Nutritional aspects of behaviour and biology during pregnancy and postpartum

Anette Lundqvist
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table of Contents</td>
<td>I</td>
</tr>
<tr>
<td>Abstract</td>
<td>III</td>
</tr>
<tr>
<td>Sammanfattning på svenska</td>
<td>VII</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>IX</td>
</tr>
<tr>
<td>Definitions used in the thesis</td>
<td>XI</td>
</tr>
<tr>
<td>Original papers</td>
<td>XIII</td>
</tr>
<tr>
<td>Preface</td>
<td>1</td>
</tr>
<tr>
<td>Introduction</td>
<td>2</td>
</tr>
<tr>
<td>Swedish antenatal care</td>
<td>3</td>
</tr>
<tr>
<td>Dietary recommendations</td>
<td>3</td>
</tr>
<tr>
<td>Dietary supplementation</td>
<td>5</td>
</tr>
<tr>
<td>Exposures to avoid or reduce</td>
<td>6</td>
</tr>
<tr>
<td>Weight gain and obesity</td>
<td>7</td>
</tr>
<tr>
<td>Behavioural changes; physical activity and diet</td>
<td>8</td>
</tr>
<tr>
<td>Vitamin D physiology and synthesis</td>
<td>9</td>
</tr>
<tr>
<td>Vitamin D in relation to pregnancy and after birth</td>
<td>12</td>
</tr>
<tr>
<td>Basic regulation of food intake</td>
<td>13</td>
</tr>
<tr>
<td>Steroid hormones, neuroactive steroids and allopregnanolone</td>
<td>13</td>
</tr>
<tr>
<td>Pregnancy and allopregnanolone</td>
<td>14</td>
</tr>
<tr>
<td>Allopregnanolone and food intake</td>
<td>14</td>
</tr>
<tr>
<td>The GABA system and allopregnanolone</td>
<td>15</td>
</tr>
<tr>
<td>Aims of the thesis</td>
<td>17</td>
</tr>
<tr>
<td>Methods</td>
<td>18</td>
</tr>
<tr>
<td>Study I</td>
<td>19</td>
</tr>
<tr>
<td>Material and methods</td>
<td>19</td>
</tr>
<tr>
<td>Participants</td>
<td>19</td>
</tr>
<tr>
<td>Interviews</td>
<td>20</td>
</tr>
<tr>
<td>Study II - IV</td>
<td>21</td>
</tr>
<tr>
<td>Settings</td>
<td>21</td>
</tr>
<tr>
<td>Sample size</td>
<td>21</td>
</tr>
<tr>
<td>Participants</td>
<td>21</td>
</tr>
<tr>
<td>The questionnaires</td>
<td>24</td>
</tr>
<tr>
<td>Estimation of dietary intake</td>
<td>24</td>
</tr>
<tr>
<td>Blood sampling</td>
<td>25</td>
</tr>
<tr>
<td>Participants - study II</td>
<td>25</td>
</tr>
<tr>
<td>Participants - study III</td>
<td>27</td>
</tr>
<tr>
<td>Participants - study IV</td>
<td>29</td>
</tr>
<tr>
<td>Analysis</td>
<td>31</td>
</tr>
<tr>
<td>Study I</td>
<td>31</td>
</tr>
</tbody>
</table>
Statistical analyses study II - IV 34
Study II 34
Study III 34
Study IV 35
Biochemical analyses study III - IV 35
Ethical considerations 36
Ethical reflections 36

Results 38
Study I 38
Study II 40
Study III 41
Study IV 46

Discussion 48
Methodological considerations 54
Study I 55
Study II - IV 57

Conclusions 62
Clinical implications and future research 62

Acknowledgements 64

References 68

Original papers
Abstract

Background
A well-balanced nutritious diet is important for the pregnant woman and the growing fetus, as well as for their future health. Poor nutrition results from both over-consumption of energy-rich foods which can lead to a higher weight gain than is healthy and under-nutrition of essential nutrients. Food intake is regulated in complex biological systems by many factors, where steroid hormone is one factor involved.

The overall aim of this thesis is to describe dietary intake, vitamin D levels, dietary information and dietary changes, and to study the relation between allopregnanolone and weight gain during pregnancy and postpartum.

Methods
Study I was a qualitative study with focus group interviews with 23 pregnant women. The text was analysed with content analysis. Study II was a quantitative cross-sectional study conducted in early pregnancy (n=209) with a reference group (n=206). Self-reported dietary data from a questionnaire was analysed using descriptive comparative statistics and a cluster analysis model (Partial Least Squares modelling). Study III had a quantitative longitudinal design. Vitamin D concentrations were analysed in 184 women, collected on five occasions during pregnancy and postpartum. Descriptive comparative statistics and a linear mixed model were used. Study IV was a quantitative longitudinal study with 60 women. Concentrations of allopregnanolone were analysed in gestational week 12 and 35. Descriptive and comparative statistics as well as Spearman’s correlation (rho) were used to describe the relationship between weight gain and allopregnanolone concentrations.

Results
The focus group interviews showed that women wanted to know more about different foods to reduce any risk for their child but the information about foods was partly up to themselves to find out. They expressed feelings of insecurity and guilt if they accidentally ate something “forbidden”. The recommendations
were followed as best as possible along with common sense to deal with diet changes. The main themes were “Finding out by oneself”, “Getting professional advice when health problems occur”, “Being uncertain” and “Being responsible with a pinch of salt”. Some differences in the dietary patterns were found among the pregnant women compared to references, with less, vegetables (47 g/day), potatoes/rice/pasta (31 g/day), meat/fish (24 g/day) and intake of alcohol and tobacco/snuff but a higher intake of supplements. Both pregnant women and references had intakes of folate through diet 45% (pregnant) and 22% (references) lower than current recommendations (500 vs 400 g/day). Vitamin D intake was 34% lower than the recommendations of 10 mg/day. At least a third of the participants had insufficient plasma levels below 50 nmol/L of vitamin D. Season was a strong factor influencing the longitudinal pattern. Gestational week, season, total energy intake, dietary intake of vitamin D, and multivitamin supplementation over the previous 14 days were factors related to vitamin D levels. A correlation between allopregnanolone concentrations in gestational week 35 and weight gain in weeks 12–35 was seen (p = 0.016). There was also a correlation between the increase in allopregnanolone (weeks 12–35) and weight gain (see above) (p = 0.028).

**Conclusions**

Dietary recommendations were described as contradictory and confusing and the dietary advice felt inadequate. The women faced their diet changes and sought information on their own but would have wished for more extensive advice from the midwife. The intake of vitamins essential for pregnancy was lower than recommended, which is also confirmed by low plasma levels of vitamin D in at least one third of the pregnant women. Vitamin D levels peaked in late pregnancy. Aside from gestational week and season which were related to plasma levels, intake from foods and supplements also affected the levels. Reasons for weight gain are complex and depend on many factors. Allopregnanolone is a factor that was seen to relate to the weight gain of the studied pregnant women.
Keywords pregnancy, antenatal care, dietary advice, qualitative, dietary intake, cross-sectional, vitamin D levels, alloprenanolone, weight gain, longitudinal
Sammanfattning på svenska

Bakgrund
En välbalanserad näringsrik kost är viktig för den gravida kvinnan och det växande fostret, så även för deras framtida hälsa. En bristfällig kost kan utgöras av både överförbrukning av energirika livsmedel vilket kan leda till högre viktuppgång än vad som är hälsosamt och bristande intag av viktiga näringsämnen. Kostintag regleras av komplexa biologiska system där flera faktorer är inblandade däribland steroidhormonet allopregnanolon.

Det övergripande syftet med denna avhandling är att under och efter graviditet beskriva kostintag, vitamin D-nivåer, kostinformation och kostförändringar och att studera allopregnanolons relation till viktökning.

Metod

Resultat
Intervjuerna i studie I visade att kvinnor ville veta mer om olika typer av mat för att minska en eventuell risk för sina barn men kostinformation var delvis upp till dem själva att ta reda på. De
uttryckte känslor av osäkerhet och skuld om de råkat äta något ”förbjudet”. Rekommendationerna följes så väl som möjligt, tillsammans med sunt förnuft för att hantera kostförändringar. Huvudteman var ”Söka information på egen hand”, ”Få professionell rådgivning när problem uppstår”, ”Känna sig osäker” och ”Ta ansvar med en nypa salt”. I studie II kunde man se vissa skillnader i kostmönster bland de gravida kvinnorna jämfört med kontrollgruppen: mindre intag av grönsaker (47 g/dag), potatis/ris/pasta (31 g/dag), kött/fisk (24 g/dag) och alkohol och tobak/snus och ett högre intag av kosttillskott. Både gravida kvinnor och kontrollgruppen hade lägre intag av folsyra via kosten med 45 % (gravida) och 22 % (kontrollgruppen) än de gällande rekommendationer som är (500 resp 400 g/dag). I studie III såg man att intaget av vitamin D var 34 % lägre än rekommendationen på 10 µg/dag. Minst en tredjedel av deltagarna hade otillräckliga plasma nivåer av vitamin D, under 50 nmol/L. Årstid var en stark faktor som påverkar det longitudinella mönstret. Graviditetsvecka, säsong, totala energiintaget, intaget av vitamin D och multivitamintillskott under de senaste 14 dagarna var faktorer som relaterade till D-vitaminnivåer. I studie IV sågs ett samband mellan allopregnanolon-koncentrationer vid graviditetsvecka 35 och viktökning från vecka 12 till 35 (p = 0,016). Det sågs också ett samband mellan ökningen av allopregnanolon (vecka 12–35) och viktökningen (se ovan) (p = 0,028).

**Slutsatser**
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>D&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Ergocalciferol (vitamin D&lt;sub&gt;2&lt;/sub&gt;)</td>
</tr>
<tr>
<td>D&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Cholecalciferol (vitamin D&lt;sub&gt;3&lt;/sub&gt;)</td>
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<tr>
<td>EDTA-plasma</td>
<td>Ethylenediaminetetraacetic acid plasma</td>
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<td>FFQ</td>
<td>Food Frequency Questionnaire</td>
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<tr>
<td>FIGO</td>
<td>International Federation of Gynecology and Obstetrics</td>
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<tr>
<td>GABA-A</td>
<td>Gamma amino butyric-acid receptor type A</td>
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<tr>
<td>LC-MS/MS</td>
<td>Liquid chromatography-tandem mass spectrometry</td>
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<td>MI</td>
<td>Motivational interviewing</td>
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<td>NFA</td>
<td>National Food Agency, Sweden (Livsmedelsverket)</td>
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<tr>
<td>NNR</td>
<td>Nordic Nutrition Recommendations</td>
</tr>
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<td>PLS</td>
<td>Partial Least Squares analysis</td>
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<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>VIP</td>
<td>The Västerbotten Intervention Programme for Health Survey</td>
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<td>WHO</td>
<td>World Health Organization</td>
</tr>
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<td>25(OH)D</td>
<td>25-hydroxy vitamin D</td>
</tr>
</tbody>
</table>
Definitions used in the thesis

**Antenatal care**

In this thesis antenatal care means health care during pregnancy provided in primary health care centres. Antenatal care is synonymous with prenatal care.

**Season**

In this thesis season at latitude 63.8° is referred to as winter 16/9–14/4 and summer 15/4–15/9 (1).

**WHO’s definition of nutrition**

Intake of food necessary for optimal growth, function and health (2).

**WHO’s definition of good and poor nutrition**

Good nutrition is a well-balanced diet that provides all essential nutrients in optimal amounts and proportions, whereas poor nutrition is a diet that lacks nutrients (either from imbalance or overall insufficient food intake) or one in which some components are present in excess (2).
Original papers


III. Lundqvist A, Sandström H, Stenlund H, Johansson I, Hultdin J. Vitamin-D Status During Pregnancy; a Longitudinal Study in Swedish Women From Early Pregnancy to Seven Months Postpartum. [Manuscript accepted]

IV. Lundvist A, Bäckström T, Sandström H. Weight gain relates to allopregnanolone levels during pregnancy – a longitudinal study. [Manuscript]

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Paper II is reprinted with permission from BMC Med.Central
Preface

During my work as a midwife, my interest in issues of health-improving factors has deepened. I experienced that food intake was marginal in conversation in antenatal care in the 1990s. The dietary recommendations in the early 1990s were not easy to interpret, with various risks to handle, and therefore quite complex for the pregnant women to follow. I remember the concrete advice I was given during my pregnancies; “eat as usual and cook in a cast-iron pot”, which was easy for me to follow.

The interest in vitamins and food intake during pregnancy ended up in a master’s thesis in nursing. My colleague AnnaLena Wennberg and I were introduced to Herbert Sandström. He was also interested and has extensive knowledge in these fields. Herbert suggested that we should make plans for a longitudinal study during pregnancy (on folic acid). We were presented to other researchers with interest and knowledge in the subject. They joined our group and we started to make plans for our new project. The project developed and took its present form over time. We worked on preparations for the data collection which started in autumn 2006. Combined with the project I worked at the Department of Obstetrics and Gynaecology, and in the beginning of the 2000s in primary care. In 2012 I finally registered as a PhD student at Family Medicine.
Introduction

Maternal lifestyle and nutritional factors before and during pregnancy can influence pregnancy outcomes which affect the child in the longer term (3). Barker et al (4) showed in the 1990s that the environment in the uterus is important for the child’s health in future life. Later studies have shown that epigenetic changes can lead to non-communicable diseases transmitted to the next generation (5,6). Healthy eating habits should also be maintained after birth and during lactation. The body needs to be rebuilt for lactation to optimize milk production and to regain weight after pregnancy.

The diet should meet the nutrient requirements of macronutrients (carbohydrates, protein, fat) as well as a variety of micronutrients such as minerals and multiple vitamins. The energy need in adults (18–30 years) is approximately 2000–2500 kcal/day depending a bit of the current physical activity level. The mother should also avoid toxins and contaminants. An unbalanced diet can occur from over-consumption of non-nutritive energy-rich foods and/or under-consumption of nutrient-dense foods (3,7–9).

The requirements for most minerals and vitamins increase during pregnancy and lactation; for some vitamins, especially folate, requirements are increased in the preconception period (10–15).

A pregnancy normally presupposes weight gain due to the growing fetus, placenta, amniotic fluid, maternal tissue and increased maternal fat stores. Well-nourished women with a mean weight gain of 12 kg have additional energy requirements of 90, 287, 466 kcal/day for the first, second and third trimester respectively (17). That corresponds roughly to a piece of fruit in months 1–3; a hearty snack and a fruit in months 4–6; two hearty snacks and a fruit in months 7–9 (16). During exclusive lactation there is an increase of energy requirements of about 454 kcal/day (17).
Swedish antenatal care

Swedish antenatal care is free of charge, financed by public health care. Each county council or region is responsible for women’s antenatal care. The antenatal clinics are organized in public or private sector as well as in hospital settings. The majority are placed in primary health care centres with a midwife as the primary caregiver. Specialist prenatal care is provided mainly through hospital clinics. There is a long tradition in Sweden of health prevention during pregnancy and the participation rate among pregnant women is high, around 99%.

The tasks for Swedish midwives are to promote health, prevent and detect health risks, contribute to good sexual and reproductive health, and minimize risk to and morbidity of women and children during pregnancy, childbirth and infancy. Pregnant women, sometimes with their partner, visit a midwife 8–10 times, starting in early pregnancy with a follow-up visit 6–12 weeks postpartum. Midwives take up a comprehensive history about somatic diseases and conditions. They provide counselling and advice based on the anamneses of psychosocial profile, lifestyle habits that include diet, physical activity, and use of alcohol, tobacco, drugs, mental health, and dietary intake, including calculating BMI. Physical and laboratory examinations are implemented, for example measurements of weight, blood pressure and laboratory tests, registrations of uterine growth and screening to detect maternal and fetal complications. In the postpartum visit both physical examinations and laboratory test are performed. Experiences of pregnancy, delivery and lactation are also in focus in the health consultations as well as sexual life issues and contraception (18).

Dietary recommendations

Nordic Nutrition Recommendations (NNR) are aimed at the general population and are a basis for national recommendations (11). A balanced Nordic diet is recommended because it contains a lot of vegetables, fruit and berries, nuts and seeds, peas and beans, whole grain, seafood, selected types
of fish, vegetable oils and low-fat dairy products (11). This Mediterranean diet gives high amounts of most micronutrients, for example a relatively high content of folate (19) zinc and several vitamins (20). Even if a healthy diet limits the need for supplementation, there are still some nutrients that need attention and may require supplementation. Iron, iodine, folate, vitamin B12, calcium and vitamin D are nutrients where deficiencies are common worldwide (3).

**Iron** is vital for all organs; for example forming the oxygen-binding part of haemoglobin that transports oxygen from the lungs to the tissues. Iron requirements are increased during pregnancy progress due to haemoglobin synthesis and transport to the fetus as well as iron losses during labour. A reduced concentration of Hb means anaemia, and the limit during pregnancy is set at 110 g/L (21,22). Deficiency has been associated with pre-term birth and low birth weight (23,24). Obese pregnant women are at a higher risk of iron deficiency compared to women with a normal weight measuring lower maternal iron stores (25). The NNR recommendations are about 15–18 mg depending on deposits and current iron values, during breastfeeding 15 mg is recommended (22). Supplementation is common during pregnancy. Anaemia is a widespread problem in many countries and puts mothers at several risks, with postpartum haemorrhage as one example (3). Dietary sources are blood-based foods, meat, fish, whole grain cereals, spinach, and some fruits (22). The concentration of iron in human milk is low but with a high bioavailability (12) which ensures that a full-term, healthy, breastfed or formula-fed infant needs no outside supply during the first 4–6 months (26,27).

**Folate** (vitamin B9) is a water-soluble vitamin that is abundant in liver, vegetables, especially in beans, green leafy vegetables, berries, fruit, and root vegetables (16). The vitamin is heat sensitive and losses in the cooking process are common (29). The synthetic form of folate is called folic acid, which is more stable and found in supplements and in fortified foods. Folate is needed in the formation of new cells, especially in rapidly growing cells and the formation of red blood cells. The vitamin is also necessary for the processes of ribonucleic acid (RNA) and
deoxyribonucleic acid (DNA) synthesis (30). Some medications (Folic Acid Antagonists) affect the body’s ability to absorb or metabolise folic acid which increases the risk of having a child with neural tube defects (31). Adequate levels of folate are required already before conception to protect against neural tube defects due to the fact that the neural tube closes very early in the pregnancy, around weeks 3–4. The vitamin also prevents megaloblastic anaemia during pregnancy (32). In several countries, certain foods are fortified with folic acid, but not in Sweden. Recommendations from the Swedish National Food Agency (NFA) are applicable to Sweden and recommendations during pregnancy and lactation are based on the NNR. NFA (33) recommends supplements with 400 µg folic acid/day to all women planning a pregnancy and continuing during the first trimester. The recommended intake of folate is 500 µg/day during pregnancy and breastfeeding due to fast-growing tissues (22,34). Folate in human milk varies over the lactation period (35) and the highest concentrations are found 3–6 months after birth (36).

NFA in Sweden has adapted and summarized the advice from the NNR about eating habits and dietary guidelines for pregnancy and lactation (16,37). Midwives are the primary caregivers in antenatal care, to whom NFA refers as the profession to give women detailed dietary advice. The advice is consistent with that of the NNR above, with a varied diet, at least 500 g of fruit and vegetables per day with a daily intake of 500 mL low-fat dairy products such as milk or yoghurt. Fish, shellfish (2–3 times per week) and vegetable oils are good sources of unsaturated fat. For some fish species there are restrictions for consumption, with a recommendation to eat a maximum 2–3 times per year due to dioxins, PCB and mercury. Meat, chicken, eggs, beans, lentils or peas are recommended as well as bread and potatoes, rice, pasta, bulgur wheat, preferably wholegrain. Water is recommended for meals and when thirsty. The recommendation is also to reduce the intake of refined sugar products such as cakes, sweets and soft drinks (16,20).
**Dietary supplementation**

Recommendations from the NFA are that anyone who might become pregnant is advised to take 400 micrograms of folic acid each day until gestational week 12, to prevent neural tube defects (16,33,38) as mentioned before. Vitamin B12 and vitamin D in supplements or fortified products are advised for vegans. Vitamin D supplements are recommended to veiled women and women who do not eat fortified foods (39). Generally, dietary supplements and herbal products should be treated with caution and some products should be completely avoided, e.g. ginseng (40).

**Exposures to avoid or reduce**

**Alcohol:** Although there are studies suggesting that light drinking during pregnancy is not linked to developmental problems in mid-childhood (41,42), the recommendation in Sweden is to abstain completely from alcohol. Spontaneous abortion, prenatal and postnatal growth restriction and neurological birth defects have been associated with alcohol exposure (43).

The restrictions during lactation allow for the ingestion of small amounts of alcohol, even though alcohol itself has no positive effects on lactation. According to current research, there is no medical risk with a moderate amount of alcohol during lactation, i.e., 1–2 glasses of wine (1 standard glass of wine=12 g alcohol=12–15 cl) or its equivalent 1–2 times a week. The amount of alcohol that is transferred to the child with the milk is small and not considered harmful (44–46).

**Caffeine:** Contradictory results in literature make it difficult for health professionals to advise pregnant women on caffeine during pregnancy. The intake of caffeine is restricted to 300 mg per day. This is equal to either three cups of coffee (1.5 dL/cup) or six cups of black tea (16). The risk of unfavourable birth outcomes has been highlighted in studies (47,48) but the latest Cochrane Review found insufficient evidence to confirm or disprove the effectiveness of caffeine avoidance on birthweight
or other pregnancy outcomes due to the quality of the included studies (49).

**Mercury** in large quantities can damage the brain and nervous system. Mercury can be passed to the baby through the mother’s placenta and breast milk. It is a metal which accumulates in fish such as perch, pike, walleye and burbot. Large predatory fish contain higher levels per gram, and thus fresh tuna, swordfish, large halibut, shark and ray should be avoided. Canned tuna belong to a different species from tuna sold fresh and do not contain high levels of mercury (28).

**Dioxin and PCBs** affect the development of the brain and nervous system, which can cause behavioural disorders. They can also affect the immune system and endocrine systems as well as reproduction. These are organic pollutants that have become widespread in the environment. The recommendation is to avoid herring and wild salmon and trout from around the Baltic Sea and the Gulf of Bothnia. This also applies to wild salmon, trout and whitefish from Vänern and Vättern. For women of childbearing age, it is especially important to minimize the intake of these toxins as they are transferred to the fetus and breastfed infants through the placenta and breast milk (50,51).

The bacteria **Listeriosis monocytogenes** is present everywhere in nature. Listeriosis is also found in food stored a long time in the refrigerator and then eaten without being heated, for example, vacuum-packed smoked and pickled fish, soft cheeses and sliced cold cuts. The Listeriosis bacteria die when foods are heated to over + 70 °C. Pregnant women may get flu-like symptoms or be symptom-free. During the infection, transmission to the fetus may in the worst cases lead to miscarriage or a severely sick child (52).

The parasite **Toxoplasma gondii** has the cat as its host and excretes the parasite in the feces. Other animals, such as lamb or pigs, can be infected and became a source of infection for us humans as cat feces is. Contaminated soil, vegetables and berries are also potential sources of infection. The parasite can spread through the placenta to the fetus and cause miscarriage,
birth defects or congenital infection. The parasite dies when heated to at least 65 °C or deep frozen at −18 °C for at least three days (16,53).

Weight gain and obesity

Weight gain during pregnancy differs substantially between women. The proportion of overweight or obese (BMI ≥25) women at enrolment in Swedish antenatal health care increased from 25% in 1992 to 38% 2012. In 2013 25% of all pregnant women in maternal care were overweight and 13% were obese (54). The guidelines from the US Institute of Medicine are the most accepted in Europe and are also used in Sweden. The guideline recommend women with a normal BMI at enrolment (BMI 18.5–25) a 11.5–16.0 kg gestational weight gain; in women with overweight (BMI 25–30) lower weight gain is recommended (7.5–11.5 kg) and in obese women (BMI ≥30) 5–9 kg (55).

The term overeating can be used when energy intake exceeds the body’s energy expenditure which leads to excess body weight and obesity in the long run (56). Factors such as increasing meal size and passing meals during the day are also linked to obesity (57). Excessive weight gain during pregnancy entails a risk of complications for both mother and child. For example metabolic complications such as gestational diabetes, hypertension, thromboembolism, implications for infant growth and adiposity are reported. Further complications are the increasing frequency of caesarean delivery and higher birth and placenta weight. Asphyxia-related outcomes in term infants are found as well as longer stay in hospital (58–62). Furthermore, lactation rates seem to be lower among obese women and early termination, which is not favourable for the mother or the child (63).

Behavioural changes; physical activity and diet

A pregnancy is a major life event that constitutes a strong motivator for women to embrace a healthier lifestyle. Motivational interviewing (MI) is a method that is used in Swedish antenatal
Introduction

care to promote motivation and behavioural change related to lifestyle factors. The idea is to support patients in their efforts to change an unhealthy lifestyle habit. The method has been influenced by Prochaska and DiClemente's theoretical model (64) and developed by Miller and Rollnick (65). The National Board of Health and Welfare has described the guidelines and educational requirements for MI (66).

Physical exercise is beneficial and safe for pregnant women (67) and has also been associated with lower weight gain. According to a meta-analysis the effect of physical exercise was lower than the effect of dietary interventions (68). Recently it was reported that women with a high gestational weight gain are more likely to have problems in managing weight retention and reaching normal weight in the postpartum period (69). Physical activities are recommended for at least 30 minutes daily to achieve and maintain a healthy weight (16,37). NNR for adults, including pregnant women are specified depending on the type of physical activity and its intensity. At least 150 minutes of moderate-intensive physical activity per week is applicable (11).

A link between Mediterranean diet and less increase in BMI during pregnancy has been observed by Silva-del Valle et al (70), and women with high adherence gained less weight and were more likely to be within the recommended BMI range from the Institute of Medicine. Adherence to a Mediterranean-like diet has also been suggested to be beneficial for fetal growth (71). Pregnancies with low adherence was associated with lower birth and placenta weight (72) and increased risk for the child to have hyperinsulinemia at birth (73). Both under- and over-nutrition can contribute to the development of gestational diabetes and also epigenetic changes that may contribute to future diabetes type 1 and 2 according to a review by Nielsen et al (74). In a study from Sweden the mean gestational weight gain was significantly reduced by 1 kg (p= 0.029) by weight interventions and education compared to a standard care group (75). Phelan et al (76) showed that behavioural lifestyle interventions reduced gestational weight gain in women with normal weight. The interventions also prevented weight retention postpartum in normal weight, overweight and obese women. The opposite result has also been seen, that eating
patterns are difficult to influence with small changes in women’s dietary intake (77). An Australian study showed that adherence to current dietary guidelines was low, and even though women perceived that they had a healthy diet, they could not meet recommendations for fruit, dairy products and other core food products (78).

**Vitamin D physiology and synthesis**

Vitamin D receptors are present in our body’s organs, in tissues and cells as well as in the placenta-decidua during pregnancy. Vitamin D is essential for the regulation of calcium and phosphorus homeostasis and is involved in many biological processes, such as cell proliferation and differentiation in target tissue (79,80).

There are two variants of vitamin D: cholecalciferol (vitamin D₃) and ergocalciferol (vitamin D₂). One source is exposure to sunlight. With the help of the sun’s UVB (ultraviolet B radiation; wavelengths 290–315 nm) rays, 7-dehydrocholesterol in the skin is converted to vitamin D₃ (81). Another source of vitamin D₃ is through food intake, fatty fish, eggs, enriched dairy products and supplements. Vitamin D₂ is also found in mushrooms and some supplements (39). Vitamin D₃ and vitamin D₂ are then hydroxylated in the liver to calcidiol 25(OH)D₂ and 25(OH)D₃. Renal handling continues the regulated conversion to the biologically active steroid hormone calcitriol (1,25(OH)₂D₃). Calcitriol synthesis is influenced by levels of calcium, phosphate, and parathyroid hormone, and it works together with parathyroid hormone to maintain calcium and phosphorus homeostasis (81,82). A brief description of vitamin D synthesis is shown below (Figure 1).
The synthesis of vitamin D in the skin is affected by many factors where amount of and exposure to sunlight, skin type, latitude and season have a strong influence on the amount of vitamin D that is synthesized (83). In the Umeå area (latitude 63.8°N), sun exposure is sufficient for vitamin D₃ synthesis from around the middle of April to the middle of September, most of this period in the hours around noon. In the remaining seven months synthesis is impossible (1).
Vitamin D in relation to pregnancy and after birth

The importance of vitamin D during pregnancy has been highlighted in recent years. The need for both calcium and vitamin D is increased during pregnancy for fetal growth and development, and during lactation due to growth of the newborns, especially for skeletal growth and mineralization. During pregnancy the body adapts by changing the metabolism with increased synthesis of calcitriol (1,25(OH)₂D) simultaneous with changes in hormones, e.g., prolactin, placental lactogen, calcitonin, and estrogen (84). The fetus accumulates about 30 g calcium, mainly in late pregnancy. The maternal intestine and kidneys facilitate maximum absorption and excretion of calcium and phosphates to meet this need (81,82).

Many studies, meta-analyses and reviews have indicated vitamin D deficiency and adverse health outcomes in pregnant women and new-born around the world. An increased risk of gestational diabetes, pre-eclampsia, preterm birth, small for gestational age and risk of reduced bone mineral content has been reported, related to deficiency of vitamin D (85–89). Contradictory results found no association with preterm birth have also been reported (90). The definition of deficiency is debated and no consensus seems to prevail about the optimal level. Vitamin D status is estimated by measuring the total concentration of 25(OH)D levels in serum or plasma, which is the sum of D₂ and D₃. The level of 25(OH)D is influenced by changes in synthesis or intake of vitamin D, otherwise the levels are stable (91). The Institute of Medicine has issued recommendations with a decision limit of serum concentrations of at least 50 nmol/L, which are adopted in this thesis (92,93).

Regarding lactation, vitamin D content in human milk is low and the infant stores of vitamin D are limited, with depletion around 8 weeks of age (84). Nevertheless lactation contributes about 200 mg/day calcium to the newborn child through breast milk (94).
Basic regulation of food intake

Food intake and appetite are strictly regulated by a complex control system. In the human body there is an interaction between the gut, brain, adipose tissue together with major organs to control the food intake for maintenance of the body’s energy metabolism. The nervous system is used to communicate these interactions together with several hormones, peptides and neurosteroids that both stimulate and inhibit the appetite (95). In the hypothalamus several nuclei influence food intake by regulating circulating hormones and neuronal activity to nuclei in the brainstem (95,96).

Regulation of food intake is also controlled by two complementary units: the homeostatic and the pleasure (hedonic) systems. The homeostatic system controls energy intake when energy stores are depleted. The pleasure signals act by reward regulation and can, for example, increase the desire to eat tasty food or use a drug. Cognitive and emotional factors can take precedence over the homeostatic metabolic system and cause an unbalanced state of energy (97,98).

In this thesis I will mainly focus on pregnancy and the relation to Gamma-butyric-acid-A (GABA-A) receptor-modulating steroids, especially allopregnanolone as this steroid increases substantially during pregnancy.

Steroid hormones and neuroactive steroids like allopregnanolone

During pregnancy large amounts of progesterone are produced by the placenta. A metabolite of progesterone, allopregnanolone, is also produced by the placenta and increases in serum parallel with progesterone. Allopregnanolone is also produced by the fetus and the concentrations are higher in the fetus than in the mother. During pregnancy the allopregnanolone levels increase considerably, up to 100 times higher than in non-pregnant women (99,100). Besides being synthesized in the placenta, allopregnanolone is synthesized in the maternal brain, adrenal and ovary, and is also high in the fetus circulation and brain (101–104).
Steroids are synthesized from cholesterol and metabolized to different types, progesterone being one primary type (105). Neuroactive steroids are small lipid-soluble molecules active in the central nervous system. They easily pass the cell membranes and blood-brain barrier and bind to receptors to exert their effect (106). Allopregnanolone binds to and acts as a potent positive modulator of the GABA-A receptor (107,108). It is now known that the GABA system and thereby allopregnanolone is central in the regulation of satiety and appetite. Activation of the GABA-A receptors in the regulation centres for food intake leads to overeating and in time to weight gain and obesity. In non-pregnant women one example is that allopregnanolone levels fluctuate during the menstrual cycle (109,110) and mean physiological values in healthy women are 0.3–1.9 nmol/L in the follicular phase and 1.1–3.7 in the luteal phase (111–113). Several studies have shown an increased energy intake during the luteal phase of the menstrual cycle and cravings have also been reported (114,115).

**Pregnancy and allopregnanolone**

Allopregnanolone has many functions related to maternal neuroendocrine stress responses. During pregnancy the levels of the sex steroids estradiol and progesterone and its metabolite allopregnanolone increase. Progesterone secretion is established and maintained during pregnancy. Allopregnanolone is important to maintain many of the necessary adaptations in the woman’s body during pregnancy. One is to protect the fetus from maternal stress hormones and unfavourable birth outcomes; another is to secure fetal growth and brain development (116).

**Allopregnanolone and food intake**

Steroid hormones such as allopregnanolone are, as mentioned earlier, highly involved in food regulation and satiety among other effects in the body (117). In conjunction, hunger, satiety hormones (e.g. ghrelin, leptin) and steroids regulate the nutritional signals with initiation, frequency and size of meals (118–120).
Allopregnanolone is well-studied and higher levels have been measured in overweight and obese humans compared to normal-weight girls, men and women (121,122). Turkmen et al found that uncontrolled eating is significantly related to allopregnanolone levels in women with polycystic ovary syndrome (123). The results of a new thesis show that allopregnanolone can act at different levels of feeding and can thus be involved in weight gain (124). The energy intake in rodents increased under the influence of allopregnanolone; both amount eaten and lengths of eating increase with increasing allopregnanolone. In addition, when a choice of food was possible, energy-rich foods were preferred. The increased weight gain was correlated to intake of energy-rich foods (125–127).

**The GABA system and allopregnanolone**

GABA is the main inhibitory neurotransmitter in the central nervous system. GABA-A receptors contain subunits forming a chloride channel. When GABA activates, chloride ions pass and hyperpolarization of the post-synaptic cell membrane follows. Allopregnanolone modulates and influences the channel’s opening time (116). This means that, depending on the concentrations of allopregnanolone, the receptor either activates for a prolonged opening time and the GABA inhibitory effect enhances (128). GABA activates different subtypes of receptors (A, B, C), GABA-A is affected by modulators such as allopregnanolone, but also by barbiturates and benzodiazepines (107,129). The subtypes of the GABA-A receptors related to food intake regulation are found in the hypothalamus, around the arcuate nucleus, the key node for energy regulation (117).

**The rationale**

A well-balanced diet and healthy weight gain in the mother provides good conditions for the well-being of both the mother and the growing baby (3). A poor diet and sedentary lifestyle may cause illness. Especially obesity and excessive weight gain entails a risk for complications associated with pregnancy, delivery and infancy. Eating habits can be difficult to change, therefore, it is advantageous to adopt and maintain good habits
already before pregnancy but in antenatal care from early pregnancy. Based on the above facts, it is interesting to further explore more about the complexity of being pregnant in relation to nutritional aspects; to adapt daily lives to restrictions in areas such as food intake and weight gain while physical and psychological changes occur. Studies on dietary intakes, including vitamins, during pregnancy are sparsely studied in a Swedish context. Variations of allopregnanolone levels and its relationship to weight gain are above all studied in rodents. There also are few longitudinal studies on vitamin D levels among pregnant women in sub-Arctic areas. Nevertheless, it is therefore important to increase knowledge and understanding about women’s nutrition in the context of being pregnant and becoming a mother.
Aims of the thesis

The overall aim of this thesis is to describe dietary intake, vitamin D-levels, dietary information and dietary changes, and to study the relation between allopregnanolone and weight gain during pregnancy and postpartum.

The specific aims of the four papers in the thesis were:

**Paper I**  To describe women’s attitudes to and experiences of dietary information and advice as well as dietary management during pregnancy.

**Paper II**  To compare dietary patterns of early pregnant women with non-pregnant women in the county of Västerbotten, Northern Sweden.

**Paper III**  To longitudinally assess vitamin D status during pregnancy and postpartum, and to identify factors associated with vitamin D status, including intake and seasonal variations, in pregnant women in northern Sweden (latitude 63.8°N).

**Paper IV**  To examine whether allopregnanolone levels relate to weight gain during pregnancy in a group of pregnant women.
Methods

The studies in this thesis are based on qualitative and quantitative methods to examine the research field from different angles and with different scientific approaches.

Table I. Material and methods used in the studies comprising this thesis.

<table>
<thead>
<tr>
<th>Paper</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Design</strong></td>
<td>Qualitative design</td>
<td>Cross-sectional cohort study</td>
<td>Longitudinal cohort study</td>
<td>Longitudinal cohort study</td>
</tr>
<tr>
<td><strong>Participants</strong></td>
<td>Pregnant women (n=23)</td>
<td>Pregnant women (n=209). Reference women (n=206)</td>
<td>Women pregnant and postpartum (n=184)</td>
<td>Pregnant women (n=59)</td>
</tr>
<tr>
<td><strong>Data collection</strong></td>
<td>Focus groups (n=6)</td>
<td>FFQ and excerption of data from medical records</td>
<td>Blood sampling FFQ Excretion of data from medical records</td>
<td>Blood sampling Weight measures Excretion of data from medical records</td>
</tr>
<tr>
<td><strong>Biochemical analysis</strong></td>
<td>–</td>
<td>–</td>
<td>25(OH) vitamin D</td>
<td>Allopregnanolone</td>
</tr>
<tr>
<td><strong>Analyses</strong></td>
<td>Content analysis: domains, subthemes, themes</td>
<td>Descriptive, comparative statistics, partial least squares analysis (PLS)</td>
<td>Descriptive comparative statistics, linear mixed model</td>
<td>Descriptive non-parametric statistics</td>
</tr>
</tbody>
</table>
Study I

Material and methods

Focus groups are well established and commonly used for data collection in health care research. In focus groups, interactions between participants are an important element when talking about experiences, beliefs and views concerning a specific phenomenon or topic chosen by the researcher. The moderator leads the discussion according to an interview guide with questions/areas related to the selected phenomenon. Follow-up questions are used to clarify and deepen the conversation. The process promotes and stimulates both depth and breadth in the discussion. Various individual experiences, new thoughts and ideas are generated and expressed among active participants, and unanticipated issues may be brought up (130,131).

Participants

We contacted midwives at five antenatal clinics in primary care and asked them to distribute written information about the study in their ongoing antenatal classes. The health care centres were located in small (n=2), midsize (n=1), and urban towns (n=3). All women were first-time pregnant except for one woman who had had a stillbirth. We visited their classes and gave further information combined with an invitation to participate in a focus group interview. The interviews were conducted in a room at the health care centre. In total 27 women agreed to participate but four of them did not show up at time of the session. Each woman participated in only one of the six focus groups, so the study included 23 women in total. Characteristics of the participating women are shown in Table 2. None of the women reported that they smoked.
Table 2. Characteristics of the participants in study I.

<table>
<thead>
<tr>
<th>Characteristics (n=23)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median (range))</td>
<td>29 (19–41)</td>
</tr>
<tr>
<td>Cohabitating/Single living (n)</td>
<td>21/2</td>
</tr>
<tr>
<td>Urban/Rural residence (n)</td>
<td>16/7</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Highest education:</td>
<td></td>
</tr>
<tr>
<td>University (n)</td>
<td>16</td>
</tr>
<tr>
<td>High school (n)</td>
<td>5</td>
</tr>
<tr>
<td>Compulsory school (n)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>No dietary preferences (n)</td>
<td>21</td>
</tr>
<tr>
<td>Lacto-vegetarian (n)</td>
<td>2</td>
</tr>
</tbody>
</table>

Interviews

In 2007, we conducted six focus group interviews at the health care centres. AnnaLena Wennberg, the first author, and I acted as either moderator or observer in all the interviews. We designed and used an interview guide with open-ended questions covering the research aim. We wanted to get more knowledge about sources of dietary information that women experienced, as well as how they experienced and handled the advice. The participating women had met previously through parental education classes and they were thus acquainted with each other before the interview. The interviews were audio-recorded, lasted about 45 minutes and were transcribed verbatim. The initial questions were followed by exploratory and follow-up questions such as “please tell more”, “what are your thoughts about that?”, “please explain how you reacted and felt”. It was easy to start a fruitful discussion in the groups. The conversation flowed and the participants’ different experiences and opinions were ventilated. Participants who were more reticent could be drawn into the conversation through the follow-up questions and the discussion thereby deepened.
Methods

Studies II–IV

Settings

All studies are based in Swedish primary care in Västerbotten County Council.

Sample size

The recruitment of the cohort proceeded from a power calculation based on Koebnick et al in a study of 39 pregnant women from first to third trimester (132). Using Koebnick’s data for vitamins B12 and folate and homocysteine with differences between first and third trimesters showed that we would have a statistical power of at least 80% if 200 women were included.

Participants

The enrolment and attendance for the cohort is described in Figure 2. The population in study II is based on the cohort on the first sampling occasion (weeks 10–12). In study III participants who provided blood samples on 3–5 occasions are included. Study IV is based on a stratified sample of participants (described later in this chapter) from the cohort on the first and third sampling occasions.

We included 226 pregnant women from five health care centres representing a socio-economic and geographical cross-sectional sample. Women at their first visit to the antenatal clinics during the recruitment period were offered information on the study by the midwives. Women who expressed an interest in participating were given verbal and written information, and were invited to participate. The exclusion criteria’s were: major medical conditions, being unable to attend the ordinary antenatal programme, and insufficient competence in the Swedish language. All data were collected in connection with ordinary antenatal visits except for the last occasion, when the women booked an appointment at the health care centres. There were no differences in the available social parameters between the dropouts and those with three or more sampling occasions.
occasions. In addition to the dropouts, 27 questionnaires scattered over the study period were not collected and six blood samples were not provided. The enrolment process was handled by the midwives and we had no face-to-face contact with the participants during the study period.
Methods

Figure 2. Enrolment and attendance of pregnant women in the cohort
Methods

The questionnaires

The self-reported questionnaire was the same as used in the Västerbotten Intervention Programme (VIP) for health survey, including a food frequency questionnaire (FFQ) of 66-items. Questions about socioeconomics, psychosocial conditions, marital status, and level of education, self-rated health, personal health history, family history, and quality of life (SF36) were also included. The questionnaire also covered social network and support, working conditions, physical activity, alcohol consumption, tobacco use, and dietary supplement use. Body weight (light clothing) and height (no shoes) were measured at the same time as blood samples were collected. The pregnant women were informed to answer the questions about the last 14 days. All data both from questionnaires and blood samples were administered and stored in Northern Sweden Biobanks in Umeå.

Estimation of dietary intake

In the FFQ, the women reported their consumption frequencies on a nine-level scale from 0 (never) to 8 (4 times/day or more). The FFQ included eight questions about the frequency of consumption of various types of fats; nine on milk and other dairy products; seven about bread and cereals, six about fruit, greens and root vegetables; six questions on soft drinks and sugar-containing snacks; and five questions on spirits, wine and beer consumption were included in a list of beverages. Twenty of the remaining 25 questions recorded intake of potato, rice, pasta, meat and fish, and five were on varied items, such as salty snacks, coffee, tea and water.

The participating women indicated their average portion of potato/pasta/rice, vegetables, and meat/fish by looking at four colour photographs illustrating four plates with increasing portion sizes of potatoes, vegetables, and meat. The reported consumption frequencies were converted to number of intakes per day. The content of energy and nutrients was calculated by multiplying daily intake frequency by the portion content according to the specific nutrient in the database provided by the NFA (Uppsala, Sweden) (133).
Methods

**Blood sampling**

Blood samples were collected after 15 minutes’ seated rest. The participants were asked to fast and 97.8% of them had at least 8 hours of fasting before blood sampling, the remaining had 4–8 hours of fasting. Peripheral venous blood was drawn, with no stasis used, into vacuum tubes, one containing EDTA, one with heparin and one serum tube suitable for trace metal analysis, total volume 25 mL. Plasma or serum was obtained by centrifugation at 1500 g for 15 minutes. Plasma and serum were aliquotted in smaller plastic tubes. Aliquots of buffy coat as well as erythrocyte fractions were also collected. All samples were stored frozen at –80 °C until analysis. Sampling procedures were handled in accordance with VIP routines (134).

**Participants – Study II**

Participating women were recruited as described previously. A reference group of 206 women was nested from the current VIP (135). A flow diagram of the study population is presented in Figure 3.

![Figure 3](image)

**Figure 3.** Participants in study II.
FFQ data were used to estimate dietary intake. VIP invites all 40-, 50-, and 60-year-old inhabitants in Västerbotten County to a health screening; 30-year-olds are invited in some municipalities. The references were selected and had participated in VIP during the same recruitment period as the pregnant women. We included all 30-year-old women from the Umeå area together with an equal-sized 40-year-old group of women (n=206). The reference women completed the same FFQ as the pregnant women but they reported their food intake during the last year while the pregnant women reported their intake during the previous two weeks. Characteristics of the participating women are shown in Table 3.
Methods

Table 3. Characteristics of the participants in study II.

<table>
<thead>
<tr>
<th></th>
<th>Pregnant women (n=209)</th>
<th>Reference women (n=206)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years):</td>
<td>30.4 (4.2)</td>
<td>35.0 (5.0)</td>
</tr>
<tr>
<td>Mean, Standard deviation (SD)</td>
<td>30.0 (21–42)</td>
<td>37.0 (30–40)</td>
</tr>
<tr>
<td>Median, min - max</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married or cohabitating (n)</td>
<td>206/3</td>
<td>170/35*</td>
</tr>
<tr>
<td>/single living (%)</td>
<td>(98.6/1.4)</td>
<td>(82.9/17.1*)</td>
</tr>
<tr>
<td>Nullipara/Multipara (n, %)</td>
<td>131/78 (62.7/37.3)</td>
<td>—</td>
</tr>
<tr>
<td>Singleton/twin pregnancy (n)</td>
<td>206 / 3</td>
<td>—</td>
</tr>
<tr>
<td>University degree (n, %)</td>
<td>126 (60.3)</td>
<td>99 (48.3)</td>
</tr>
<tr>
<td>High school/Compulsory school (n, %)</td>
<td>83 (39.7)</td>
<td>106 (51.7)</td>
</tr>
<tr>
<td>Born in Sweden/other origin (n)</td>
<td>197/12</td>
<td>Not available</td>
</tr>
<tr>
<td>Starting BMI (kg/m² (mean (SD))</td>
<td>24.2 (4.8)</td>
<td>—</td>
</tr>
<tr>
<td>BMI groups (n, %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 24.9</td>
<td>144 (68.9)</td>
<td>127 (61.7)</td>
</tr>
<tr>
<td>25–29.9</td>
<td>47 (22.5)</td>
<td>50 (24.3)</td>
</tr>
<tr>
<td>≥ 30.0</td>
<td>17 (8.1)</td>
<td>29 (14.1)</td>
</tr>
<tr>
<td>Smokers/snuff users (n)</td>
<td>1/2</td>
<td>13/26</td>
</tr>
</tbody>
</table>

*stated as unmarried.

Participants – Study III

Study III included 184 women recruited as described previously. They participated in three to five sampling occasions during the study period. The number of blood samplings varied depending on the different participation at each sampling occasion, for example; a participant who provided three blood samples could have provided them in week 12, week 21 and 12 weeks postpartum. EDTA-plasma was used to analyse concentrations of vitamin D and FFQ for dietary intake. A flow
Methods

diagram of the study population is presented in Figure 4 followed by characteristics (Table 4).

**Figure 4.** Participants included in study III.
Methods

Table 4. Characteristics of the participants in study III.

<table>
<thead>
<tr>
<th>Characteristics (n=184)</th>
<th>Mean (SD)</th>
<th>Proportions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (21–42 years)</td>
<td>31 (4.4)</td>
<td></td>
</tr>
<tr>
<td>Born in Sweden</td>
<td></td>
<td>93.5</td>
</tr>
<tr>
<td>Married or cohabitant</td>
<td></td>
<td>98.7</td>
</tr>
<tr>
<td>University education</td>
<td></td>
<td>59.4</td>
</tr>
<tr>
<td>Nullipara/multipara</td>
<td></td>
<td>62.2/37.8</td>
</tr>
<tr>
<td>Singleton/twin pregnancy</td>
<td></td>
<td>97.8/2.2</td>
</tr>
<tr>
<td>Gestational length (weeks)</td>
<td>39.4 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Starting BMI (kg/m²)</td>
<td>24.1 (3.8)</td>
<td></td>
</tr>
<tr>
<td>Reported total energy</td>
<td>1,629 (476)</td>
<td></td>
</tr>
</tbody>
</table>

1Energy intake was increased by 25% to adjust for underreporting due to the short FFQ as described in study II (136).

Participants – Study IV

For study IV a sample of 60 pregnant women was included from the larger cohort of 226 women. The women were stratified on the basis of weight gain and they were divided into two weight gain groups based on the median value of 11 kg: <11 kg (n=29), ≥11 kg (n=30). EDTA-plasma was used to analyse allopregnanolone from gestational week 12 and 35. The internal loss is represented by three women who did not provide blood sample in week 35 which means that 56 blood samples are analysed in week 35. The aim in the recruitment was to have a spread in the weight increase during pregnancy and to cover the weight gain area. A flow diagram of the study population is presented in Figure 5 followed by characteristics (Table 5).
Methods

Pregnant women recruited at enrolment in antenatal care (n=226)

Recruitment of 60 women with weight gain under (n=30) and over (n=30) 11 kg

Women included in the study (n=59)

No blood samples on both occasions (n=1)
No blood samples at week 35 (n=3)

Weight gain group < 11 kg from week 12 – 35 (n=29)

Weight gain group ≥ 11 kg from week 12 – 35 (n=30)

Figure 5. Participants included in study IV.
### Table 5. Characteristics of the participants in study IV.

<table>
<thead>
<tr>
<th>Characteristics (n=59)</th>
<th>Median (min–max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.0 (21–39)</td>
</tr>
<tr>
<td>Married or cohabitant (%)</td>
<td>98.3</td>
</tr>
<tr>
<td>Born in Sweden (%)</td>
<td>91.5</td>
</tr>
<tr>
<td>University education (%)</td>
<td>66.1</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3550 (2445–4640)</td>
</tr>
<tr>
<td>Placenta weight (g)</td>
<td>575 (340–890)</td>
</tr>
<tr>
<td>Weight total (kg)</td>
<td></td>
</tr>
<tr>
<td>Gestational week 12</td>
<td>66.0 (47–120)</td>
</tr>
<tr>
<td>Gestational week 35</td>
<td>78.0 (56–127)</td>
</tr>
<tr>
<td>BMI total (kg/m²)</td>
<td></td>
</tr>
<tr>
<td>Gestational week 12</td>
<td>23.7 (18–41)</td>
</tr>
<tr>
<td>Gestational week 35</td>
<td>28.0 (22–43)</td>
</tr>
<tr>
<td>Starting weight (kg) week 12</td>
<td></td>
</tr>
<tr>
<td>Weight group &lt; 11 kg</td>
<td>66.0 (47–120)</td>
</tr>
<tr>
<td>Weight group ≥ 11 kg</td>
<td>65.5 (50–86)</td>
</tr>
<tr>
<td>Follow-up weight (kg) week 35</td>
<td></td>
</tr>
<tr>
<td>Weight group &lt; 11 kg</td>
<td>72.0 (56–127)</td>
</tr>
<tr>
<td>Weight group ≥ 11 kg</td>
<td>79.5 (62–103)</td>
</tr>
</tbody>
</table>

### Analysis

#### Study I

Qualitative content analyses focuses on the subject and the material to analyse both manifest and latent content. The analysis deals with interpretation but the interpretations vary in depth and level of abstraction. Differences between and similarities within codes and categories are sought (137, 138).

The transcribed text (verbatim) was analysed according to procedures described by Graneheim and Lundman (137). First
Methods

we listened to the recordings for comparison with the text mass to perceive any non-verbal expressions or signs that could be applied to the text, e.g. laughs and breaks. Notes had also been written down during the interviews by the observer, which were also available for completion. The interviews were read several times to get an idea of the whole picture, such as a general perception of what has emerged, and we compared and discussed this in our research group. The text was then divided into meaning units according to the aim. A meaning unit is words, sentences or paragraphs with content and context related to one another close to the text (137). The next step was to shorten the meaning units, to condense but keep the core. Then the meaning units were labelled into codes, with the whole context in mind. Various codes were compared based on similarities and differences individually and then we compared and discussed them together with the research group. Codes were then labelled and linked to domains based on the subject areas. According to Graneheim and Lundman (137), a domain sheds light on a specific topic of content with marginal interpretation from parts of the text. The codes were further abstracted by content, creating 11 subthemes and four themes. The themes answers the question “How?” and have a thread of an underlying meaning from meaning units to subthemes with a higher level of interpretation (137). The whole process of abstraction from codes to subthemes and themes was reflected on and discussed recurrently in the research group until agreement was reached. In this process we went back to the text several times to check that we had understood and interpreted correctly so nothing contradictory had emerged on the path to the themes that were found. The analysis process on one of the themes, “Being responsible but with a pinch of salt”, is seen in Table 6.

My contribution

I am the second author of this paper. I participated in the planning, design of the study and collection of data. I also participated actively in the analysis process and critical revision of the manuscript.

32
### Table 6. An example of the analysis process from meaning unit to subthemes and theme.

<table>
<thead>
<tr>
<th>Condensed meaning units</th>
<th>Codes</th>
<th>Subthemes</th>
<th>Theme</th>
</tr>
</thead>
<tbody>
<tr>
<td>“more careful with vegetables, fruit almost manic”, “observant out in cafés, what’s in the sandwich?”, “I buy Swedish products”, “I eat self-caught trout”, “know the origin and the handling feels healthy”</td>
<td>More observant of what is eaten. Want to know food content</td>
<td>Checking food content</td>
<td>Being responsible but with a pinch of salt</td>
</tr>
<tr>
<td>“there is much to watch out for if you want”, “but it depends on how much one absorbs”, “I have used my common sense”, “mother said, ‘In my time there was no rule like that – use your common sense.’”</td>
<td>Much to concern about. Choose what you bother about. Use common sense. Relate to the past</td>
<td>Using common sense</td>
<td></td>
</tr>
<tr>
<td>“I eat as I used to”, “eat almost as usual”</td>
<td>Eat as usual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“I watch out and eat nothing inappropriate”, “I keep away from what’s forbidden”, “it is not so awkward for me…”</td>
<td>No fuss for me</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“I take it with a pinch of salt...have avoided to some extent”</td>
<td>Take with a pinch of salt</td>
<td></td>
<td>Making exceptions</td>
</tr>
<tr>
<td>“sometimes can’t do any harm”, “I make exceptions sometimes with ham”, “small deviations in general ... follow the advice later”</td>
<td>Make a few exceptions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“I put it aside during Christmas”, “I let loose on the Christmas table...otherwise not been eating anything”</td>
<td>Put aside on grand occasions (Christmas)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“take a small slice because it’s tasty”, “if weight gain is normal you can indulge in things”</td>
<td>Want to enjoy good food</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“the body tells you and gives signals about what it wants”, “my taste has changed”, “I fancy snacks”, “I’m hungry for milk products”, “I had a need for juices, fruit and vegetables”</td>
<td>The body tells you what it wants</td>
<td>Following body signals</td>
<td></td>
</tr>
</tbody>
</table>
Methods

Statistical analyses in studies II–IV

IBM SPSS Statistics version 20/22 (IBM Corporation, New York, NY, USA) was used for all calculations in studies II–IV.

Study II

Multivariate PLS modelling was used to search for clustering of subjects and to identify factors associated with being a pregnant or reference women. PLS handles all variables, including those that co-vary, simultaneously, after auto scaling to unit variance and transformation of non-normally distributed variables, and creates a pooled point estimate for each woman. The method searches for structures between the x-swarm (here diet and some subject characteristics) and the outcomes (here being a pregnant or reference women) (139). Detailed information on variables in the independent (x-data) matrix are presented in Paper II.

Dietary variables were estimated amounts eaten per day and presented as mean with 95% confidence intervals. For alcohol median and range (minimum-maximum) was set. Categorical variables were presented in percentages. To test for differences between groups we used Student’s t-test and a Mann Whitney U test for alcohol. For categorical variables we used Pearson Chi-square test; when there were five or lower in each cell Fisher’s exact test was used. Mean values between all pregnant and reference women were adjusted for BMI, education, smoking and age group in a generalized linear model. Macronutrients (fat, protein, carbohydrates) are presented as energy percent (E%). E% means the proportion of macronutrients provided as percentages of the total energy intake. The level of significance was set at 0.01, two-sided.

Study III

A failure analysis was made of the 42 women who provided ≤ 2 samples which showed only marginal/no differences in characteristics. Levels of vitamin D were also analysed in those women who had a miscarriage, abortion or missed abortion and
the mean value was 60.5 nmol/L compared to 57.7 nmol/L in the 184 women participating in the study.

Mean value and standard deviations are presented for continuous variables. Categorical variables are presented as percentages. To compare mean values of different variables with vitamin D levels at each sampling occasion we used Student’s t-test. To investigate correlations between BMI and vitamin D levels at each sampling occasion Spearman’s correlation coefficient ($r_s$) was used. A linear mixed model was used for both univariate and multivariate analyses to test relationships between vitamin D concentrations in plasma and different factors. The vitamin D levels were not linear, for each pregnancy week the levels slightly increased. Therefore a square term of gestational week was used in the linear mixed model. The level of significance was set at 0.05, two-sided.

**Study IV**

Non-parametric statistics were used. Continuous variables were presented with median and range (minimum and maximum). Categorical variables were presented with percentage. Correlations by Spearman’s coefficient ($r_s$) were used to test relationships between the weight gained (wk 12–35) and the allopregnanolone increase (wk 12–35) and between allopregnanolone concentrations at the end of pregnancy and the weight gained (wk 12–35). The participants were divided into weight gain group low (<11 kg) or high (≥11 kg). The differences in distribution between weight increase, allopregnanolone, birth and placenta weight at each sampling occasion (e.g., wk 12, 35) were tested with a Mann Whitney $U$ test. The level of significance was set at 0.05, two-sided.

**Biochemical analyses studies III–IV**

In study III, concentrations of 25(OH)-vitamin D in EDTA-plasma were analysed with liquid chromatography-tandem mass spectrometry (LC-MS/MS). The method allows for the separation of D$_2$ and D$_3$ forms of 25(OH)-vitamin D.
In study IV, allopregnanolone in EDTA-plasma was analysed using Radio-Immuno Assay (RIA) technique, previously described in detail by Timby et al (113). To quantify the plasma samples, 0.4 mL was extracted with diethyl ether (Merck KGaA, Darmstadt, Germany). Purification and separation of allopregnanolone from cross-reacting steroids was done with celite chromatography. Allopregnanolone was measured by RIA using a polyclonal rabbit antiserum raised against 3 α-hydroxy-20-oxo-5α-pregnan-11-yl-carboxymethyl ether coupled to bovine serum albumin, provided by RH Purdy (The Scripps Research Institute, La Jolla, CA, USA) (140). The sensitivity of the assay was 25 pg. The intra-assay coefficient of variation was 6.5%, and the inter-assay coefficient of variation for the allopregnanolone assay was 8.5%.

**Ethical considerations**

The study was approved by the Regional Ethical Review Board at Umeå University, Sweden (Dno 04-171 M).

The ethical application included permission from the Data Inspectorate regarding data storage and handling of sensitive data. In study I we collected informed oral approval before the interviews were booked and information was given about protection of confidentiality due to the recorded interviews. For studies II–IV, information was given both orally and in writing about the whole procedure, confidentiality, data handling and the assurance that participants could withdraw at any time. Written consent was collected at the start in early pregnancy and also for each sampling occasion according to Biobanks routines.

**Ethical reflections**

Different ethical considerations were brought up when planning and collecting the data. One reflection concerns participation; it is possible that the women found it difficult to decline participation as the midwife also performed routine medical care. The ability to say no without any negative consequences was clearly expressed in the oral and written information. We
talked about that issue with the midwives before starting so they would be aware of different reactions. Neither I nor the other researcher were involved in the care of the women, which reduced the risk of dependence on the researcher. I have also thought of the questionnaire, which is quite extensive and could be perceived as a privacy violation, but we heard no such views. However, what came to our attention was that it was comprehensive, which could be perceived as a hassle. I would also like to bring up the food registration as a risk for concern about women’s own composition of the diet. The midwives received certain nutritional training from the researchers so they could be aware and be prepared for participants’ questions about diet. A small but still important reflection is about blood sampling and fasting, which can feel painful and tough if one has morning sickness. The staff offered women juice to drink and biscuits after sampling. Waiting times at the blood samples and other practical inconveniences were also the reason for some women to dropout after 1–2 occasions as we were told. All blood samples were frozen and stored until analysis. Any deficiencies such as low haemoglobin levels would be discovered in blood tests by the antenatal base program.
Results

Study I

Table 7 briefly presents the themes.

**Table 7.** A summary of findings in study I.

<table>
<thead>
<tr>
<th>Domains</th>
<th>Subthemes</th>
<th>Themes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary information</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gain</td>
<td>Having private talks</td>
<td>Finding out by oneself</td>
</tr>
<tr>
<td></td>
<td>Reading by oneself</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Getting guidance for deviances</td>
<td>Getting professional advice when health problems occur</td>
</tr>
<tr>
<td></td>
<td>Getting guidance for symptoms</td>
<td></td>
</tr>
<tr>
<td>Reactions to dietary</td>
<td>Being confused</td>
<td>Being uncertain</td>
</tr>
<tr>
<td>information</td>
<td>Feelings of fear and guilt</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Being monitored</td>
<td></td>
</tr>
<tr>
<td>Dietary management</td>
<td>Checking food content</td>
<td>Being responsible with a pinch of salt</td>
</tr>
<tr>
<td></td>
<td>Following body signals</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Using common sense</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Making exceptions</td>
<td></td>
</tr>
</tbody>
</table>

The themes “Finding out by oneself” and “Getting professional advice when health problems occur” were interpreted from the women’s described experiences.

**Finding out by oneself:**
“You read through these booklets you get from the midwife, but especially about diet, I think there is very little written in the material you get. So it’s mostly on the Internet you find something.”

**Getting professional advice when health problems occur:**
“My levels of iron began to decline and I heard (from the midwife) that I should eat liver paté and black pudding.”

The women were interested and wanted information and support about healthy food for themselves and for the child. They were also concerned about risks and closely followed the recommendations. However, they experienced that dietary
information was partly up to them to find out by searching in the media, talking with friends and relatives. They were given some booklets by the midwife but not enough to interpret and show how to handle their everyday food choices. When deficiencies or symptoms occurred advice was given by the midwife. The women experienced different reactions related to the information or advice according to the theme “Being uncertain”.

**Being uncertain:**
“You should eat this fish but not that one, which leads you to avoid all fish so you don’t risk eating the wrong kind, but then you don’t get enough Omega 3... so it’s a bit confusing.”

They described positive and negative experiences of feeling watched over by other people, for example what they were eating. Feelings of fear and guilt arose when eating something they should not have, related to the risk of harming the baby. Situations that could cause emotions of confusion were when dietary advice felt contradictory or different in various contexts and they didn’t know how to find out what is the right thing to do.

This theme deals with women’s ways of managing the dietary advice about what to eat and what to avoid due to health risks.

**Being responsible with a pinch of salt**
“Well, it’s like this; I have handled it with a pinch of salt, because I really don’t bother to find out about everything; at the same time, I pay attention, of course, and don’t eat anything inappropriate.”

The women talked about being stricter by following the advice particularly in the early phase of pregnancy with a more easy-going style in late pregnancy. Minor exceptions from recommendations were regarded as acceptable and mentioned in all groups, on special occasions like Christmas. They also talked about knowing the origin of the food, organic or homemade food. To listen to and follow your body signals was a way to make the right food choices; the body give signs about what they wanted to eat and preferred not to eat. Most women
said that they wanted to use their common sense in decisions about food choices, selecting among the large flow of information to make their own sensible decisions.

**Study II**

When we compared the dietary pattern of the pregnant women to the reference women, the pregnant group differed to some extent, which is illustrated in the PLS analysis (Figure 6). Clustering of participants is displayed in a score loading plot with $t[1]$ and $t[2]$ on the x- and y-axis. The two axes are significant components used for the projection, $t[1]$ and $t[2]$ with the optimal separation of the variables in the model. The differences in dietary intake between the two groups are quantified and stated in percentages and adjusted mean values, is presented in Table 8. Both groups reported a lower intake from foods of folate and vitamin D compared to the NNR (Table 9) (11). Pregnant women had lower intake of alcohol and use of tobacco/snuff than reference women.
Results

**Figure 6.** Clustering of dietary patterns for pregnant and reference women, a) <35-year-old and b) ≥35-year-old women.

**Table 8.** Dietary intake compared between groups of women.

<table>
<thead>
<tr>
<th></th>
<th>Early pregnant women</th>
<th>Reference women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multivitamin (%)</td>
<td>42.6</td>
<td>14.1</td>
</tr>
<tr>
<td>Multimineral (%)</td>
<td>17.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Iron supplement (%)</td>
<td>11.0</td>
<td>5.8</td>
</tr>
<tr>
<td>Vegetables (g/day)</td>
<td>97.7</td>
<td>144.7</td>
</tr>
<tr>
<td>Potatoes/rice/pasta (g/day)</td>
<td>167.1</td>
<td>197.9</td>
</tr>
<tr>
<td>Meat/fish (g/day)</td>
<td>103.3</td>
<td>127.0</td>
</tr>
<tr>
<td>Iron by food (mg/day)</td>
<td>13.0</td>
<td>14.5</td>
</tr>
<tr>
<td>Folate intake by food (µg/day)</td>
<td>276.5</td>
<td>312.8</td>
</tr>
<tr>
<td>Vitamin D intake by food (µg/day)</td>
<td>6.6</td>
<td>6.6</td>
</tr>
</tbody>
</table>

**Study III**

Mean levels of vitamin D increased by gestational length at each sampling occasion, 55.2, 60.2, 64.6, 54.2, 53.5 nmol/L, with the highest levels observed in late pregnancy. The distribution in categories showed that at least a third of the women had insufficient plasma levels of vitamin D (<50 nmol/L) during the study period (37.2, 38.0, 33.3, 38.0, 42.0%).

Box plots in Figure 7 visualize how median values are distributed over the study period, **a)** plasma concentrations of 25(OH)D, **b)** self-reported estimated dietary intake of vitamin D by foods. Vitamin D concentrations show a greater variation than dietary intake, which shows a more stable pattern.
Figure 7. Box plots showing total plasma concentration and estimated dietary intake of vitamin D on sampling occasions 1–5.
The linear mixed model analyses showed that gestational week, season, total energy intake, dietary intake of vitamin D, and multivitamin supplementation over the previous 14 days were factors related to vitamin D levels of the participating women. The individual patterns show that seasonal variation influenced the baseline values and longitudinal patterns of vitamin D levels during and after pregnancy.

Figure 8 visualizes how median values of plasma concentrations of 25(OH)D are distributed over the study period divided by season i.e., a) winter and b) summer. This figure shows the impact of summer season with substantially higher vitamin D levels at each sampling occasion.

Figure 9 visualizes how median values from dietary intake of vitamin D by foods are distributed over the study period divided by season i.e., a) winter and b) summer. The estimated intake by foods is quite stable, although we can notice a slight increased intake on some occasions during the study period.
Results

a) Winter season 16/9–14/4

![Box plot for winter season](image)

b) Summer season 15/4–15/9

![Box plot for summer season](image)

**Figure 8.** Box plots showing total plasma concentration of vitamin D on sampling occasions 1–5 divided by season.
Results

a) Winter season 16/9–14/4

![Box plots showing estimated dietary intake of vitamin D by foods on sampling occasions 1–5 divided by season.]

b) Summer season 15/4–15/9

![Box plots showing estimated dietary intake of vitamin D by foods on sampling occasions 1–5 divided by season.]

**Figure 9.** Box plots showing estimated dietary intake of vitamin D by foods on sampling occasions 1–5 divided by season.
Study IV

The differences in distribution showed that the group of women who gained ≥11 kg during pregnancy showed significantly higher plasma concentrations of allopregnanolone (nmol/L) in the 35th gestational week compared to the woman who gained < 11 kg (61.7 vs 45.4, \( p=0.006 \)). Spearman’s \( \rho \) (rho) showed a correlation coefficient of 0.288, (\( p=0.028 \)), indicating a positive relationship between the weight increase and the allopregnanolone increase. The correlation coefficient of 0.320, (\( p=0.016 \)), also indicating a positive relationship by correlation analysis between allopregnanolone in the 35th gestational week and weight increase.

Figure 10 shows that the increase in allopregnanolone (nmol/L) from week 12–35 was significantly larger with a weight gain of ≥ 11 kg compared women with a weight gain of <11 kg (median 36.5 vs 50.9, \( p=0.011 \)).
Figure 10. a) illustrates the increase of allopregnanolone between the two weight groups and b) illustrates the weight increase between the two weight groups. Median increase 6 and 14 kg respectively in groups low and high weight increase.
Discussion

The overall aim of this thesis is to describe dietary intake, vitamin D levels, dietary information and dietary changes, and to study the relation between allopregnanolone and weight gain during pregnancy and postpartum.

This thesis shows that pregnant women found dietary recommendations confusing and difficult to interpret and deal with. Pregnant women showed a desire for increased knowledge about suitable safe food and there were demands for more support from midwives on this issue. In early pregnancy, intake by foods rich in iron was on the low side, folate and vitamin D intake seemed to be insufficient compared to recommendations. Dietary patterns of pregnant women reflected a lower intake of vegetables, meat/fish, potatoes/rice/pasta, alcohol, and tobacco/snuff compared to reference women. This was also confirmed by low serum levels of vitamin D (<50 nmol/L) in at least a third of the participants. Vitamin D levels increased to a peak in late pregnancy and we found several factors that related to levels of vitamin D, with season being the strongest. Finally, we also found that higher weight gain was related to higher allopregnanolone levels. Allopregnanolone seems to be a factor involved in the complex mechanisms of weight increase.

In study I women described their experiences of gaining control over food content, by closely following the list of “approved” food and checking the food content when shopping or when eating in restaurants. Even at dinners with friends and among the family they were quite thorough with what they ate and conscious exceptions were occasional, as for example at Christmas. The reason for their concern was the risk of harming the child that was also expressed. This is in agreement with Lupton et al, who stated that women are expected to deliver a healthy baby by monitoring and regulating their own actions with the goal of creating a safe environment in the uterus in which nothing harmful can happen. However, risk thinking can take over and create feelings of pressure and anxiety (141), which seems to correspond with our findings even though our women described ways to handle it.
The findings about the lack of dietary advice in study I are supported by Lucas et al (142) who concluded in a systematic literature review that women generally do not receive adequate nutrition education during pregnancy. Routinely having assistance to facilitate informed decision is also questionable. The reason for that could be speculated about but according to Arrish et al (143) nutrition education content in midwifery programmes is inadequate, which leads to a lack of knowledge in midwives regarding nutrition requirements. Wennberg et al interviewed midwives who talked about their lack of knowledge on nutritional issues in the care of pregnant women in need of counselling. Midwives also had difficulties controlling or evaluating sources of information on the Internet in their ambition to support women to interpret various health information (144). Another explanation could be unclear guidelines on what should be communicated and how. In 2007, the WHO published standards for maternal and new-born care to secure nutritional education for pregnant women (145), and recently a guide for pregnancy, childbirth, postpartum and new-born care has been published by the WHO for further implementation in antenatal care (146). In a Swedish context antenatal guidelines recommend midwives to give dietary advice in early pregnancy but how the education should be given is not further specified (18).

Tradition, social environment and cultural beliefs are factors that influence eating habits. Food and its consumption culture is one of the most important factors associated with the unique culture of various ethnic groups or nations (quote from Nam et al) (147). Knudsen et al (148) found age differences and regional differences for alcohol consumption as well as for lactation plans and food intake. Emmet et al (149) found differences in nutrient intake and dietary pattern associated with maternal educational level, smoking habits, and economic aspects.

There are conflicting results in studies due to improved diet during pregnancy and lactation. Wennberg et al used a food index to compare the healthiness of reported food habits. During pregnancy women reported relatively unchanged food habits with a lack of nutritious diet. Those who reported a healthy food index at the beginning of pregnancy reported
healthy eating throughout pregnancy. After birth generally poorer diet content was reported, with less fruit and vegetables (150). Findings by Smedley et al (151) showed a positive trend towards a better diet and decreased fast food intake during pregnancy.

I believe that becoming a parent should imply opportunities to reflect on food-related issues with health care professionals who are knowledgeable and inspire confidence. Larsson found in a cross-sectional study that the Internet is a well-used source among Swedish pregnant women. The women in their study sought information on different issues related to pregnancy. The women seldom discussed the information with their midwives, however. The author suggests that midwives should take the opportunity to discuss information with the women during antenatal visits, and this is in line with the needs among the women in study I who expressed uncertainty about nutrition issues and a need for more knowledge (152). Having midwives as a guide to good nutrition is important for parents to help them to make informed choices. It should be easy for parents to find out which food is beneficial and feel confident about it. In a focus group study by Wise et al (153), pregnant adults expressed their views about the development of a nutrition intervention. A modern and youth-focused way of education was advocated that motivates and encourages behavioural changes. Methods of learning were video formats, peer discussion and hands-on cooking with active participation cooking. Education should be an expert-focused, competency-based and interactive display with simple, comfortable and affordable cooking.

The low alcohol and tobacco/snuff usage found in study II can be interpreted as adherence to current recommendations. The sufficient intake of calcium could be explained by food habits and taste depending on what you like to eat or drink, which was also expressed in the study by Wise et al (153). Branum et al found in a US study the lowest folate levels in the first trimester and the use of folic acid or iron-containing supplements in 55–60% of women in early pregnancy. In addition to supplements, the American food supply had been enriched with folic acid since 1997 (154). Both studies II and III reflect an inadequate
intake of foods with important vitamins such as folate and vitamin D. In early pregnancy (study II) intake of multivitamins was reported in less than 50% of the pregnant women. This supports the idea of a need for more knowledge and understanding among health care professionals and coming parents to prevent insufficient levels in important stages of fetal development. When divided by season, we found a low but quite stable intake of vitamin D by foods over the year, which suggests that the nutritional content is roughly unchanged among the participants. The intake among participants is comparable with Jensen et al (155), who suggest supplements for pregnant women to improve health. Plasma concentration in study III, on the other hand, was significantly higher during the summer season as expected and in line with other Swedish studies (156,157). Some dairy products in Sweden such as milk and margarine are fortified with vitamin D. A global summary of maternal and new-borns vitamin D status found a European maternal prevalence of low vitamin D levels in 57% with <50 nmol/L. For new-borns the prevalence was 73% and the concentrations between mother and child were highly correlated (86).

The iron balance requires stores of approximately 500 mg iron in the beginning of pregnancy, according to NNR (22). In late pregnancy an increasing amount of iron is transferred to the fetus. This means that an additional amount of iron is required usually in late pregnancy in order not to develop iron deficiency or even to suffer from iron deficiency anemia (24). Study II reflected an iron intake by food on the low side. In a Swedish context, severe anemia during pregnancy with related complications is fortunately uncommon. The reasons for this are many, including social welfare, good public health and access to proper antenatal care.

Results consistent with ours, as reported in a review by Blumfield et al (158), found low intake of micronutrients in developed countries. This is also supported by Emmet et al (149). It is difficult to ensure adequate intake of folate and vitamin D only from the diet depending on differences in intake of food and exposure to sunlight. Vegetables, including beans, fruit, berries, green leaves, liver, dairy products and fat fishes
Discussion

are rich in folate and vitamin D, but consumption of these food groups varies a lot between pregnant women in different countries.

In a Nordic perspective, we have quite good access to and opportunities for necessary nutritional intake as well as following food and lifestyle recommendations. Sweden is considered to have a generally high level of knowledge with respect to acquiring, understanding and using health information. This is often referred to as health literacy and facilitates the potential to make informed health decisions (159). The women in study I had what I consider to be a high educational level which they also used to make choices about food content. Although variations occur among countries, Lupatelli et al (160) conducted a multinational Internet-based study on pregnant women which showed an association between low health literacy and poor adherence to prescribed medication. The International Adult Literacy Service (161) reported that in Sweden about 10% of the adult population were at the elementary level of health literacy. According to Mårtensson and Hensing, a person with generally good health literacy can, in some situations, have this literacy lowered due to stress or unease (159). A pregnancy could be an example of such a situation when certain requirements are made on one’s lifestyle and changes to adapt to. The women in study I talked about being watched over by others in the selection of different foods. That also included their desire to find enough information about suitable food, to feel safe and make the right choices. There are many considerations that have to be made which can cause pressure on the women. Too rapid a flow of information can lead to a poorer ability to use health information received. One example is recent research which presents and considered as the “truth” but the next day is replaced by another “truth” (159). Feelings of uncertainty and confusion about what is right may come up and put one’s health literacy out of order. These are feelings that women expressed in study I. They also spoke of a need to reflect and discuss difficulties about food choices, also suggested by Mårtensson and Hensing as a tool to deal with worries (159). Areas of concern could be, for example, whether to eat fish or not, take supplements or not, carefully weighing benefits versus risks. In
the debate, pregnant women can end up confused about what is best, as they don’t want to take any risks for the baby’s sake. There are some target groups who are generally recommended to take extra supplements such as vegans and veiled women. The low levels of vitamin D we found among the pregnant women raise the question of how to achieve a sufficient vitamin D for women throughout the year.

In study IV we found that allopregnanolone levels were associated with weight increase in pregnancy. What use can health care professionals have for these findings? I hope it will give a greater understanding for women’s feelings of cravings and taste for different food. Pregnant women in study I wished more comprehensive information about food issues and how to deal with food changes which the findings in study IV can hopefully contribute to. Healthcare professionals who meet pregnant women can confirm women’s need for increased food intake with knowledge of physiological processes and at the same time point out the importance of good food choices. According to an interview study by Wennberg et al, midwives find situations very challenging when women are obese, have eating disorders or practise other diets such as vegan. Midwives then used strategies to convey their message to the women (162).

According to Atkinson, overeating is a result of increased appetite and lack of satiety which can lead to development of obesity (163). Experiences from earlier studies highlight that allopregnanolone can inhibit satiety neurons by modulating the GABA-A receptors and thereby regulate food intake (98,107). We can only speculate but not declare any causal explanation for our findings of any increased food intake in the study group, as this remains to be investigated. It is essential for infant growth that mothers increase their food intake during pregnancy. Allopregnanolone levels are normally increased during pregnancy due to synthesis by the fetus and in the placenta (101,102), with a possible influence on the feeling of satiety. Allopregnanolone, like other steroids, has favourable protecting effects for the fetus (116) but it could also be one mechanism that ensures sufficient food intake when the body needs it. A problem nowadays is that many people eat too much
energy-rich food, but may lack essential nutrients. In the richer part of the world we have access to a lot of tasty energy-rich food which may not have an optimal content of nutrients needed during pregnancy and lactation.

As mentioned above, weight gain is individual and recommendations are based on pre-pregnancy BMI. However, excessive weight gain and obesity are established as risk factors for medical complications. Risks relates to weight before conception, weight gain during pregnancy and maternal glucose metabolism during pregnancy and lactation (164). A recently published Swedish study shows that the risk of stillbirth increases linearly with increasing weight (165). Compared to two stillbirths/1000 pregnancies among women with stable BMI, the number of stillbirths is estimated at three/1000 pregnancies among women who increase their BMI by 2–4 BMI units. Adjustment was made for factors associated with increased risk of fetal death, such as smoking, low education, mother born abroad (165). In the post-pregnancy period it is important to return to the body’s normal state, rebuild body stores and return to pre-pregnancy weight. Davis et al found that short intervals between pregnancies as well as excessive weight gain are risk factors for lasting maternal obesity (166). Weight loss reduces the risk of remaining overweight. To reduce weight gain and prevent adverse outcomes, lifestyle interventions with counselling combined with weight control programmes are suggested by Phelan et al (76,167).

Methodological considerations

I have some reflections about the project that I want to highlight. This project required an extensive planning before the data collecting could be started and furthermore during the collection period. Our project also required a lot of skills and patience by all involved to complete the study in a credible way. We informed and educated the midwives and laboratory personnel at the participating health care centres. A lot of information and clarifying of routines was developed. All links in the chain had to work from data collection and further reading of the questionnaire and handling of specimens for properly handling in accordance with established procedures.
The midwives showed great commitment, which was also needed to deal with study procedures. Midwives and researchers had continuous contact with each other to reconcile how everything worked during the study period.

My pre-understanding as a midwife has naturally followed me throughout the project. It is unavoidable but important to be aware of this. During the interviews it was something I kept in mind when listening to what the women expressed and how they talked about a current issue. Staying open for unexpected thoughts and views was important, but I cannot rule out that my prior understanding affected this negatively or positively in some way. I could easily understand what situations the women talked about and it was easy to put follow-up questions.

An inductive approach is used in qualitative methods in which conclusions are drawn from observations and narrated experiences. Qualitative methods are often used in complex issues where the researcher intends to understand a phenomenon (138). In quantitative research a hypothetical-deductive approach is used to test assumptions, i.e., hypotheses. When we discuss the validity of both qualitative and quantitative studies we use different concepts, but the purpose is to evaluate the extent to which the studies have reached a trustworthy conclusion (168).

**Study I**

The reason that study I was made was that we first did a pilot study on 20 volunteer women to test our questionnaires. When we made the follow-up call for evaluation, almost all women commented that the questionnaire did not cover all issues about diet. There were more things that they liked to talk about that felt relevant for them, for example snacks between meals, questions about dietary and practical consequences in daily life.

The focus group interviews were made in 2007 but, as we know, conditions continuously change over time and have naturally changed since then. Guidelines for antenatal care and dietary recommendations are updated. The result should be interpreted with that in mind. However, increased access to information
Discussion

through the Internet can contribute to continuing confusion about how to manage diet changes in the best way. Various discussions about nutrition from different countries can be questioned by the pregnant women themselves.

Data were collected together with the first author and we alternated roles of being moderator and assistant at the interviews. We also filled in for each other when needed in order to cover all the information we were aiming for. Participants in each group were acquainted with each other, which might have made it easier for them to speak freely with one another (131).

There are some limits I would like to point out. In the written aim “attitudes” were included. In hindsight, it was an unfortunate way to express the women’s experiences, which it was our aim to study. However, it can be difficult to tell whether an experience cannot include an opinion on the subject. Also, the last sentence in paper I says: “Therefore, this study increases the knowledge about effective, preventive antenatal care”, a statement which I think may be far-reaching. Further, I can ask myself afterwards why we overruled the role of the partner and did not go further into this issue. We noticed that few women talked about their partner, which surprised us.

There is also a limited discussion about how to achieve trustworthiness in the paper. On the discussion about transferability to other contexts or other groups of people, Hamberg et al state that it is up to the reader to decide whether the results are transferable (169).

The transferability; of our results to a broader multicultural context has to be assessed with care since only Swedish-speaking women, born in Sweden, were interviewed. On the other hand, wanting the best for your child and not taking risks, having thorough information to handle diet changes can be assumed to be fundamental to all pregnant women.

Credibility highlights how well the procedure (data collection and analyses) captures the research focus. We wanted to shed light on the research question of pregnant women’s experiences
of receiving dietary information and handling food changes. It was a task for my co-researcher and me to inspire confidence, ask as much or as many open questions to follow up and deepen the discussion. The participating women knew that we were midwives, and even though we were not working in antenatal care at that time it might have prevented them from speaking freely. Credibility also means how well similarities and dissimilarities have been judged during the interpreting process and that all relevant data are included. The recurrent discussions in our research group to find an agreement improved the credibility.

**Dependability;** the degree of changes that happen over time during the data collection or analyse process can make a study unstable. Open dialogues with co-researchers are ways to handle questions of dependability (137), and we did indeed discuss this. We read the previous interviews to see whether certain areas needed more light, we discussed that and if needed put more specific questions in the next interview.

**Studies II–IV**

Both internal and external validity are discussed in this section.

*Internal validity* refers to the extent to which a study’s results reflect the true situation of the study population (168).

*External validity* refers to the extent to which a study’s results are applicable to other populations (168).

**The FFQ in relation to studies II–III;** A shortened version of the original FFQ (66 vs 84 items) was used since that is the available instrument used in the VIP. To balance the inevitable increment of underreporting, energy and nutrient intakes were adjusted by +25% to facilitate comparisons (in relation to other studies and recommendations) with what would have been obtained with the longer version.

**The FFQ instrument in general**
The VIP-FFQ has been frequently used for many years and a longer version with 84 questions has also been validated against different biomarkers and nutrients (170–172), which ensured a reliable interpretation of estimated nutrients. The VIP questionnaire is mainly focused on risk factors for diabetes and cardiovascular disease and not specially developed for pregnant women. The FFQ is not particularly designed to record certain vitamin intake, but rather to take monitor overall dietary intake. On the other hand, the FFQ contains all food groups for the general population, which includes pregnant women. A self-reported FFQ has limitations associated with recording of diet in general and the use of FFQ especially. Errors may occur depending on the precision of the information that the women reported and also instrument errors (173).

To what extent is the study group comparable with the reference group in study II?

To search for similarity between the study group and the reference group in study II, the data were collected within the same age span, in the same time period and geographical region and with the same questionnaire in both groups. The difference is that the reference women attended a health check-up and the pregnant women visited antenatal care. Further, the references answered by reflecting their diet intake in the last year and the study group the last 14 days to give an idea of the current dietary intake during pregnancy. It still cannot be ruled out that some degree of recall of the pregnant women’s previous circumstances has been biased as regards intake of healthy and unhealthy food, alcohol and tobacco. Recall bias has been found in the VIP cohort as a source of error (174). Both groups participated voluntarily and their decision to participate was probably motivated by similar concerns since the VIP also carries out research which is well known in Västerbotten. I believe that this difference did not affect the outcome significantly.

The ambition was to invite a sufficient number of participants in the cohort, representing a socio-economic and geographical cross-sectional sample, based on a power calculation. A potential selection bias may be present in study II as 60% of the pregnant women had a university degree compared to 48% for
the references. That may be a result of a skewed selection of antenatal clinics or women declining participation, which they are fully entitled to do, and also a consequence of a consecutive recruitment method. A failure analysis was conducted in study III in order to draw fair conclusions. In study II, by presenting the characteristics of both pregnant women and references the reader can also assess the generalizability of the data/results (168).

The representativeness both within and outside the respective population
In study II participants were divided into two age groups (<35 and ≥35-year-old women) to illustrate any differences in food habits depending on age in both groups. The overall differences in evaluated nutrient intake between references and pregnant women were not so great. One explanation is that women were in an early phase of pregnancy when adaptation to pregnancy changes are in progress. It is also possible that food habits during this stage of life do not change so much and continue almost as usual. Exceptions, such as differences in intake of alcohol, tobacco/snuff and supplements show that the need for immediate situation-specific adaptations is observed. The size of the cohort is considered sufficient for the results to be transferable to other populations living under similar conditions. The study group in study IV was quite small. A strength was that we were able to demonstrate significant correlations even though a larger study group would have been preferable. Information on food intake was not included in the study, which could have been a possible explanatory factor for allopregnanolone.

The project design was to follow each woman individually with repeated sampling for approximately 1.5 years, monitor changes in dietary intake, plasma vitamin D levels and allopregnanolone levels in plasma. There were quite a few pregnant women who ended their participation in general; some dropouts were inevitable as a result of moving or having a miscarriage. Variations by dietary intake have been reported, and blood levels were measured in all seasons of the year, which is a strength. The study group was ethnically homogenous, which limits bias due to skin pigmentation and clothing habits which
could have affected the levels of vitamin D for example. **Reliability:** the repeated measurements of vitamin D indicated a steady intake over the year, which can be seen as a credible result. The allopregnanolone levels in our sample followed the same increased level as in other comparable studies (99,100) which supports the reliability of the measurements and results. I believe that the results in study III could be transferred to pregnant women living in similar environments. In study IV it is likely that weight increase relates to increased allopregnanolone levels in pregnant women in other populations as well.

**Standardization and controlling for confounding variables**
Potential confounding variables were statistically adjusted for in study II and study III by regression models. In study II, the divided age groups were standardized as mentioned above, to adjust for differences in food habits by age and because of the imbalance in the different groups. Energy was standardized to specify E% by macronutrients. Other nutrients were standardized by residuals from regression which is a form of energy standard that explains how much of a nutrient is ingested in relation to all energy intake, described in detail by Willett (173). The use of residuals was intended to circumvent potential errors from underreporting or overreporting, as underreporting has been found due to the instrument in smoking, low education and high BMI (175). In study IV there was no control for confounding variables as this was a descriptive study and the purpose was not to explain any factors that cause either weight gain or allopregnanolone concentrations.

**The biochemical analyses**
In study III the method used to analyse levels of vitamin D<sub>2</sub>, D<sub>3</sub> and total vitamin D level (25(OH)D) is regarded as the gold standard LC-MS/MS (Perkin Elmer, Waltham, MA, USA). The current method reduces the problems usually found with immunological methods. The analytical reliability was ensured by external controls from DEQAS.

In study IV, Radio Immuno Assay (RIA) for the measurement of allopregnanolone was used, described and referred to in the method section. RIA has been validated versus GC-MS/MS (gas
chromatography tandem mass spectrometry) and LC-MS/MS (liquid chromatography tandem mass spectrometry) (99).
Conclusions

I. Pregnant women were eager to know about healthy diet to reduce the risks of eating something “forbidden”. They searched for information by themselves and could manage dietary changes quite well. They described dietary recommendations as confusing as they felt inadequate and contradictory and would have wished for more comprehensive advice from the midwife.

II. Early pregnant women had a different dietary pattern to some extent, with more supplements, less alcohol and tobacco/snuff compared to reference women. Intakes of folate and vitamin D by food were low compared to recommendations and intake of iron was on the low side.

III. Vitamin D levels peak in late pregnancy but levels were <50 nmol/L in at least a third of the participants. Aside from gestational week and season which were related to plasma levels, intake from foods and supplements also affected the levels.

IV. Allopregnanolone was associated with weight gain during pregnancy.

Clinical implications and future research

The findings in this thesis suggests that midwives in primary care have an important role to support and educate pregnant women about healthy eating habits and to improve dietary intake.

Obesity and excessive weight gain during pregnancy is an increasing problem. Further studies are needed to describe the role of allopregnanolone during pregnancy and the postnatal period.
Based on this thesis I have some suggestions for future research:

- Longitudinal studies on dietary intake during pregnancy and postpartum period including subgroups in a multicultural context.
- Exploring the role of allopregnanolone, its significance and consequences during pregnancy including measurement of food intake.
- Investigate experiences from a family perspective of the pregnant woman, with respect to dietary intake as well as eating habits.
- Exploring fetal outcome in relation to maternal dietary parameters.
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71


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82


