78.9 (20.5)</td>
<td>25.2 - 157.3</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>1026</td>
<td>73.6 (23.2)</td>
<td>25.1 - 216.2</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>1026</td>
<td>259.2 (69.5)</td>
<td>74.5 - 662.2</td>
</tr>
<tr>
<td>Vitamin A (µg)</td>
<td>1026</td>
<td>1206.5 (871.0 - 1580.25)</td>
<td>268.0 - 17489.0</td>
</tr>
<tr>
<td>Beta-carotene (µg)</td>
<td>1026</td>
<td>3372 (2225.8 - 5269.0)</td>
<td>289.0 - 31748.0</td>
</tr>
<tr>
<td>Vitamin D (µg)</td>
<td>1026</td>
<td>7.9 (4.9 - 12.3)</td>
<td>0.5 - 104.2</td>
</tr>
<tr>
<td>Vitamin E (alfa-TK)</td>
<td>1026</td>
<td>14.2 (9.5 - 20.1)</td>
<td>1.8 - 223.2</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>1026</td>
<td>178.0 (131.0 - 250.0)</td>
<td>15.0 - 1450.0</td>
</tr>
<tr>
<td>Tiamin (mg)</td>
<td>1026</td>
<td>1.5 (1.2 - 1.9)</td>
<td>0.4 - 29.0</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>1026</td>
<td>1.9 (1.4 - 2.5)</td>
<td>0.3 - 33.4</td>
</tr>
<tr>
<td>Cobalamin (µg)</td>
<td>547</td>
<td>5.4 (3.9 - 6.9)</td>
<td>1.2 - 28.5</td>
</tr>
<tr>
<td>Folic acid (µg)</td>
<td>1023</td>
<td>410.0 (257.3 - 584.0)</td>
<td>88.0 - 4620.0</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1026</td>
<td>903.9 (334.9)</td>
<td>132.0 - 2528.0</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>707</td>
<td>10.8 (8.8 - 14.3)</td>
<td>4.1 - 286.6</td>
</tr>
<tr>
<td>Iron (mg)a</td>
<td>100</td>
<td>9.9 (8.3 - 12.1)</td>
<td>4.1 - 199.7</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>707</td>
<td>318.7 (82.8)</td>
<td>112.0 - 630.0</td>
</tr>
</tbody>
</table>

*a Median (25-75 percentile) when non-normally distributed  
*b Iron intake in the iron study group

Most of the women had an adequately mean intake of micronutrients compared to the NNR for pregnant women, with exception of some nutrients. Vitamin A intakes are too high and well beyond the strictly, recommended daily intake of 800 µg/day, with a median value of 1206 and 1184 µg in gestation week 14-16 and 30-32, respectively. Vitamin D and iron intake are below the recommended intake, with a median of 8 µg and 11 mg in gestation week 14-
16, and a median intake of 8 µg and 12 mg in gestation week 30-32, respectively. The median folic acid intake was within the recommended intake in gestation week 14-16, but was too low in gestation week 30-32 (median 269 µg).

The protein-, β-carotene-, vitamin C-, folic acid- and iron intake changed significantly from gestation week 14-16 to gestation week 30-32. The intake decreased for every nutrient except for iron, which slightly increased.

During gestation week 14-16, 2.2-76.8 % of the women did not meet the daily recommendations, depending on the micronutrient. The different frequencies are noted in Table 8.

Table 8: Percentage of how many pregnant women who had an intake below the lower limit of intake, how many who met the RDI and how many of the women that had an intake above the upper limit of intake.

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>% &lt; LL&lt;sup&gt;a&lt;/sup&gt;</th>
<th>How many did not reach RDI</th>
<th>% &gt; RDI</th>
<th>% &gt; UL&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gestation week 14-16</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>3.9 %</td>
<td>14.7 %</td>
<td>78.5 %</td>
<td>2.9 %</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>6.1 %</td>
<td>64.0 %</td>
<td>35.7 %</td>
<td>0.3 %</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>ND</td>
<td>22.8 %</td>
<td>77.2 %</td>
<td>0.0 %</td>
</tr>
<tr>
<td>Tiamin</td>
<td>ND</td>
<td>46.8 %</td>
<td>53.2 %</td>
<td>ND</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>ND</td>
<td>34.9 %</td>
<td>65.1 %</td>
<td>ND</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>3.9 %</td>
<td>5.8 %</td>
<td>94.2 %</td>
<td>0.0 %</td>
</tr>
<tr>
<td>Calcium</td>
<td>ND</td>
<td>41.8 %</td>
<td>58.1 %</td>
<td>0.1 %</td>
</tr>
<tr>
<td>Iron</td>
<td>ND</td>
<td>76.8 %</td>
<td>23.2 %</td>
<td>5 %</td>
</tr>
<tr>
<td>Magnesium</td>
<td>ND</td>
<td>32.5 %</td>
<td>67.5 %</td>
<td>ND</td>
</tr>
<tr>
<td>Cobalamin</td>
<td>ND</td>
<td>2.2 %</td>
<td>97.8 %</td>
<td>ND</td>
</tr>
<tr>
<td>Folate</td>
<td>ND</td>
<td>48.2 %</td>
<td>51.8 %</td>
<td>3.3 %</td>
</tr>
<tr>
<td><strong>Gestation week 30-32</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>3.1 %</td>
<td>18.0 %</td>
<td>79.8 %</td>
<td>2.2 %</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>5.8 %</td>
<td>60.0 %</td>
<td>39.8 %</td>
<td>0.2 %</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>ND</td>
<td>22.9 %</td>
<td>77.1 %</td>
<td>0 %</td>
</tr>
<tr>
<td>Tiamin</td>
<td>ND</td>
<td>49.7 %</td>
<td>50.3 %</td>
<td>ND</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>ND</td>
<td>27.3 %</td>
<td>72.7 %</td>
<td>ND</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>3.9 %</td>
<td>7.5 %</td>
<td>92.5 %</td>
<td>0.0 %</td>
</tr>
<tr>
<td>Calcium</td>
<td>ND</td>
<td>53.9 %</td>
<td>41.5 %</td>
<td>4.6 %</td>
</tr>
<tr>
<td>Iron</td>
<td>ND</td>
<td>63.5 %</td>
<td>29.5 %</td>
<td>7.3 %</td>
</tr>
<tr>
<td>Magnesium</td>
<td>ND</td>
<td>32.3 %</td>
<td>67.7 %</td>
<td>ND</td>
</tr>
<tr>
<td>Cobalamin</td>
<td>ND</td>
<td>0.6 %</td>
<td>99.4 %</td>
<td>ND</td>
</tr>
<tr>
<td>Folate</td>
<td>ND</td>
<td>77.1 %</td>
<td>22.9 %</td>
<td>3.1 %</td>
</tr>
</tbody>
</table>

<sup>a</sup> LL = Lower limit of intake: 500 µg for vitamin A, 2.5 µg for vitamin D, 75 mg for vitamin C.
<sup>b</sup> UL = Upper limit of intake: 3000 µg for vitamin A, 50 µg for vitamin D, 1000 alfA-TE for vitamin E, 1000 mg for vitamin C, 2500 mg for calcium, 50 mg for iron and 1000 µg for folate.
ND = No data available
Few of the women reached the lower- and upper daily limits in gestation week 14-16, 3.9-6.1% and 0.0-5.0% respectively. In gestation week 30-32, 0.6-77.1% did not meet the RDI, depending on the nutrient. Between 3.1-5.8% and 0.0-7.3% had an intake below the lower limit or above the upper limit, respectively. Of most concern are the vitamin D-, iron-, calcium- and folic acid intakes, where as many as 48.2-76.8% of the women had an intake too low to meet the daily recommended intakes.

Analysis of under- and overreporting in the STORK population showed that 42.4% of the women underreported their dietary intake in gestation week 14-16, where 19.9% of the underreporters had an intake inconsistent with life over time. In gestation week 30-32, 62.3% underreported their intake and 37.7% of these had an intake inconsistent with life over time. Only 2.1 and 0.4% overreported their intake in gestation week 14-16 and 30-32, respectively.

5.3 Characteristics in the iron study group

The women’s iron status (based on plasma concentrations of iron, transferrin, soluble transferrin-receptor, transferrin-saturation and ferritin) is summarized in Figures 2-6 with mean (SD) for normally distributed data, or with median (25-75 percentile) for non-normally distributed. As expected iron, transferrin-saturation and ferritin values decreased significantly from early to late pregnancy, whereas transferrin (Tf) and soluble transferrin-receptor (s-TfR) concentrations increased along with the reduced iron supplies.

P-iron levels (µmol/L) at gestation week 14-16 and 30-32 were 9 (7-11) and 5 (4-7), respectively, The concentration changed significantly from early to late pregnancy (p<0.001), where min and max values were 4-18 and 2-16, respectively (Figure 5). P-Tf (g/L) at gestation week 14-16 and 30-32 were 2.6 (0.5) and 3.7 (0.6), respectively, and changed significantly from early to late pregnancy (p<0.001). Min and max values were 0.9-4.3 and 2.4-5.3 at gestation week 14-16 and 30-32 (Figure 6). P-Tf saturation (%) at gestation week 14-16 and 30-32 was 13.0 (0.7) and 6.0 (0.4), and changed significantly from early to late pregnancy (p<0.001). Min and max values were 0.01-0.29 and 0.01-0.22, respectively (Figure 7). P-TfR (mg/L) concentration at gestation week 14-16 and 30-32 were 1.9 (0.4) and 2.9 (0.9), respectively. There were a significantly change from early to late pregnancy (p<0.001),
where min and max values were 0.7-3.1 and 1.3-5.7, respectively (Figure 8). P-ferritin (µg/L) concentration at gestation week 14-16 and 30-32 were 40 (25-76) and 8 (6-15), and changed significantly from early to late pregnancy (p<0.001). Min and max values were 6-374 and 3-227, respectively (Figure 9).

Figure 5: Box-plot (n=100). P-iron levels (µmol/L) at gestation week 14-16 and 30-32.

Figure 6: Box-plot (n=100). P-Tf (g/L) at gestation week 14-16 and 30-32.
Figure 7: Box-plot (n=100). P-Tf saturation (%) at gestation week 14-16 and 30-32.

Figure 8: Box-plot (n=100). P-TfR (mg/L) at gestation week 14-16 and 30-32.

Figure 9: Box-plot (n=100). P-ferritin (µg/L) at gestation week 14-16 and 30-32.
In addition to the results mentioned above, daily nutrient intake among the women in the iron study group showed that 82 % and 71 % did not reach the recommended daily intake of iron (15 mg) during early and late pregnancy, respectively, and that 2 % and 4 % had an intake above 50 mg/day. When looking at the iron (μmol/L) concentration, 77 % and 94 % had levels below the lower limit of 12 μmol/L during gestation week 14-16 and 30-32. With regards to the transferrin levels (g/L), 21 % had concentrations indicating iron deficiency (p-Tf > 3.9) in late pregnancy, whereas 11 % had values indicating iron overload in early pregnancy, respectively. For soluble transferrin-receptor (mg/L), 6.1 % had levels above 4.4 mg/L in gestation week 30-32, which indicates tissue iron deficiency. For transferrin-saturation (%), 60 % and 95 % had a saturation of transferrin below 15 % in gestation week 14-16 and 30-32, which indicates iron deficiency. During gestation week 14-16, 4.0 %, 7.1 %, 13.1 % and 62.6 % of the women had ferritin levels below 12, 15, 20 and 60 μmol/L, whereas only 4.0 % and 1.0 % had levels above 150 and 300 μmol/L. During gestation week 30-32, 62.6 %, 78.8 %, 89.9 % and 99.0 % of the women had ferritin levels below 12, 15, 20 and 60 μmol/L, whereas only 1.0 % had levels above 150 μmol/L. This means that only 37.4 % had proper iron storage in early pregnancy to meet the iron demands during pregnancy (~500 mg).

When using WHO´s definition (51) of iron deficiency based on ferritin values (< 15 μg/L), 7.1 % had iron deficiency in the beginning of the pregnancy, whereas 78.8 % had iron deficiency in 3rd trimester. WHO´s definition of severe iron deficiency (P-ferritin < 12 μg /L implies that 4 % of the women went into the pregnancy with empty iron stores (but not necessarily iron-deficient erythropoiesis (52)), and that 62.6 % had empty iron stores in 3rd trimester.

Whether or not the women had iron deficiency depends on which biomarker is used for the definition of iron deficiency and anemia. Figure 10 illustrates how many of the pregnant women that had a value below the reference level of the given biomarker, how many that had a value above the reference level, and how many that had an adequate iron status (defined by the given biomarker alone).
5.4 Correlation between intake of micronutrients and birth weight

Significant correlations were observed between infant birth weight and calcium-, magnesium- and iron intakes (Figure 11-12). Iron was positively correlated ($r_p: 0.085, p=0.024$) with birth weight in gestation week 14-16, whereas calcium where positively correlated with birth weight in gestation week 30-32 ($r_{sp}: 0.102, p=0.008$). Magnesium was positively correlated with birth weight in both gestation week 14-16 and 30-32 ($r_{sp}: 0.077 (p=0.042)$ and $r_{sp}: 0.107 (p=0.006)$, respectively). No significant correlation was observed between the other micronutrients and infant birth weight.

Figure 10: Iron status defined by the different iron biomarkers. The lower limit intake of iron is 15 mg, whereas the upper limit intake is 50 mg. The lower and upper limit of P-iron (μmol/L) is 12 and 35 μmol/L, respectively. The lower and upper limit of P-Tf (g/L) is 2.2 and 3.8 g/L, respectively. The lower and upper limit of P-TfR (mg/L) is 1.9 and 4.4 mg/L, respectively. The lower and upper limit of P-Tf-saturation (%) is 15 and 45 %, respectively. The lower and upper limit of P-ferritin (μg/L) is 15 and 150 μg/L, respectively.
Iron’s correlation with infant birth weight could have been a coincidence based on a large study sample (n=1031). However, a division of the study sample in three groups (subjects 1-500, 250-750 and 500-1030, respectively) was done and the correlation remained even more statistically significant in all subgroups, compared to the whole sample (subjects 1-1031) (data not shown). This suggests that the positive results are not due to the large study sample alone.
5.5 Correlation between iron status in blood and birth weight

None of the blood status variables (iron, transferrin, soluble transferrin-receptor, transferrin-saturation or ferritin) showed any correlation with infant birth weight. Nor did the iron intake correlate with any of the iron status biomarkers (data not shown).

5.6 Regression analysis

The results of the linear regression analysis are detailed in Table 9, with crude and adjusted values. Univariate linear regression showed that iron (in gestation week 14-16), magnesium (in gestation week 14-16 and 30-32) and calcium (in gestation week 14-16) were significant correlated with infant birth weight. For every 10 mg increase in iron, calcium or magnesium intake, the infant birth weight increased with 19.1, 1.40 and 5.52 g, respectively.

When using a multivariate linear regression model, all effects of iron, calcium and magnesium on infant birth weight ceased. After the adjustments for confounders (total energy intake at gestation week 14-16 and 30-32), no changes in the results were seen (data not shown).

Table 9: Multiple regression model with both crude and adjusted values. Infant birth weight (g) explained by iron-, calcium- and magnesium intakes (n=1031).

<table>
<thead>
<tr>
<th></th>
<th>Crude</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Iron intake (mg)</strong></td>
<td><strong>b</strong></td>
<td><strong>(95 % CI)</strong></td>
</tr>
<tr>
<td></td>
<td>1.91</td>
<td>(0.13, 3.70)</td>
</tr>
<tr>
<td><strong>Calcium intake (mg)</strong></td>
<td>0.14</td>
<td>(0.01, 0.27)</td>
</tr>
<tr>
<td><strong>Magnesium intake (mg)</strong></td>
<td>0.55</td>
<td>(0.04, 1.07)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Significant at 0.05 level
Furthermore, the multiple regression model was checked for variables known to affect infant birth weight, but with uncertain impact on micronutrient intake (smoking (10), gestational age, glucose values (118), weight gain during pregnancy, gender of the child, pregestational weight/BMI, protein intake (119) and the mothers physical activity level). After the adjustments, the effects of iron, calcium and magnesium on infant birth weight increased slightly, but remained non-significant (data not shown).

5.7 Summary of the results

The average infant birth weight in this thesis corresponds to the distribution in Norway (120). However, the proportion of infants born with low- or high birth weight was lower in this study population compared to the general population.

Intakes of many of the micronutrients were too low compared to the NNR 2004 (40), whereas only few of the women had intakes above the recommendations. Of most concern is the high vitamin A consumption during pregnancy, and the low iron-, folic acid- and vitamin D intakes.

This thesis showed a slight increase in infant birth weight for every mg iron, calcium or magnesium the mothers consumed (either through diet, supplements or both) in univariate analyses. No other correlations between micronutrients, iron status biomarkers and infant birth weight were found.

Many of the women displayed iron deficiency trends at early pregnancy, which worsened during 3rd trimester. Very few of the women had an iron status adequate for a pregnancy without developing iron deficiency.
6 Discussion

The purpose of this master thesis was to investigate if maternal intake of micronutrients and maternal iron status had an effect on infant birth weight, by analyzing the micronutrient intake and iron status biomarkers in gestation week 14-16 and 30-32. We found that micronutrient deficiency and low iron stores is common in healthy, Norwegian pregnant women. Significant correlations between iron, calcium and magnesium and infant birth weight were observed in univariate analyses.

Whether or not it was the dietary intake, the supplements or both that contributed to the positive univariate associations found between iron, calcium, magnesium and birth weight in this thesis, remains uncertain. Unfortunately, how much the supplements contributed to the nutrient intakes in STORK was not possible to see since the dataset only included a variable that recorded both dietary and supplement intake.

Beneficial health effects on fetal development of nutrient supplementation in well-nourished pregnant populations are only documented for folic acid supplementation in the prevention of NTD’s (124) and for iron in the prevention of anemia (125). Most of the studies on micronutrients intake and fetal development are carried out in developing countries, and are comparing multivitamin-mineral (MVM) supplementation vs iron/folic acid supplementation during pregnancy. Many studies imply that pre- and perinatal multiple micronutrients supplements may improve fetal growth, and that the effect on fetal growth is cumulative (17, 126). Meta-analyses of antenatal MVM supplementation in the developed world reveal a modest but significant increase in birth weight, reduction in low birth weight, SGA and preterm birth (95, 121). Some studies have found no impact on preterm birth or perinatal mortality (16, 127), whereas others have found a lower risk of perinatal mortality (96).

For the remaining micronutrients, the findings indicate no association with birth weight, at least within the range of intake encountered in this investigation. With respect to the vitamin A, -C, -D and -E, some studies have found positive or negative correlation to birth weight, whereas the majorities have reported no association (110, 112, 113). For the vitamins tiamin, riboflavin, niacin, pyridoxine, pantothenic acid and cobalamine, most studies have found no association, which is in accordance with this thesis. However, negative correlation between niacin and birth weight has been reported, and positive correlations between pantothenic
acid, biotin, cobalamine and birth weight has been reported (110). With respect to folate intakes, most studies have found positive associations, but some studies report absence of an association or negative association (96, 114).

The same applies for maternal intakes of mineral- and trace elements as with vitamins and their association with infant birth weight: the results of the studies are largely contradictory. Thus, positive, negative and no associations have been reported for iron, calcium, copper, magnesium, sodium and zinc (110, 115), which reflect the findings in this study.

As seen in the results, a large part of the women did not reach RDI for micronutrients. Similar results are seen in Norwegian Mother and Child Cohort (2008) and in NORKOST 1997 (24, 122). The intake of the remaining micronutrients did not give rise to concern.

6.1 Vitamin A intake and correlation with infant birth weight

No correlation between vitamin A intakes and infant birth weight was found in our study. Nevertheless, many studies have found associations between the vitamin and birth weight. Positive, negative and no correlation have been noted (128, 129). The results from the different studies are not consistent, and further research on the subject is needed.

The median intakes of vitamin A were high at both early and late pregnancy, and 80.4 % of the women had an intake above 800 µg/day, where 2.9 % of these had an intake above the upper limit of 3000 µg/day. This is in accordance with findings in other Norwegian studies regarding both pregnant and non-pregnant women (24, 122).

Numerous studies have documented deleterious effects of retinol deficiency on fetal development. The most critical period is during organogenesis in early pregnancy. In animal studies, the likelihood that teratogenicity occurs depends on the level of exposure as well of the time of exposure. The available epidemiological studies indicate that intakes up to 3 mg vitamin A per day during pregnancy do not increase the risk to give birth to a malformed child. Available data do not allow for clear-cut definition of vitamin A intakes during
pregnancy. However, fertile women should be encouraged to limit their vitamin A intake from food and supplements to below 3000 mg/day (123, 130).

6.2 Vitamin D intake and correlation with infant birth weight

No correlation between vitamin D intakes and infant birth weight were found in our study. Nonetheless, some studies have found associations between the vitamin and birth weight. Most of the data have indicated positive or no effects (13, 45, 128, 131).

It is ominous that as many as 64.0 and 60.0 % of the women did not reach the recommended daily intake in gestation week 14-16 and 30-32. In contrast to our findings, MoBa found that the dietary intake of vitamin D did reach the RDI for pregnant women. The median intakes were 10.4 µg, where supplements provided more than 50 % of the total intake of vitamin D. Since several studies have showed that approximately 80 % of the pregnant women take MVM supplements (24, 132, 133), it is of even greater surprise that so many pregnant women in STORK did not meet the daily requirements. However, this might be due to the limitations regarding FFQ, rather than an actual low vitamin D intake among STORK.

As mentioned in the background, low maternal vitamin D intake and status has been associated with complications for both mother and infant, which makes the focus on an adequate intake during pregnancy even more important. The results are inconsistent, and blinded randomized trials of vitamin D supplementation during pregnancy with long-term follow-up are needed to determine the benefits, and possible risks, on offspring growth and development.

6.3 Folic acid intake and correlation with infant birth weight

No correlations between folate intake and infant birth weight were seen in this study. Nevertheless, other studies have found positive, negative and no correlations to birth weight
in well-nourished pregnant women (134, 135, 136), and no increase in the risk of neonatal mortality.

In STORK, 48.2 % of the women did not reach the recommended intake of 400 µg/d in gestation week 14-16. However, the currently folic acid intake is probably higher nowadays, with the increased focus on the importance of folic acid supplements, the intensified governmental information to the public, and because of the limitations regarding the FFQ used in STORK. In contrast to our findings, MoBa found that the dietary intake of folate did reach the RDI for pregnant women. The median intake was 607 µg, where supplements provided more than 50 % of the total intake (24).

As mentioned earlier, folate is critically important for fetal development. The need for folate increases during times of rapid tissue growth: nucleic acid synthesis and cell division. Animal models have showed that epigenetic features, for example DNA methylation, can be altered periconceptionally by nutritional interventions such as folate supplementation, thereby changing the phenotype (38). The evidence in humans is scant. What we know is that during pregnancy, carbon-1 metabolism has to cope with high fetal demands for folate needed for neural tube closure and normal development (137). Nonetheless, numerous studies have showed the benefits of an adequate intake of folate (either through diet and/or supplements) and reduced risk of spontaneous abortion, preterm delivery, low birth weight, intrauterine growth restriction and NTD’s (95, 138, 139). However, some studies have also found increased rates of infants born with low birth weight and spontaneous abortion when women took folic acid supplements periconceptionally (139, 140). The conclusions about folate’s role in the amplified frequency remain unclear.

6.4 Calcium intake and correlation with infant birth weight

A minor, but significant positive correlation between calcium intakes in late pregnancy and infant birth weight was found in STORK before adjusting for confounding variables. This is in accordance with some other studies, however, the results are diverse: both positive and no associations between an adequate calcium intake and birth weight have been found (69, 131)
The calcium intake can only explain a minimal part of the increased infant birth weight \( r = 0.102 \), and the clinical relevance is debatable. For pregnant women following the recommendations for dietary intakes, the increase in birth weight may be insignificant. However, for women taking a noteworthy amount of calcium supplements periconceptionally, an increase of 1.4 g in birth weight for every increase in intakes of 10 mg of calcium might have clinical relevance. Most Norwegian supplements contain 200-500 mg of calcium, which means an increase in infant birth weight of approximately 28-70 g. Should medical personnel and nutritionist working with pregnant women advice them to be careful with high-doses of supplements, in the prevention of macrosomia? Until further studies have confirmed the final conclusions about calcium intake and the safety of supplements during pregnancy, the results must be applied with caution.

Many of the women had an intake below the RDI in early and late pregnancy, although the average intake of calcium was satisfactory. Few women reached the upper daily limit. This is in accordance with other Norwegian studies. In MoBa, the median intake was 1270 mg, where supplements provided with less than 20% of the total amount of calcium (24). In conclusion, it seems clear that women should be encouraged to eat in accordance with RDI periconceptionally, and to increase their intake of calcium rich foods.

### 6.5 Magnesium intake and correlation with infant birth weight

Analyses in this thesis showed that magnesium was correlated with infant birth weight in both late and early pregnancy. However, this effect disappeared after correction of possible confounding variables. Nevertheless, other studies have found positive association between the mineral and birth weight (128, 143, 144), whereas others have found no associations (141, 145). Before adjustment of confounding variables, the intake of 1 mg magnesium lead to an increase in birth weight of 0.55 g. However, with a mean intake of 318 mg, this seems of no clinical relevance unless the women are taking high doses of magnesium supplements.

A fair amount of the women did not reach RDI for magnesium, and very few reached the upper limit. In contrast to our findings, MoBa found that the dietary intake of magnesium did
reach the RDI for pregnant women. The median intake was 467 mg, where supplements contributed with less than 20 % of the total amount of magnesium (24). The different findings might be due to the huge underreporting in STORK, since MoBa used a FFQ designed specific for pregnant women. However, no data on underreporting are published from the Norwegian Mother and Child Cohort study.

### 6.6 Iron intake and association with infant birth weight

The observed intake of iron in STORK was significantly positive related to infant birth weight. However, the effect disappeared when controlling for other dietary factors and other possible confounding variables. Nevertheless, considering birth weight in grams as an outcome, a change of 16.6 g per 10 mg/day increase in iron intake may be of clinical relevance if the women are taking high-dosage supplements of the mineral. The change is of most importance in those mothers with risk of having a child born SGA, since many studies have showed that iron/folic acid supplements decrease the risk. Some studies have found multivitamin-mineral (MVM) supplements to be more efficient than iron/folic acid supplements alone, regarding decreasing the risk of having an infant born with low birth weight (95, 96). There is an unclear consensus concerning the effects of iron intake and/or supplements during pregnancy on infant birth variables. Available data suggest only a modest effect of iron’s effect on infant birth weight in developed countries (92, 146).

Intake of iron in STORK population varied largely. The results from this thesis showed that many of the women did not manage an intake up to the RDI, and that few women had very high intakes, most probable due to intake of iron supplements. The low intake may be explained by the fact that many of the women underreported their dietary intake. In addition, the awareness of the importance of iron during pregnancy might be low in the Norwegian population. Health cares might be reluctant to advice a higher intake of supplements due to the fact that iron could be toxic in high doses, and because of the lack of strong evidence on positive or negative effects. Furthermore, due to the new guidelines from 2005 (22), extra attention to the iron intake- and status are only given when the hemoglobin (Hb) concentration is too low. This might allow women in the risk zone of iron deficiency (with a normal Hb-concentration) to carry on with the same dietary pattern, with an iron intake
insufficient to meet the demands during pregnancy, which might lead to iron deficiency in 3rd trimester. Norwegian women do not make major changes in their dietary habits when they become pregnant (23, 122). The same applies for Danish and English women as well. Studies from Denmark (23, 147) and UK (92) have showed that no great dietary changes are done during pregnancy, and that both populations have a mean dietary intake of 9 mg/d and 10 mg/d, which is somewhat equal to the iron intakes in STORK.

Canada, USA and Germany recommend a dietary iron intake of 23-30 mg/day during pregnancy (23, 67). This is almost twice of what is recommended in Norway and Denmark. To meet the iron demands during pregnancy, women would need to make significant changes in their diet that may be unrealistic, hence the recommendation of iron supplements. A low iron intake over time will lead to iron deficiency if one does not increase the intake (70). As mentioned earlier, iron deficiency has many consequences for the pregnant women. On the other side, iron overload could lead to oxidant-mediated tissue injury. However, Pearson et. al found that children born to mothers with iron overload did not lead to an overload in the newborns (148).

After revising findings from this thesis and other studies, the effect of iron on birth weight remains unclear. Data from randomized, clinical, double blind trials (RCT) are needed to examine the true effect.

### 6.7 Maternal iron status

#### 6.7.1 Iron status and association with birth weight

No association between dietary iron intake and iron status biomarkers was found. This could be due to the fact that some of these blood indices are only affected when iron deficiency is pronounced. However, since many of the women in STORK showed signs of iron deficiency, this seems less probable. Most likely, the FFQ used in this thesis is insensitive for registering the true iron intake. In addition, no associations between the iron biomarkers and infant birth weight were found. At present, the full context between infant birth weight and maternal iron status is indefinite: both positive, negative and no effects of adequate maternal iron status on infant birth weight have been found (50, (121).
6.7.2 Iron status in STORK and comparison with other studies

The number of women with iron deficiency increased over 11-fold by 3rd trimester, whereas the number of women with depleted iron stores increased over 15-fold from early to late pregnancy. This is in accordance with the low iron intake in STORK. However, analysis of under- and overreporting is suggesting that the true intake is higher. Few women had high iron stores, which means that the risk of iron overload and possible adverse effects in this study group remains low. The observations in this study are in agreement with other Scandinavian studies. Fertile, non-pregnant women have low iron status with a median ferritin value of 31-40 µg/L, whereas only 14-20 % have ample iron stores ≥ 500 mg (measured as ferritin levels > 60 µg/L) (23, 50, 122). Several studies have shown that mothers with a low ferritin concentration give birth to infants with lower iron stores compared to infants born by mothers with adequate stores (26, 51). The concentration of soluble transferrin-receptor increased with almost a 2-fold from early to late pregnancy, which is in some accordance with the ferritin levels. However, the reported values for soluble transferrin-receptor in different studies show a wide range of discrepancies, and many studies have found soluble transferrin-receptor concentration in healthy pregnant women to be around 4-6 mg/L (53, 123, 124), which differs substantially from the results in this thesis. One study has found similar results as in our results, with soluble transferrin-receptor concentrations around 2-5 mg/L (122).

It is difficult to distinguish physiologic anemia from iron deficiency anemia in 3rd trimester. Thus, the best time to detect any risk associated with maternal anemia might be in 1st trimester. Studies have confirmed the beneficial effects of adequate iron status or iron supplementation and reduced risk of anemia (123). Given the risk factors and consequences of iron deficiency and iron deficiency anemia (IDA) during pregnancy, these results highlight the importance of adequate iron intake throughout pregnancy, and possibly supplementation to help achieve the recommendations of 15 mg/day. However, studies have also found no reduction in overall prevalence of iron deficiency and IDA (124).

6.7.3 The usage of iron status biomarkers

The iron concentration is best viewed in relation to each woman’s normal concentration, and not alone as a marker of iron deficiency. Walsh et.al. found that the WHO recommendation of anemia cut-off (hemoglobin < 10 mg/L) were insensitive to biochemical iron deficiency and missed over 90 % of the low ferritin values (< 12 µg/L), which were mostly associated with
much higher hemoglobin (Hb) concentrations. There are major limitations for the use of Hb as an indicator of iron status during pregnancy, as it does not represent specific or sensitive measures of iron body stores (23, 123). In addition, the haemodilution in the women varies widely, which makes the interpretation of the Hb concentration even more difficult during pregnancy. Ferritin concentration is a good marker for mobilizable body iron reserves, whereas soluble transferrin-receptor values yields information about iron deficiency at the cellular level. Both appear to be sensitive markers of iron deficiency at pregnancy (53, 127). However, the concentration of soluble transferrin-receptor starts only to decrease when the iron reserves are exhausted (125), which means that it will not capture those women who have not yet developed iron deficiency, but who are in the risk zone. Nevertheless, when used in conjunction with ferritin, it gives a better picture of the iron status and how much iron supplements that is needed during pregnancy, especially in the presence of coexisting disease (126). This is reflected by the results in this thesis.

Randomized studies of iron prophylaxis (~ 30-40 mg Fe^{2+}) during pregnancy have demonstrated positive effects on reducing low hemoglobin and hematocrit, and increasing serum ferritin, serum iron and other measures, including bone marrow iron (67, 125, 126). However, the concern about intake of iron supplements in non-anemic women remains. It has been suggested that such use may build up the mother’s iron stores and increase blood viscosity so that utero-placental blood flow is impaired or that iron excess could cause other toxic reactions. Scholl et al. found that ferritin concentration at or above the 90th percentile in 3rd trimester was associated with preterm delivery, preeclampsia and gestational diabetes mellitus (127). However, this risk changed if the concentration declined from entry.

6.8 Characteristics of the women and newborns

Since no significant distinctions were found between the cohort and the iron study group, data from the latter should be relatively representative of the cohort.

More infants were born with high birth weight rather than low birth weight. However, the observed percentage of infants born with low- and high birth weight in STORK was low. This could mean that no hard end points related to the intake of micronutrients and correlation with extreme infant birth weights can be made. The strength of the results is thus highest in the
newborns born with ‘optimal weight’. Compared to findings in national surveys, the percentage of infants born with low birth weight in this study group is much lower than the incidences found nationwide. This could be due to that the study population only consisted of mostly healthy, pregnant women. Another explanation could be that many Norwegian pregnant women take multiple vitamin-mineral (MVM) supplements during pregnancy, which several authors have found to increase infant birth weight (15-17, 128). However, most of the studies are performed in developing countries where micronutrient deficiencies are more common. The percentage of infants born with high birth weight in this study group is in accordance with findings nationwide, with a proportion slightly higher than the ones observed in the Norwegian population.

6.9 Macronutrient intake

The average pregnant woman eats in consensus with the NNR 2004 in regards of the mean intakes of macronutrients: 51 E % from carbohydrates, 32 E % from fat and 15 E % from protein, respectively. This is in accordance with findings in the Norwegian Mother and Child Cohort (2008) and the last national dietary survey (NORKOST 1997). The former found that the mean energy distribution of the macronutrients were 53.6 E % from carbohydrates, 31.0 E % from fat and 15.1 E % from protein. While the latter found that the mean intakes in non-pregnant Norwegian women (in the age range of 20-29 and 30-39) were 52.7 and 50.6 E % from carbohydrates, 30.7 and 32.6 E % from fat, and 14.6 and 15.5 E % from protein, respectively (24, 129). It seems that pregnant- and non-pregnant women have an almost equally diet based on the mean intakes of energy giving nutrients. However, the time difference between 1997 and 2002-2008 might change the picture.
The study population

6.10.1 Participation

There are several reasons to believe that the participants in STORK may differ from the nationwide population. Firstly, the extra hospital visits in association with participation in STORK may have been perceived as time consuming, stressful and invasive at the time, especially with regards to experiences as fatigue, tiredness and nausea that often follows a pregnancy. Secondly, selection bias might have occurred if women accepted the study based on extra follow-up. The participants were offered three ultrasound scans and four extra visits, in contrast to the one ultrasound scan and eight antenatal controls offered in the standard national antenatal care program (77). Thirdly, one might speculate if the participants had a higher education level than the non-participants and greater interest in science and health practices. Furthermore, perhaps the women generally took better care of themselves and their unborn child in a health related perspective, was older, sicker or had any other reasons for close follow up during pregnancy. However, no conclusions can be made, but it is likely that there is a socio-economic gradient that influences estimates and observed dietary patterns.

Only one third of the eligible women giving birth at the hospital (Rikshospitalet) were invited due to exclusions criteria, limited resources and capacity. No investigation was done to find out why 70% of the invited women declined to participate. However, it is thought that most of the women failed to respond to the request, rather than actively refuse to participate in STORK. The women were given no compensation for participation, and could not legally take the day off from work (which is possible on the eight antenatal doctor visits in the care program).

6.10.2 Comparing the STORK population to Norwegian pregnant women

Data from Medical Birth Register in Norway (MBRN) showed that pregnant women and their infants differ slightly from STORK population (120). Mean infant birth weight, mean maternal age and gestational age in Norway and Oslo were somewhat lower than our study.
group (data not shown), and could be a contributor to the increased infant birth weight seen in STORK compared to the general population.

Smoking is associated with low socioeconomic status (130), and is consequently a relevant comparable indicator. During the pregnancy, 7.2 % smoked in Norway, whereas 6.4 % smoked in Oslo. In STORK and the iron study group, this number was 3 and 2 %, respectively, which might indicate that the participants came from a higher socio-economic level than the general population.

The study population consisted exclusively of women with Scandinavian heritage living in an urban area. It is important to note that approximately 6 % living in Norway and 19 % living in Oslo have a non-western background (131). Hence, the data from this study might not be representable either nationwide or for women living in Oslo, since all the subjects in STORK are native Scandinavian. However, the women in STORK have been compared to a corresponding native Scandinavian group of 151 women giving birth at Rikshospitalet-Radiumhospitalet medical centre (non-participants), and a second corresponding group of 85 pregnant native Scandinavian women at Ullevål University Hospital (Oslo): no significant differences were found (6, 27). Thus, the results may be extrapolated to other pregnant native Scandinavian women from urban areas living in the western world.

6.11 Methods

6.11.1 Dietary assessment

The food frequency and amount questionnaire NORKOST 1997 was chosen in STORK as the best available method to collect dietary data. A weighed diet record seems to reflect the true intake more accurately and give a better estimate of the nutrient intake. However, weighed records acquire more from the participant and administrator, greater available resources and might give a lower completion rate (partly because of the extra load on the participants) (132).

Food frequency questionnaires (FFQ) are suitable for large-scale surveys; they are cost-effective, relatively easy to use for both the participant and administrator and have the advantages that one can focus on specific nutrients with few food sources (132). There are
several limitations with this nutritional assessment method. Firstly, the FFQ form requires validation in relation to both reference measure and study group. Secondly, literacy and numeracy skills are needed if self-completed. Thirdly, errors might occur if false check marks are noted in the forms and if the auto readings of the forms misinterpret these marks. In addition, since it is a retrospective method, the memory accuracy of the participants might be low, and give origins to under-and overreporting of different food groups.

6.11.2 Validation of the NORKOST questionnaire

NORKOST 1997 has been evaluated in several studies. They have concluded that it measures macro- and micronutrient intake in an adequate way in Norwegian men, and that the FFQ is suitable for Norwegian nutrition surveys, compared to other methods (113, 133, 134). Unfortunately, no questionnaires designed for pregnant women were available when the collection of the data started in 2002. Hence, the same scheme was used throughout the whole STORK study (2002-2008), rather than use NORKOST in STORK 1 and a better option in STORK 2, based on argumentation about comparability.

The Norwegian Mother and Child Cohort Study (2008) designed a questionnaire specific for pregnant women after the initiation of STORK (114, 135). This FFQ has been analyzed and compared with NORKOST in relation to correlation in a small sample of 86 women. The energy- and protein intake had a correlation coefficient of 0.69 (p<0.01) and 0.71 (p<0.01), respectively. This might indicate that NORKOST is a suitable tool when measuring energy and macronutrients in a population of pregnant women.

One validation of NORKOST has shown that the correlation between this questionnaire and a subgroup with weighed records lies between 0.44-0.62 r, with regards to micronutrients. The correlation coefficients of iron, magnesium and calcium were 0.44, 0.45 and 0.50, respectively. In the same evaluation, only 34-51 % of the micronutrients were correctly classified by quartiles in the FFQ. For iron, magnesium and calcium, the percentage was 38, 36 and 39 %, respectively. In addition, 4, 6 and 2 % was grossly misclassified by quartiles in iron-, magnesium- and calcium intake. Generally, the questionnaire used gave higher median values and more imprecise classification than the weighed diet records for micronutrients (113). If these correlations apply to STORK as well, any conclusions about the relationship between iron, calcium and magnesium and infant birth weight may be erroneous, if they are based on dietary intake from FFQ’s alone.
6.11.3 Evaluation of the instructions and usage of FFQ

The participants were only giving a written notice on how to fill in the forms, and had to have good skills relating to the perception and conceptualization of food size. When we double-checked some of the FFQ’s, several of the participants had false check marks, especially in the last pages that consisted of the questions about dietary supplements. This may have led to a number of errors in the nutrient calculations that might have clouded the results, depending on how the optical readable machine interpreted this. Furthermore, ‘social desirability’ and ‘social approval’ bias could also occur. Moreover, participants that do not have regular eating habits might have had difficulty with describing the usual frequency of consumption. In addition, the use of food composition tables will introduce errors into the estimates of energy and nutrient intake.

NORKOST is originally designed to measure the mean intake in the last 6-12 months. This means that the reported intake in gestation week 14-16 might have overlapped with the intake in week 30-32, since the participants completed the two questionnaires within a time period of 3-4 months. Hence, the differences in intake between late and early pregnancy might be less evident in the analyses than in reality.

The primary problem with NORKOST in relation to usage in this master thesis (in addition to the above mentioned), was that its main focus was on energy and macronutrient intake, while our main focus was on micronutrients. In addition, the FFQ was not designed to assess and distinguish between the different time periods during pregnancy, by trimester (1st, 2nd or 3rd) or by critical windows for fetal organ/tissue development. However, a major advantage was that the dietary intake of supplements was taken into account in NORKOST and therefore a part of the analyses. Supplements provide a substantial amount of vitamins and minerals (24), and no estimations of nutrient intake would have given a true picture without the supplements included.

6.11.4 Under- and overreporting

The problem of underreporting in nutritional assessment methods has been noted in a couple of studies (136, 137). There are two types of underreporting: ‘under-eating’ and ‘under-registration’. When participants ‘under-eat’, they eat less than normally because they are more conscious of their diet when they are reporting. The participants that are ‘under-registering’
neglect reporting what they actually consumed. These types of errors could have occurred at both visits in STORK. Studies have concluded that underreporting of all food groups appears, but the degree of underreporting can vary significantly between food groups. Furthermore, underreporting tends to increase with increased intake, and second administrations of FFQ show greater underreporting than first administration (138), which is in accordance with this thesis.

Level of dietary consciousness might severely bias reported food intake in dietary surveys, and could conceal true effects between diet and maternal- and infant health risks. A large proportion of women in STORK underreported their intake, while a small fraction reported an energy intake (EI) higher than probable. However, there are several pitfalls with this estimation. Firstly, EI/BMR does not take into account the true energy expenditure, only the intake. However, this estimation method is less expensive and considerably more suitable for use among large samples than other estimations like double labelled water method. Secondly, EI is typically lower when evaluated by dietary records than by dietary history, which means that dietary surveys may include systematic errors when food consumption is being registered. Thirdly, there are many factors that could contribute to the under-and overreporting of certain food groups. Such biases could be age, sex, smoking, educational level, health consciousness, dieting and degree of overweight and obesity (117). If the degree of biased reporting is unevenly distributed within the population, the search for associations between diet and health variables might be disturbed. This means that the correlation between micronutrients and infant birth weight may be false: the relationship might be decreased, enhanced or masked.

6.11.5 Birth weight as a method for predicting neonatal outcomes

Infant birth weight alone will only tell us something about fetal under-, normal- or overweight. It will not tell us anything about fetal body composition and amount of fat-free mass, which might be better indicators of understanding possible metabolic changes and short-and long-term consequences. Some studies have used body mass index (BMI) or ponderal index (PI) instead of infant birth weight when reviewing different fetal outcomes. The results are diverged: some studies have found that BMI or PI are superior, while some
have found that birth weight relative to gestational age is the better predictor of short-and long-term conditions (139, 140).

It is clear that more studies on this subject will be needed. Biophysical techniques are available (dual energy X-ray absorptiometry (DXA), among other examinations) for more exact measures of body composition, but data on the issue are limited. DXA was used in STORK 2 on 207 of the infants, hoping this would add more information about infant birth weight in terms of differentiating between genetically small, genetically large but lean infant, morbid small infants and the morbid large infant with excessive fat tissue. They found that DXA measurements of body composition demonstrated good reliability, provide a fair estimate of fat mass, and can be used as a reference method in neonates (141).

6.11.6 Blood samples

Fasting glucose levels measured at gestation week 14-16 had an unexpected increase (0.6 mmol/L) over time during the longitudinal recruitment in STORK, and were de-trended based on independent control values. Possible explanations can be a real change in characteristics of the study population, selection bias in the participants, an unintended change in reporting routines or systematic bias in measurement equipment or procedures. Although the prevalence of metabolic syndrome-like symptoms have increased over the last years (142), it is less likely that the change in fasting glucose values in these healthy pregnant women have a biological cause. Firstly, no corresponding increase in the women’s BMI, insulin or age was found. In addition, the same personnel performed all laboratory analyses and calibration at an accredited laboratory. Furthermore, measurement routines, storage and use of the manufacturer’s reference values were in accordance with the manufacturer’s instructions. Frøslie et al. concluded that the observed trend in both women’s blood glucose values and the control solution values was due to an unknown effect of long-term use of the Accu-Chek measurement system (111).

Most of the studies on women’s iron status have considered concentrations of hemoglobin, transferrin, soluble transferrin-receptor, transferrin-saturation and ferritin in analyses. It would have been very desirable to measure hemoglobin as well in the iron study group, and to have an even greater basis of comparison of our findings with others discoveries. In addition, since the Norwegian recommendation of monitoring iron status in pregnant women is based exclusively on the hemoglobin (Hb) concentration, it would have been of great interest to see
whether Hb really did capture women’s iron status alone, and the correlation between Hb and the other biomarkers. However, both soluble transferrin-receptor and ferritin has proven to be good candidates for identifying women’s iron status during pregnancy, so in the consensus of investigating this issue, it might be sufficient (22, 23, 53, 125, 126, 143).

The iron status biomarkers were determined by methods with high specificity and sensitivity, with intra-assay coefficients of variation (CVs) < 10% for all assays. For detection of transferrin the expected values lies between 2.0-3.6 g/L and the lower limit of detection is <0.1 g/L, with a CV of 4.8%. For soluble transferrin-receptor the measuring range is 0.5-40 mg/L with a lower limit of detection and CV of 0.068 mg/L and 5%, respectively. No data exist on the CV of transferrin-saturation, but the expected values range between 0.10-0.50%. For ferritin the expected values are 33-193 µg/dL with lower limits of measurement of 5 µg/dL, and a CV of 9.8% (144).

The major limitation lies in the interpretation of the results, mainly because of the uncertainty regarding changes in the biomarkers during pregnancy. Few data exist on international optimal plasma concentrations, which means that comparability to other studies are difficult when different definitions are used. Reference values are usually developed on basis on healthy non-pregnant women (20, 51, 144).

6.11.7 Number of participants in the iron study group

Different approaches to calculate the number of participants (n) were done, with diverse clinical relevant changes in infant birth weight and different study strengths. The randomized sample size of 100 women were chosen based on available resources, the time limit in the master thesis, a clinical relevant difference and clinical experience. Increasing n to above 100 participants did not increase the probability of finding a greater clinical relevant difference; the same applied by decreasing the number of participants. Other studies have used clinical relevant differences in infant birth weight ranging from 10 g-100 g, depending on the studied independent variables (17, 145, 146). Therefore, we assumed that findings between these values would be sufficient to describe a relationship between nutrient intake and infant birth weight.

Since we lacked registered dietary intake of iron in many of the participants in STORK, we decided to extract the randomized sample size of the 706 women that we had dietary iron data
on from gestation week 14-16 and 30-32. This was due to the fact that we wanted good comparability between early and late pregnancy.

6.11.8 Statistical analyses

The outliers and extreme values in the original dataset and in the women’s individual FFQ were thoroughly investigated. The most common cause of the extreme values was intake of vitamin- and mineral supplements, which means that their high intake most likely reflected their actual intake and not false check marks. Therefore, with intention-to-treat (ITT) in mind, no exclusions of participants with extreme values or outliers were done. Efficacy subset analysis can introduce biases to the statistical analysis and inflate the chance of a false positive. However, full application of intention to treat can only be achieved where there are complete outcome data for all randomized subjects. Unfortunately, many of the women did not submit both dietary forms, of unknown reasons.

Various micronutrients were missing in the original STORK data set, since micronutrients were not seen as an explanatory variable in the main hypotheses concerning STORK. Yet, several studies have indicated that many of these excluded micronutrients might correlate with infant birth weight (97, 108, 147-149).

Controlling for confounders are essential to delineate the causal relationship between exposure and disease. However, statistical adjustment is a double-edged sword. Adjustment for a common consequence (collider) of the exposure and the outcome can lead to as much bias as absence of necessary adjustment for a confounder (common cause of both exposure and outcome/disease) (150). Due to lack of evidence that smoking, glucose values, weight gain during pregnancy, gender of the child, pregestational weight, BMI, physical activity, protein intake and gestational age affects both intake of micronutrients and birth weight, they were excluded in the final regression model. Nevertheless, forward analysis with these potential confounders was done, and no significant changes arose in any of the multiple regression models.
6.12 Diluted results – possible explanations of absence of effects

The significant effects of the maternal intake of magnesium, calcium and iron on infant birth weight in this thesis are not very strong. In addition, none of the iron biomarkers were correlated to infant birth weight or dietary iron intake. There could be many reasons to the absent effects. To begin with, birth weight is influenced by multiple factors, such as the infant’s gender, gestational age, maternal BMI among others. Thus, the effects of nutritional variables and iron status might be masked by the other determinants. Furthermore, the study population consists of healthy women and few of the infants in STORK had extreme birth weights. Several studies are indicating that the dietary intake of micronutrients or iron status first and foremost affects extreme birth weight in either direction. Lack of significant results in the multivariate analyses may be explained by the fact that the true physiological effect may work in a dose-response manner.

When it comes to disparities that are seen between this thesis and different studies, two underlying reasons might explain this. Firstly, there is a great variance and divergence between the study designs, especially when it comes to the number of participants, ethnicity and whether the studies are being performed in developing or developed countries. In addition, the recommendations for the micronutrient intake and the boundaries for normal concentrations of the iron status biomarkers are very fluctuating. This makes the comparability between studies difficult. In addition, control for confounding and interfering variables vary greatly (e.g. from parity, maternal BMI, gestational age, ethnicity, maternal height, maternal weight, energy intake, protein intake) between the studies, which could mean that some studies have taken colliders into account, whereas others have failed to control for actual confounders, whereas some others again have controlled for unlikely many variables just to be on the safe side.

6.13 Future directions in research and antenatal care

Until recently, many have believed that the micronutrient intake in developed countries have been adequate. In addition, the prevalence of iron deficiency and overload has been assumed
to be low. However, this thesis and other studies have shown that many Scandinavian pregnant women have low intakes of several micronutrients, and that many have low iron stores pre-conceptionally. Furthermore, several of the women develop iron deficiency during pregnancy.

Optimization of the micronutrient intake and a good iron status throughout the pregnancy could possible contribute to prevention of maternal and fetal complications and reduction in adverse pregnancy outcomes such as small for gestational age (SGA), low and high birth weight, stillbirths, and perinatal- and neonatal mortality. The exact role of the variety of micronutrients in fetal growth and development has yet to be explored in detail.

To be able to guide the pregnant women health workers should be more aware of what food items are contributing to deficiency and excess of micronutrients. In the STORK population, iron, vitamin D and folic acid intake should be increased, whereas vitamin A intake should be decreased. Since most of the vitamin A intake seems to come from supplements, women should be guided to take supplements without vitamin A, since hypovitaminose A is not a problem in Nordic countries (123).

More research is needed to address the safety, efficacy and effectiveness of maternal micronutrient supplementation. A follow up study of women before, during and after pregnancy to assess the effect of pre-pregnant nutritional- and iron status on relevant maternal and fetal outcomes would be ideal. The study should include nutrient intakes from the diet alone, intakes from supplements, physical activity, iron status biomarkers (ferritin, hemoglobin and soluble transferrin-receptor), complication rates for the mother and child and other related factors. A multi centre study with participants not only limited to Scandinavian heritage would be better. In addition, usage of specific methods designed for the pregnant population (i.e. FFQ) would be preferable and necessary. Studies like these are expensive and time consuming, but may increase the knowledge of the connections between maternal state and future health of the child.

A great effect might come from promoting the already existing recommendations: thus, how to influence pregnant women to change their diet and life style should be addressed. Dietary advice given to pregnant women may have positive health effects beyond her and the fetus’s personal health. It may also give origin to a change in the family’s dietary habits.
7 Conclusion

Dietary factors seem to have a small effect on birth weight in univariate analysis; iron, calcium and magnesium intake have positive effects on infant birth weight, but the association disappeared. Therefore, there is insufficient evidence for any firm conclusion about the effects of diet or iron status on infant birth weight and pregnancy outcome.

Iron deficiency occurs commonly in Norwegian pregnant women, however, the optimal method for its detection is still debated. This master thesis suggests that multiple biomarkers should be used to evaluate pregnant women’s iron status. Given the limitations of transferrin, iron and transferrin-saturation during pregnancy, perhaps the concentration of soluble transferrin-receptor in conjunction with ferritin may present the best picture of iron deficiency at various levels.

Too many Norwegian pregnant women show trends toward an unbalanced diet that is not within the recommended guidelines: especially in regards to the low intake of iron, vitamin D and folic acid, and the high vitamin A intake. In addition, many of the women appear to have iron deficiency in gestation week 14-16, and even more develop iron deficiency at the end of pregnancy. Extra attention should be given to the women about the importance of an optimal micronutrient intake and iron status during pregnancy.

Knowledge of how to prevent micronutrients deficiencies- and overload, and how to advice the pregnant population with the right information is a challenge, and must be focused on in the future. More research is needed to make any firm conclusions and add new information, so that we can modify individual and population based advices to the pregnant women.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>Amino acids</td>
</tr>
<tr>
<td>As</td>
<td>Arsenic</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>Cd</td>
<td>Cadmium</td>
</tr>
<tr>
<td>CMR</td>
<td>Chylomicrons remnants</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CP</td>
<td>Ceruloplasmin</td>
</tr>
<tr>
<td>Cr</td>
<td>Chrome</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotrophic releasing hormone</td>
</tr>
<tr>
<td>CT</td>
<td>Calcitonin</td>
</tr>
<tr>
<td>Cu</td>
<td>Copper</td>
</tr>
<tr>
<td>CP</td>
<td>Ceruloplasmin</td>
</tr>
<tr>
<td>DBP</td>
<td>Vitamin D binding protein</td>
</tr>
<tr>
<td>DMT1</td>
<td>Divalent metal transporter</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyriboonucleic acid</td>
</tr>
<tr>
<td>DOHaD</td>
<td>Developmental Origins of Health and Disease</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual energy X-ray absorptiometry</td>
</tr>
<tr>
<td>ECF</td>
<td>Extracellular fluid</td>
</tr>
<tr>
<td>FAD</td>
<td>Flavin adenine dinucleotide</td>
</tr>
<tr>
<td>Fe</td>
<td>Iron</td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
<td>Divalent iron (ferrous iron)</td>
</tr>
<tr>
<td>Fe$^{3+}$</td>
<td>Trivalent iron (ferric iron)</td>
</tr>
<tr>
<td>FMN</td>
<td>Flavin mononucleotide</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>HCP1</td>
<td>Heme-binding protein 1</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>Hg</td>
<td>Mercury</td>
</tr>
<tr>
<td>IF</td>
<td>Intrinsic factor</td>
</tr>
<tr>
<td>IGF2</td>
<td>Insulin-like growth factor II</td>
</tr>
<tr>
<td>ITT</td>
<td>Intention-to-treat</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>LGA</td>
<td>Large for gestational age</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
</tr>
<tr>
<td>Mg</td>
<td>Magnesium</td>
</tr>
<tr>
<td>Mo</td>
<td>Molybdenum</td>
</tr>
<tr>
<td>MTHFR</td>
<td>5,10-methyltetrahydrofolat</td>
</tr>
<tr>
<td>NAD</td>
<td>Nicotinamide adenine nucleotide</td>
</tr>
<tr>
<td>NADP</td>
<td>Nicotinamide adenine nucleotid phosphate</td>
</tr>
<tr>
<td>NTD</td>
<td>Neural tube defects</td>
</tr>
<tr>
<td>PI</td>
<td>Ponderal index</td>
</tr>
<tr>
<td>PL</td>
<td>Pyridoxal</td>
</tr>
<tr>
<td>PM</td>
<td>Pyridoxamine</td>
</tr>
<tr>
<td>PN</td>
<td>Pyridoxine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>PUFAs</td>
<td>Polyunsaturated fatty acids</td>
</tr>
<tr>
<td>RA</td>
<td>Retinoic acid</td>
</tr>
<tr>
<td>RAH</td>
<td>Retinal</td>
</tr>
<tr>
<td>RBP</td>
<td>Retinol binding protein</td>
</tr>
<tr>
<td>RE</td>
<td>Retinyl ester</td>
</tr>
<tr>
<td>RES</td>
<td>Reticuloendotheliale system</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonuclei acid</td>
</tr>
<tr>
<td>ROH</td>
<td>Retinol</td>
</tr>
<tr>
<td>SGA</td>
<td>Small for gestational age</td>
</tr>
<tr>
<td>T3</td>
<td>Triiodothyronine</td>
</tr>
<tr>
<td>T4</td>
<td>Thyroxine</td>
</tr>
<tr>
<td>TC 1</td>
<td>Haptocorrin</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricarboxylic acid</td>
</tr>
<tr>
<td>Tf</td>
<td>Transferrin</td>
</tr>
<tr>
<td>TTR</td>
<td>Transferrin receptor</td>
</tr>
<tr>
<td>TTR</td>
<td>Transthyretin/retinol binding globulin</td>
</tr>
<tr>
<td>VLBW</td>
<td>Very low birth weight</td>
</tr>
</tbody>
</table>
# 9 Definitions

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anencephalus</td>
<td>Severe neural tube defect where the foetus is born without a head.</td>
</tr>
<tr>
<td>Anophtalamia</td>
<td>Congenital disorder where the eye is severely underdeveloped or not developed at all.</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index is a proxy for human body fat, and is defined as the individual's body mass divided by the square of his or her height.</td>
</tr>
<tr>
<td>Cheiloshisis</td>
<td>Cleft lip</td>
</tr>
<tr>
<td>Embryopathy</td>
<td>Fetal malformations.</td>
</tr>
<tr>
<td>Endemic cretinism</td>
<td>Disease that affects the fetus if the mother has extreme hypothyroidism. Characterized by mental impairment, deaf and dumb, spasms, and growth retardation. Easily avoided by supplements.</td>
</tr>
<tr>
<td>Exencephaly</td>
<td>A rare malformation of the neural tube, which leads to absent of the bones of the cranial vault, with protrusion of brain tissue into the amniotic cavity.</td>
</tr>
<tr>
<td>Goiter</td>
<td>Abnormal enlargement of the thyroid gland.</td>
</tr>
<tr>
<td>Hydrocephalus</td>
<td>A buildup of fluid inside the ventricles that leads to brain swelling.</td>
</tr>
<tr>
<td>Hydronephrosis</td>
<td>Swelling of a kidney due to a backup of urine.</td>
</tr>
<tr>
<td>LGA</td>
<td>Large for gestational age.</td>
</tr>
<tr>
<td>Micrognathia</td>
<td>Underdeveloped, small jaws.</td>
</tr>
<tr>
<td>Micromelia</td>
<td>Unusual small or short arms and legs.</td>
</tr>
<tr>
<td>Mineral</td>
<td>Essential minerals are defined by their RDI &gt; 100 mg/d, and that it’s contents in the body is in the order of grams.</td>
</tr>
<tr>
<td>Omphalacele</td>
<td>A birth defect in which the infant's intestine or other abdominal organs stick out of the belly button.</td>
</tr>
<tr>
<td>Palatochisis</td>
<td>Cleft palate</td>
</tr>
<tr>
<td>RDI</td>
<td>Recommended daily intake</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>A general term that refers to some form of persistent (i.e. inflammation) or acute (i.e. photon beam radiation) damage to the retina of the eye.</td>
</tr>
<tr>
<td>SGA</td>
<td>Small for gestational age.</td>
</tr>
<tr>
<td>Spina bifida</td>
<td>Congenital disorder caused by the incomplete closing of the embryonic neural tube.</td>
</tr>
<tr>
<td>Syndactyly</td>
<td>Congenital malformation with webbing or fusion of two or more digits, a result of failure of early interdigital tissue to degenerate.</td>
</tr>
<tr>
<td>Trace element</td>
<td>Defined by a RDI &lt; 100 mg/d, where the content in the body is in the order of mg or µg.</td>
</tr>
</tbody>
</table>
Reference list


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10 Appendix
10.1 Written consent from study subjects
Til deg som er gravid og har fødeplass på Rikshospitalet

**Forespørsel om å delta i STORK-prosjektet**
(maternelt metabolsk syndrom, store barn og svangerskapskomplikasjoner)

Stork-prosjektet søker å finne svar på hvorfor forekomsten av store barn er økende. Fra 1990 til 1999 har andelen av barn med fødselsvekt over 4kg økt fra 16% til vel 20%. Dette er en uheldig utvikling. Det er kjent at store barn kan medføre økt risiko for mor og barn under fødselen.


Undersøkelsen er kun ute etter å samle informasjon og vil **ikke ha noen påvirkning på svangerskapet eller barnet ditt.**

Undersøkelsene:

Hvis du velger å delta, vil det bety fire kontroller på Rikshospitalet i løpet av svangerskapet. Noen av disse vil kunne være i stedet for kontroller hos egen lege / jordmor.


1. gang ved ca. 14-16 ukers svangerskapsvarighet
   - Kostholdsskjema som du har fått i posten skal leveres
   - Fastende blodsukkerbelastning. Du får drikke 75 g. druesukker. Deretter tas det blodprøve fire ganger (hver ½-time) for å måle hvordan kroppen reagerer på en viss mengde sukker. Vi setter inn en veneflon (et tynt plastrør) i en blodåre på armen, slik at det bare blir ett stikk. Prøven tar litt over to timer, så det er lurt å ta med lesestoff og evt. en matpakke til å spise like etterpå. Svaret vil foreligge med en gang, og du vil få vite resultatet.

2. gang ved 22-24 uker.
   - Det blir tatt blodprøve til nedfrysing. Det tar ca. 15 minutter.

3. gang ved 30-32 uker-
   - Sukkerbelastning som første gang, pluss
   - ultralyd som andre gang
   - nytt kostholdsskjema

4. gang ved 36-38 uker
   - Blodprøver som andre gang, pluss
   - ultralyd som tidligere
   - fysisk aktivitetsskjema

Det vil hver gang tas blodprøver som skal friyes ned. Disse prøvene vil bli analyseret først etter at hele prosjektet er gjennomført.

Resultatene av undersøkelsen vil bli offentliggjort i godkjente tidsskrifter.

Studien er vurdert av datatilsynet og regional etisk komite.

Med vennlig hilsen

.....................
Nanna Voldner
jordmor / stipendiat
Hvis du ønsker å være med i prosjektet er det fint hvis du så raskt som mulig gir en tilbakemelding til Kvinneklinikken.

Du kan ringe til sekretær Tone Hassel 23 07 29 27 eller mobil 93 02 22 54 og si at du skal være med i STORK-prosjektet og du kan få time over telefon.

Du kan også sende en e-post til tone.hassel@rikshospitalet.no og få time tilbake på mail.

Spørsmål kan rettes til Nanna Voldner tlf: 23 07 29 26, mobil 99 73 82 90 eller på mail, nanna.voldner@rikshospitalet.no

Jeg har mottatt skriftlig informasjon om denne undersøkelsen og samtykker i å delta.

_________________________   ____________________________________________________
Dato                         Underskrift

Jordmors erklæring:

Jeg bekrer at kvinnen har fått skriftlig og muntlig informasjon om hva det innebærer å delta i prosjektet.

_________________________   ____________________________________________________
Dato                         Underskrift (jordmor Nanna Voldner)
10.2 Ethical approvement of STORK
To whom it may concern

Dato: 14.12.06
Deres ref.: S-01191 – approval

Title of protocol:
S-01191 Maternal metabolic syndrome, macrosomic newborn and pregnancy complications

Principal investigator: Chief physician dr.med. Tore Henriksen, Rikshospitalet.

The protocol was reviewed and approved by The Regional Committee for Medical Research Ethics, Southern Norway, Oslo, Norway, on 30 August 2001.

Sincerely yours,

Kristian Hagestad
Fylkeslege cand.med., spes. i samf.med
Chairman

Jørgen Hardang
Secretary
10.3 Food Frequency Questionnaire (NORKOST 1997)