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"Assessment of the micronutrient status of Austrian pregnant women via chemical analysis of spot urine samples."

verfasst von / submitted by

Christian Luipersbeck, BSc

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Wien, 19.12.2023 Christian Luipersbeck

Ort, Datum Unterschrift

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List of abbreviations

µg – microgram(s)
µL – microliter(s)
ANC – antenatal care
ANR – Austrian Nutrition Report(s)
APO – adverse pregnancy outcome(s)
BMI – body mass index
Ca – calcium
Cl – chloride
CRM – certified reference material
CV – coefficient of variation
d – day
D-A-CH – D: Deutschland/Germany, A: Österreich/Austria, CH: Confœderatio Helvetica/Switzerland
DGE – German Nutrition Society
dL – deciliter(s)
DL – detection limit(s)
DNA – deoxyribonucleic acid
EFSA – The European Food Safety Authority
ESÖSM - Ernährungsstatus von schwangeren gebürtigen Österreicherinnen im Vergleich mit Frauen mit Migrationshintergrund
Fe - iron
FFQ – food frequency questionnaire
g – gram(s)
GDM – gestational diabetes
GRADE – Grading of Recommendations Assessment, Development and Evaluation
GW – gestational week
H_2SeO_3– selenous acid
H_2Se – hydrogen selenide
HCl – hydrochloric acid
HELLP – hemolysis, elevated liver enzymes, low platelet count
Hg – mercury
HNO_3 – nitric acid

I – iodine

ICD-11 – International Classification of Diseases 11th Revision

IFA – iron and folic acid (supplementation)

IQR – interquartile range

ISA – ionic strength adjustment

ISE – ion selective electrode

$KClO_3$ – potassium chlorate

L - liter

LBW – low birthweight

LGA – large for gestational age

LMIC – lower- and middle-income countries

mg – milligram(s)

Mg – magnesium

mL – milliliter(s)

mm – millimeter(s)

MMS - multiple micronutrient supplements

mV – millivolt

Na – sodium

$NaBH_4$ – Sodium borohydride

ng – nanogram(s)

PB – preterm birth/premature birth

PE – pre-eclampsia

PIH – pregnancy induced hypertension

PIHD – pregnancy induced hypertensive disorders

ppm – parts per million

ppt – parts per trillion

PTFE – Polytetrafluoroethylene

RCT – randomized controlled trial(s)

RNA – ribonucleic acid

SD – standard deviation

Se - selenium

SGA – small for gestational age

UIC – urinary iodine concentration

WHO – World Health Organization

Zn - zinc

1. Scope and Purpose

Pregnancy is considered a vulnerable period for both the mother and the unborn child. A diet should contain appropriate amounts of all nutrients necessary which can be challenging due to higher maternal and fetal needs. According to the WHO (World Health Organization), these increased needs can often not be met and may result in micronutrient deficiencies which can negatively affect the health of both the mother and the fetus. (1)

As it is important to ensure that pregnant women have a healthy micronutrient supply status, the aim of this master thesis is to assess the nutritional status of the dietary minerals iron, iodine, zinc, selenium, sodium, chloride, calcium, and magnesium for Austrian pregnant women through chemical analyses of urine samples. This thesis will evaluate if analysis of spot urine samples can be an efficient way for the determination of the micronutrient status of a population group and answer the question of how many pregnant women in Austria exhibit micronutrient deficiencies. For this purpose, urine levels will be compared with data on the intake of micronutrients recorded via self-reporting measures.

To determine the nutritional status of pregnant women in Austria, urine samples of 53 pregnant women, previously collected for a project titled “Ernährungsstatus von schwangeren gebürtigen Österreicherinnen im Vergleich mit Frauen mit Migrationshintergrund" (ESÖSM)” were analyzed.

Relevance and functions of dietary minerals during pregnancy, recommendations for the intake and possible consequences of the nutritional status on adverse pregnancy outcomes (APO) will be discussed in detail within the literature review of this master thesis. Additionally, evidence of possible beneficial effects of supplementation on these outcomes will be summarized and discussed.

2. Literature review

2.1. Introduction

Maternal over- and undernutrition can negatively affect the health of the mother and their babies and increase the risk of adverse pregnancy outcomes. Nutritional interventions may reduce the repercussions of these circumstances. The WHO recommendations on antenatal care (ANC) for a positive pregnancy experience published in 2016 has the objective to improve the health care for women during pregnancy to achieve a positive pregnancy experience. ANC is defined as care given to pregnant women and adolescent girls by health-care professionals to maintain the health of mother and baby.

In this guideline the WHO evaluated the evidence on dietary and nutritional interventions, including supplementation of dietary minerals during pregnancy, and their possible beneficial effects on pregnancy outcomes.

Other recommendations regarding “maternal and fetal assessment, preventive measures, interventions to improve the utilization and quality of ANC”, etc. are also described in the guideline. (2) All recommendations not specifically addressing micronutrients are out of scope of this thesis and will not be discussed in this review. A summary of all recommendations for nutritional interventions are shown in **Table 1**.

A clear recommendation is given by the WHO only for the supplementation of iron and folic acid (IFA). Every other recommendation for micronutrients is “context-specific”. For most of the nutritional interventions, this means that the intervention should only be applied in deficient or undernourished populations. (2)

As iron deficiency in mothers is the most prevalent micronutrient deficiency during pregnancy worldwide, supplementation with iron is considered essential and recommended by the WHO since the 1950s. (3)

Daily folic acid is also a routine antenatal supplement to prevent neural tube defects. Therefore, both micronutrients are often given as a combined IFA supplementation. (1)

Table 1: Summary list of WHO recommendations on antenatal care for a positive pregnancy experience. Modified from source (2).

These recommendations apply to pregnant women and adolescent girls within the context of routine ANC

A. Nutritional interventions		
	Recommendation	**Type of recommendation**
Dietary interventions	**A.1.1:** Counselling about healthy eating and keeping physically active during pregnancy is recommended for pregnant women to stay healthy and to prevent excessive weight gain during pregnancy.[a]	Recommended
	A.1.2: In undernourished populations, nutrition education on increasing daily energy and protein intake is recommended for pregnant women to reduce the risk of low-birth-weight neonates.	Context-specific recommendation
	A.1.3: In undernourished populations, balanced energy and protein dietary supplementation is recommended for pregnant women to reduce the risk of stillbirths and small-for-gestational-age neonates.	Context-specific recommendation
	A.1.4: In undernourished populations, high-protein supplementation is not recommended for pregnant women to improve maternal and perinatal outcomes.	Not recommended
Iron and folic acid supplements	**A.2.1:** Daily oral iron and folic acid supplementation with 30 mg to 60 mg of elemental iron[b] and 400 µg (0.4 mg) of folic acid[c] is recommended for pregnant women to prevent maternal anaemia, puerperal sepsis, low birth weight, and preterm birth.[d]	Recommended
	A.2.2: Intermittent oral iron and folic acid supplementation with 120 mg of elemental iron[e] and 2800 µg (2.8 mg) of folic acid once weekly is recommended for pregnant women to improve maternal and neonatal outcomes if daily iron is not acceptable due to side-effects, and in populations with an anaemia prevalence among pregnant women of less than 20%.[f]	Context-specific recommendation
Calcium supplements	**A.3:** In populations with low dietary calcium intake, daily calcium supplementation (1.5–2.0 g oral elemental calcium) is recommended for pregnant women to reduce the risk of pre-eclampsia.[g]	Context-specific recommendation
Vitamin A supplements	**A.4:** Vitamin A supplementation is only recommended for pregnant women in areas where vitamin A deficiency is a severe public health problem,[h] to prevent night blindness.[i]	Context-specific recommendation

a. A healthy diet contains adequate energy, protein, vitamins and minerals, obtained through the consumption of a variety of foods, including green and orange vegetables, meat, fish, beans, nuts, whole grains and fruit.
b. The equivalent of 60 mg of elemental iron is 300 mg of ferrous sulfate hepahydrate, 180 mg of ferrous fumarate or 500 mg of ferrous gluconate.
c. Folic acid should be commenced as early as possible (ideally before conception) to prevent neural tube defects.
d. This recommendation supersedes the previous recommendation found in the WHO publication *Guideline: daily iron and folic acid supplementation in pregnant women* (2012).
e. The equivalent of 120 mg of elemental iron equals 600 mg of ferrous sulfate heptahydrate, 360 mg of ferrous fumarate or 1000 mg of ferrous gluconate.
f. This recommendation supersedes the previous recommendation in the WHO publication *Guideline: intermittent iron and folic acid supplementation in non-anaemic pregnant women* (2012).
g. This recommendation is consistent with the *WHO recommendations for prevention and treatment of pre-eclampsia and eclampsia* (2011) and supersedes the previous recommendation found in the WHO publication *Guideline: calcium supplementation in pregnant women* (2013).
h. Vitamin A deficiency is a severe public health problem if ≥ 5% of women in a population have a history of night blindness in their most recent pregnancy in the previous 3–5 years that ended in a live birth, or if ≥ 20% of pregnant women have a serum retinol level < 0.70 µmol/L. Determination of vitamin A deficiency as a public health problem involves estimating the prevalence of deficiency in a population by using specific biochemical and clinical indicators of vitamin A status.
i. This recommendation supersedes the previous recommendation found in the WHO publication *Guideline: vitamin A supplementation in pregnant women* (2011).

Table 1: Summary list of WHO recommendations on antenatal care for a positive pregnancy experience, continuation. Modified from source (2).

Zinc supplements	**A.5:** Zinc supplementation for pregnant women is only recommended in the context of rigorous research.	Context-specific recommendation (research)
Multiple micronutrient supplements	**A.6:** Multiple micronutrient supplementation is not recommended for pregnant women to improve maternal and perinatal outcomes.	Not recommended
Vitamin B6 (pyridoxine) supplements	**A.7:** Vitamin B6 (pyridoxine) supplementation is not recommended for pregnant women to improve maternal and perinatal outcomes.	Not recommended
Vitamin E and C supplements	**A.8:** Vitamin E and C supplementation is not recommended for pregnant women to improve maternal and perinatal outcomes.	Not recommended
Vitamin D supplements	**A.9:** Vitamin D supplementation is not recommended for pregnant women to improve maternal and perinatal outcomes.[j]	Not recommended
Restricting caffeine intake	**A.10:** For pregnant women with high daily caffeine intake (more than 300 mg per day),[k] lowering daily caffeine intake during pregnancy is recommended to reduce the risk of pregnancy loss and low-birth-weight neonates.	Context-specific recommendation

j. This recommendation supersedes the previous recommendation found in the WHO publication *Guideline: vitamin D supplementation in pregnant women* (2012).
k. This includes any product, beverage or food containing caffeine (i.e. brewed coffee, tea, cola-type soft drinks, caffeinated energy drinks, chocolate, caffeine tablets).

In 2020 an updated version of the ANC recommendations guideline was published by the WHO with the focus on the potential benefits of multiple micronutrient supplements (MMS) during pregnancy and comparing them with the standard and recommended IFA supplements. The new guideline is based on a Cochrane review that was also updated in 2019 to include new evidence.

The recommendation for MMS including IFA changed from “not recommended” in the ANC guideline of 2016 to a recommendation “in the context of rigorous research”. The WHO states that this research should include high-quality methods such as controlled clinical trials to determine gestational age, maternal and perinatal outcomes and research evaluating the repercussions of switching from IFA to MMS.

The updated Cochrane review found no significant beneficial effects of MMS regarding maternal, fetal, or neonatal outcomes, except a slightly more pronounced reduction in the outcome low birthweight. No data on the maternal pregnancy outcomes preeclampsia/eclampsia, gestational diabetes mellitus or infections were present in the review and subsequently no recommendations regarding those outcomes were given by the WHO in their guideline. All randomized controlled trials (RCT) in the review were performed in lower- and middle-income countries (LMIC). (1) According to the WHO, pregnant women in LMIC are especially susceptible to nutritional deficiencies as the dietary intake is

often insufficient to meet the increased needs. (2) Therefore, it is questionable how the results can be transferred to populations of high-income countries which are often not at risk of deficiencies.

In both ANC guidelines, the WHO defined the outcomes as relevant to nutritional interventions that are shown in **Table 2**. (1)

Table 2: Adverse pregnancy outcomes relevant to nutritional interventions. From source (1).

Box 1. ANC nutritional interventions outcomes of interest

Maternal outcomes	Fetal/neonatal outcomes
Infections	Neonatal infections
Anaemia	Small for gestational age
Pre-eclampsia/eclampsia	Low birthweight
Gestational diabetes mellitus	Preterm birth
Mode of delivery	Congenital anomalies
Excessive weight gain	Macrosomia/large for gestational age
Side effects	Fetal/neonatal mortality
Maternal mortality	
Maternal satisfaction	

2.2 Adverse pregnancy outcomes:

In this chapter definitions will be given for adverse pregnancy outcomes for which evidence will be reviewed in **chapter 2.3** and **chapter 2.4**. Effects of the nutrient status/deficiency or supplementation/intake of dietary minerals on possible APO will be summarized in **Table 4** and **Table 5** for trace elements and in **Table 6** and **Table 7** for macro elements.

2.2.1 Maternal outcomes:

Infections:

Maternal infections are defined as infectious and parasitic diseases that complicate pregnancy, childbirth or puerperium. (4)

Anemia:

The ICD-11 (International Classification of Diseases 11th Revision) describes anemia complicating pregnancy, childbirth, or the puerperium as “a condition of the circulatory system affecting pregnant females, characterised by a

haemoglobin level below 11 grams per decilitre". (4) The definition in the WHO ANC guideline of 2016 places more emphasis on the trimesters. Anemia during pregnancy is diagnosed if the hemoglobin concentration is less than 110 g/L during the first and third trimester or less than 105 g/L during the second trimester. Anemia is a major global health problem and severe anemia is associated with maternal and infant mortality. (2)

Pre-eclampsia/eclampsia:

Pre-eclampsia (PE) and eclampsia are hypertensive disorders which are major causes of maternal mortality and morbidity.

The criteria for the diagnosis of pre-eclampsia are a new episode of hypertension characterized by a systolic blood pressure above 140mm Hg (mercury) and/or a diastolic blood pressure at or higher than 90 mm Hg, measured twice with at least four hours apart, in addition to the onset of proteinuria or another maternal organ dysfunction. (4) Substantial proteinuria is defined by the WHO as a protein level above 0.3 g/24h. (5) Eclampsia in pregnancy is characterized by a new onset of seizures and convulsions in pregnancy accompanied with anxiety, epigastric pain, severe headaches, blurred vision, and edema (4) often in addition to the already existing pre-eclampsia symptoms. (5)

Gestational diabetes mellitus:

According to the definition in the ICD-11, gestational diabetes mellitus (GDM) is any degree of glucose intolerance which was first detected during pregnancy regardless of whether it is treated by administering insulin or via diet modifications or if the condition persist after pregnancy. (4)

However, in the ANC guidelines of 2016, the WHO defines "diabetes mellitus in pregnancy" as hyperglycemia that is more severe and persists after pregnancy whereas the hyperglycemia of GDM will recede.(2)

As the ICD-11 classification is more current than 2016 ANC guideline, the understanding regarding the persistence of hyperglycemia in GDM may have changed in recent years.

2.2.2 Fetal/neonatal outcomes:

Small for gestational age:
The outcome small for gestational age (SGA) is defined when a mother gives birth to an infant with a birth weight below two standard deviations of the mean or the 10th percentile following intrauterine growth charts. (4)

Low birthweight:
Low birthweight (LBW) is defined as a child born with a weight between 1500 and 2499 g. (4) The outcome LBW is associated with infant morbidity and mortality and is a major public health problem more common in LMIC (6).

Preterm birth:
The outcome preterm birth or premature birth (PB) is defined as a birth after less than 37 completed weeks (or less than 259 days) of pregnancy. (4)
PB is associated with neonatal mortality. (2)

Macrosomia/large for gestational age:
The ICD-11 description for macrosomia is when the neonate is exceptionally large having a birth weight of > 4500 g, regardless of gestational age. A large newborn for gestational age (LGA) is an infant born with a birth weight between 4000 and 4499 g or above the 90th percentile for gestational age. (4) Various definitions for macrosomia/LGA can be found in the literature. Sometimes it is just described as “high birth weight”. (2)

Fetal/neonatal mortality:
The outcome fetal death is defined as the death of the fetus prior to its birth, irrespective of pregnancy duration. Another outcome resulting in fetal death is miscarriage/spontaneous loss which is defined as a loss of pregnancy before the completed 22nd gestational week. Stillbirth will also be added to these outcomes, which is defined in the ICD-11 as the complete expulsion or extraction of the fetus following its death prior to delivery. (4) There are various definitions for stillbirth, neonatal, perinatal mortality, etc. in the literature which also overlap to some degree. (7, 8) Therefore, all causes that result in the death of the baby will be included in this outcome.

In the next chapter, this master thesis will review the importance of all dietary minerals, that were selected for chemical analysis. Then the evidence of the nutrient status and supplementation on adverse pregnancy outcomes will be discussed, with a focus on the ones given in **Table 2**. Evidence will be summarized after each chapter.

2.3. Trace Elements:

2.3.1. Iron:

Iron (Fe) is a component of hemoglobin, myoglobin, and enzymes such as cytochromes and ribonuclease which are responsible for oxygen and electron transport as well as their storage and are also involved in redox reactions. The majority or iron in the human body (60%) is bound to hemoglobin. The rest is bound to ferritin which stores and transports iron, to hemosiderin, myoglobin, or a component of the enzymes mentioned before.

Even though during pregnancy the blood and iron loss resulting from menstruation is halted, the daily recommendation for pregnant women is increased. This is because there are considerable additional requirements of iron for the fetus, the placenta, and due to the increased maternal blood volume. Therefore, the daily recommendation is doubled, from 15 mg/d (milligrams per day) for non-pregnant and non-lactating women below the age of 50, to 30 mg per day for pregnant women. This increased demand cannot be reached by the diet alone. (9)

The bioavailability of heme iron which is in animal products is above 20% whereas the absorption of non-heme iron in plant-based foods is impaired by substances such as phytates (found in whole grain foods, legumes, seeds), polyphenols like tannins (in coffee, tea, legumes), some proteins (soy products) and calcium (dairy products). Calcium inhibits both the absorption of non-heme and heme iron. (10)

The major contributors to the iron intake are meat and sausage products but also bread and vegetables can be important sources if fruits containing ascorbic or citric acid are also consumed which increases the absorption of non-heme iron.(9)

Nutrient status/deficiency:

Iron deficiency is the most common micronutrient deficiency during pregnancy. It is causing anemia and negatively affecting 40% of pregnant women worldwide.(3)

Regarding the prevalence of iron deficiency in Austria, a study published in 2021 by Zeisler et al. measured the serum ferritin level of 425 pregnant women. The cutoff point to detect iron deficiency was set to 10 µg/L of serum ferritin which was also used in the Austrian Nutrition Report (ANR) of 2012. Using this cutoff point Zeisler et al. detected a prevalence of 12,17% of iron deficiency for women at their first visit which was considerably lower than the 17.2% that were found in the ANR 2012. The prevalence of iron deficiency was also shown to increase significantly throughout the duration of pregnancy. Women with a gestational age of 38 weeks or above were found to have a prevalence of 41.76%. (11)

In the ANR of 2017, it was shown that 85.1% of 19 to 24-year-old women and 84.8% of 25 to 50-year-old women did not reach the recommendations for the iron intake. (12)

For the prevalence of anemia, a study conducted in Styria, a federal state in Austria, was researching pregnant women aged 15 to 45 between 2006 to 2014. Hemoglobin levels were measured once before the 16th week of gestation and then between week 25 and 28 for the second time. During the study, anemia, defined as a hemoglobin level below 11 g/dL, was detected in either measurement in 13.7% of all pregnancies. (13) According to the WHO, an anemia prevalence between 5 and 19% is considered a “mild public health problem”. (14)

Iron deficiency negatively affects the functions of the immune system, muscle activity and work performance (2) as well as disrupts the thermoregulation and increases the risk of contracting malaria. (9) Anemia during pregnancy can lead to various APO. A systematic review and meta-analysis found that maternal anemia is associated with low birthweight infants. (6)

Another systematic review revealed that maternal anemia increases the risk of premature birth. Subgroup analyses showed that this association was significant for the first trimester, but not for the second and third trimester. The authors conclude that maternal anemia in the first trimester is a risk factor for premature birth and that this relationship is more pronounced in developing countries. (15)

A systematic review done by Young et al which included 95 observational studies in their meta-analyses researched the influence of maternal hemoglobin concentration during pregnancy on several maternal and infant APO. A low maternal hemoglobin level which was defined as a concentration lower than 110 g/L was shown to be significantly associated with preterm birth, LBW and SGA

neonates. The authors also found significantly increased odds for stillbirth, neonatal and perinatal mortality.

Regarding maternal outcomes, the meta-analyses showed a significant association between low maternal hemoglobin levels and higher odds of PE and postpartum hemorrhage and a nonsignificant trend for reduced odds of gestational diabetes. It was also shown that high maternal hemoglobin levels (> 130 g/L) are associated with the outcomes SGA, stillbirth, PE, and GDM. For other adverse pregnancy outcomes, not enough studies were identified in the systematic review, so no meta-analyses were conducted. (7)

Supplementation/intake:

Iron supplementation is often given in combination with folic acid which is recommended to prevent anemia and neural tube defects. Daily iron supplementation may also reduce the risk of PB and LBW infants and reduce the chance for maternal infections. The WHO recommends daily oral supplementation of 30 mg to 60 mg of iron for prevention of adverse pregnancy outcomes. (2). For populations with a prevalence of anemia of 40% or higher, supplements containing 60 mg of iron are recommended. (1)

The systematic review conducted by Pena-Rosas et al. which was used by the WHO as evidence to derive the recommendation for iron supplementation from, concluded that iron supplementation was able to reduce the risk of maternal anemia and iron deficiency but beneficial effects on other adverse pregnancy outcomes for mothers and infants were unclear. Associations between iron supplements and LBW neonates, PB and neonatal deaths were nonsignificant. This review included 61 RCT conducted in low-, middle- and high-income countries in the meta-analyses. (16)

A systematic review that was published five years later which included 72 trials for the meta-analyses researched the effects of supplementation of various dietary minerals during pregnancy compared with placebo. Regarding iron supplementation, the authors found significant associations with maternal anemia and having a LBW infant. No significant effects were found for the outcomes perinatal mortality, neonatal mortality, and PE. This systematic review conducted by Oh et al. included only studies done in LMIC (17) in which according to the WHO, iron deficiency is more common among the populations. (2)

As described earlier, iron deficiency is affecting considerably less pregnant women in Austria than pregnant women worldwide. According to the German Nutrition Society (DGE) and The Healthy Start – Young Family Network, iron supplementation is only recommended, after iron deficiency was diagnosed by health professionals. Additional iron intake can increase the risk for pregnancy complications if pregnant women are already adequately supplied. (18)

This is in accordance with a guideline for the care of healthy pregnant women which was published by the "Österreichisches Hebammengremium" in 2019 which is the representative body for midwives in Austria. The guideline recommends a screening for anemia as early as possible in pregnancy. If anemia is detected, following the WHO criteria in **chapter 2.2**, supplementation should be started. Women without diagnosed anemia should refrain from taking iron supplements. (19) However, a study published in 2019 by Spary-Kainz et al. showed that the actual situation in Austria is different. 325 pregnant women completed a survey including questions on their current supplementation and if they had been diagnosed with anemia. Among the 287 participants who were not diagnosed with anemia before becoming pregnant, 185 (64.5%) reported taking a supplement containing iron, the majority of which was recommended by their physicians.

The authors concluded that these recommendations for iron supplementation may not be evidence-based but possibly influenced by marketing strategies, as women not diagnosed with iron deficiency must pay for the supplements themselves. (20)

2.3.2 Iodine:

Iodine (I) is a component of the thyroid hormones and connected to the functions of selenium via the selenium-containing deiodinases. Most of the iodine in the human body is inside of the thyroid gland. (9)

During pregnancy, the iodine requirements are increased as the transfer of iodine and thyroid hormones to the fetus is essential for its development. (21) Additional iodine is also required due to the increased renal blood flow and subsequently a higher excretion of iodine through the urine. Therefore, the recommendations for the iodine intake for pregnant women in Austria are 230 micrograms per day.

There are considerable variations in the iodine content in foods, as the amount of iodine in plant-based and animal products is dependent on the iodine concentration in the soil and in animal feed. Foods rich in iodine are salt-water fish and sea food as well as dairy products and eggs if iodine was added to the animal

feed. In Austria, iodization of salt was able to improve the iodine status of the population. (9)

Nutrient status/deficiency:

As pregnancy is a period of rapid growth, pregnant women and especially the fetus are very vulnerable to the effects of iodine deficiency. Iodine deficiency during pregnancy leads to decreased thyroid hormone synthesis and subsequently decreased transfer of hormones to the fetus. Deficiency during the early phase of pregnancy impairs its neurological development. Other disorders associated with maternal iodine deficiency are abortion, stillbirth, or perinatal mortality. According to the WHO the best measure to combat iodine deficiency is universal salt iodization. However, iodization of salt is only voluntary in most European countries which decreases the effectiveness. (22)

For the situation in Austria, the ANR 2017 showed that the iodine intake of 87.1% of participants did not reach the D-A-CH recommendations (D-A-CH stands for D: Deutschland/Germany, A: Österreich/Austria and CH: Confœderatio Helvetica/Switzerland). The authors also added that an exact calculation of the iodine intake is not feasible as no data on the use of iodized salt in food manufacturing or out-of-home consumption are available yet. (12)

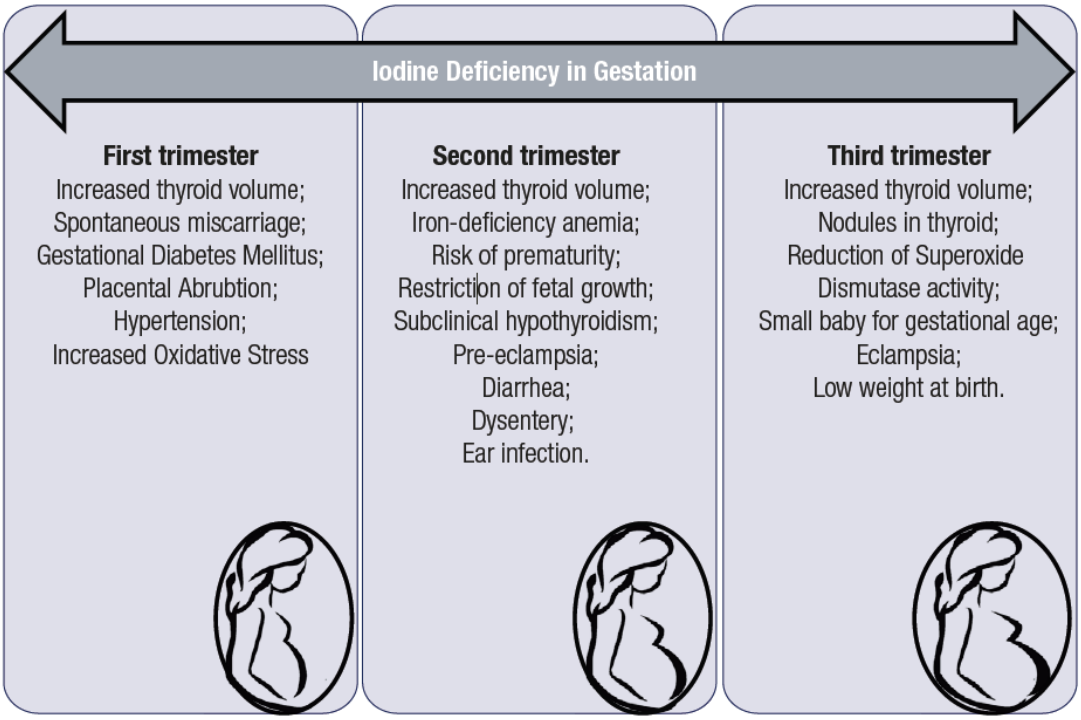
A study by Lindorfer et al. published in 2014 researched the iodine status of 246 Austrian pregnant women by analyzing spot urine samples collected in the morning. The authors found a median UIC (urinary iodine concentration) of 87.0 µg/L which indicated a “mildly insufficient iodine intake”. (23)

Urinary iodine concentration is an effective indicator to determine the iodine status of populations. According to the WHO, the iodine intake of pregnant women is considered “insufficient” if the median UIC is below 150 µg/L. The median is used, as iodine levels in the urine tend to not follow a normal distribution. (24) Lindorfer et al. found that the UIC for 81.2% of the participants was below 150 µg/L and concluded that pregnant women in Vienna showed a “potentially clinically significant iodine deficiency”. The authors also stated that recommended doses of iodine supplementation may not be insufficient and pregnant women should start iodine supplementation before pregnancy. (23)

Regarding the effects of iodine deficiency in pregnancy, a systematic review researched negative pregnancy outcomes for all trimesters. The authors included

11 observational studies and summarized the outcomes investigated in the included studies. All identified APO are shown in **Figure 1**.

Figure 1: Implications caused by iodine deficiency in each gestational trimester. From source (25).



According to the authors, negative effects of iodine deficiency vary across the trimesters, are still reversible in the first or second trimester and therefore need to be diagnosed early to prevent damage to the health of mother and child. (25)

A systematic review investigated the influence of iodine status of pregnant women with normal thyroid function on adverse pregnancy outcomes. The meta-analysis which included six cohort studies, found, that the iodine levels of euthyroid pregnant women measured in the first half of pregnancy were not associated with the prevalence of the adverse pregnancy outcomes PB, LBW or hypertensive disorders. The authors stated that the association between iodine deficiency causing maternal hypothyroidism and adverse pregnancy outcomes is very strong. This study showed that this is not the case for pregnant women in an euthyroid state which means that iodine deficiency in the first half of pregnancy does not affect pregnancy outcomes independently of the thyroid state. The authors concluded that the iodine status alone is an insufficient indicator for predicting APO. (26)

Supplementation/intake:

A Cochrane review by Harding et al. researched the effects of iodine supplementation that was administered preconceptual, during pregnancy or postpartum on several maternal and infant outcomes. In the meta-analyses which included eleven randomized controlled trials no significant effects of iodine supplementation on the adverse pregnancy outcomes spontaneous miscarriage, PB, perinatal mortality, LBW and SGA were found.

The authors concluded that data were insufficient to make any conclusions regarding the positive or negative effects of iodine supplementation before conception, during pregnancy or postpartum. (27)

The overview conducted by Croce et al. included additionally to the one done by Harding et al. two other meta-analyses on iodine supplementation, both of which found no effects of iodine on any researched adverse pregnancy outcomes. The authors stated that there is some evidence on the correlation between iodine status of pregnant women and adverse maternal and neonatal outcome, but this is not the case for supplementation. According to Croce et al. the main problem is that the majority of studies were conducted on pregnant women with only mild to moderate iodine deficiency and future supplementation studies should assess the baseline iodine status. (28)

However, a randomized controlled trial comparing iodine supplementation with a placebo in a population that is known to be severely iodine deficient may be unethical. (27)

2.3.3. Zinc

The trace element zinc (Zn) is bound to thousands of enzymes such as alcohol dehydrogenase, oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases and is imperative for various functions in the human metabolism. Zinc is also important for the storage of insulin in the pancreas as well as a component of transcription factors and therefore essential for regulation of gene expression.

The absorption of dietary zinc is dependent on the content of phytate in the diet which inhibits the uptake of zinc. Foods which include a high amount of phytate are whole grain cereals and legumes. There are different recommendations for the daily intake of zinc in relation to the amount of phytate consumed.

During pregnancy there is a higher demand of zinc due to the growth of the fetus and the uterus, the increased blood volume and the formation of the placenta

which is partially compensated through an increased absorption rate. To warrant a sufficient zinc supply during pregnancy, an additional total intake of 100 mg of zinc over the whole pregnancy duration is recommended. Considering the weight gain during pregnancy, the zinc recommendations for pregnant women are shown in **Table 3**.

Table 3: Zinc recommendations for pregnant women for each trimester and different intakes of phytate. Modified from source (29).

	Low phytate	**Medium phytate**	**High phytate**
1st trimester	7 mg/d	9 mg/d	11 mg/d
2nd trimester	9 mg/d	11 mg/d	13 mg/d
3rd trimester	9 mg/d	11 mg/d	13 mg/d

Over 80% of zinc is inside of muscle tissue and the skeleton. However, as there is no real storage organ for zinc in the human body, dietary intake must include enough zinc to prevent deficiency. Foods containing high amounts of zinc are oysters, wheat and rye germs, offal, beef, pork, cheese, pecan nuts and cashews. The main contributors to dietary zinc are meat, sausage products, bread, and dairy products. (29)

Nutrient status/deficiency:

Iron is competing with zinc for absorption and IFA is a routine supplement during pregnancy, which makes it difficult for pregnant women to meet the increased requirements. As it is also not possible for the human body to store zinc, many pregnant women are at risk of deficiency, especially in LMIC. (30)

The ANR 2017 showed that 31.5% of all participants did not reach the DACH recommendations for the zinc intake. (12) For pregnant women, a study by Rust et al. assessed dietary habits of 190 pregnant women via two 24h-recalls and found that the intake of zinc was unsatisfying. (31) No further evidence on the zinc status of Austrian pregnant women or prevalence of deficiency is available.

Zinc deficiency disrupts functions of the immune system which decreases the protection against infections and other diseases. (29) During pregnancy, zinc deficiency may also be associated with several APO such as PE, PB, and

postpartum hemorrhage but the current evidence is weak according to the WHO. (30)

A systematic review of observational trials measuring maternal zinc levels during pregnancy assessed the effects of zinc status on various adverse pregnancy outcomes as for example PE, gestational hypertension, gestational diabetes, SGA and LBW infants. The authors concluded that there may be trends between maternal zinc status and infant birthweight as well as the development of severe PE but the present evidence on the associations between zinc status and APO is insufficient. A major limitation of this review is that only few of the studies researched women who were zinc deficient or populations at a high risk of zinc deficiency. Most of the studies were conducted in Europe or the United States where the prevalence of zinc deficiency only ranges from 3.9% to 12.7%. The authors stated that future studies need to analyze women at risk for zinc deficiency and assess dietary zinc intake to determine the effects of the zinc status during pregnancy. (32)

He et al. researched the serum levels of zinc, magnesium, and calcium of pregnant women with pregnancy induced hypertension (PIH) and compared them with healthy pregnant women in their systematic review which included 15 observational trials in the meta-analyses. The authors found that the levels of zinc, magnesium, and calcium were significantly lower in women with pregnancy induced hypertension and concluded that those dietary minerals should be assessed for patients with PIH. Fourteen of the studies included in this review were done in China, Turkey, or India and one study in South Korea. (33)

There was no information on the zinc status or the prevalence of zinc deficiency in the study populations or if the fact that most of the studies were conducted in LMIC may have had any influence on the results.

Supplementation/intake:

In 2021 the WHO reevaluated the evidence on the recommendation for the zinc supplementation given in the ANC guideline from 2016. For the new recommendation an updated Cochrane review published in 2021 was used as a scientific basis. (30)

In the meta-analyses, 25 RCT comparing zinc supplementation with a placebo administered before the start of the third trimester were included. Study

populations of all studies were healthy pregnant women of which most presumably were zinc deficient. (34)

Zinc supplementation was shown to have little or no effects on all researched APO including PE, maternal infections, LBW, SGA or PB infants and no effects on neonatal mortality, stillbirth, and perinatal mortality. Therefore, the WHO does not recommend a routine zinc supplementation and the recommendation only in the context of rigorous research is retained. (30)

2.3.4. Selenium

Selenium (Se) is an essential trace element mainly present in the human body in the form of selenocysteine which is the central component of several enzymes. selenium containing enzymes such as glutathione peroxidases, thioredoxin reductases and deiodinases are important for many redox reactions. Through those enzymes, selenium is essential for various functions such as male fertility, cellular processes, DNA (deoxyribonucleic acid)-synthesis, regulation of thyroid hormone homeostasis, antioxidant defense, regulating the immune system and inflammatory processes. The selenium demand during pregnancy is slightly increased due to the demands of the fetus which amounts to two additional micrograms per day. As this additional demand is very low, the recommended dose of selenium for pregnant women is 60 mg per day which is the same for all adults. Selenium content in plant-based foods is dependent on the selenium content in the soil. As European soils are poor in selenium, a reliable contributor to selenium intake are animal products such as meat and eggs. Some plant-based foods are accumulating selenium and subsequently contain relatively high amounts of selenium. Therefore, in a vegetarian diet, emphasis should be placed on the consumption of foods such as Brazil nuts, broccoli, onions, garlic, mushrooms, and asparagus to reach the recommended intake. (9)

Nutrient status/deficiency:

During pregnancy, the health of the fetus is very vulnerable to oxidative stress which can impair its development. Selenium protects the fetus through antioxidant-enzymes, especially through the glutathione peroxidase activity. A deficient status may disrupt the antioxidant system, lead to various adverse pregnancy outcomes, and impair the development of the fetus. (35)

In a single center observational study conducted in Slovenia, associations of the selenium concentration in the amniotic fluid during the second trimester (week 16/17 of pregnancy) and the development of SGA were researched. The authors found that selenium levels in the amniotic fluid of women with SGA newborns were significantly lower when compared with women giving birth to infants appropriate for gestational age. Pregnant women in the SGA group were also more likely to develop PE but the authors stated that the selenium levels were inadequate to statistically predict that outcome. Additionally, no significant association between amniotic selenium levels and preterm birth was found. According to the authors, maternal blood selenium concentration was correlated with amniotic fluid levels and concluded that analysis of amniotic fluid could be an additional method and tool to reduce the risk of APO. (36)

A systematic review done by Monangi et al. researched the effects of selenium levels in pregnant women before the third trimester on preterm birth. The meta-analysis including all 17 cohorts found a significant association between selenium concentration and PB, specifically that an increase in selenium serum concentration (by 15 ng/mL) was associated with decreased odds for PB (odds ratio = 0.95). However, the authors found significant heterogeneity between the studies not only explicable by geographic location. There may be unknown site-specific and population-specific confounding factors responsible for the strong statistically significant associations that were found only at some sites. The authors concluded that their results did not support an association between maternal selenium levels and the risk for preterm birth. (37)

Hamdan et al. researched the association between selenium and PE in their systematic review with meta-analysis including observational trials published until April 2022. The authors found that selenium levels of pregnant women with PE were significantly lower than those of normotensive healthy controls. According to the GRADE (Grading of Recommendations Assessment, Development and Evaluation) certainty of evidence analysis, this study generated evidence of low certainty. A possible explanation may be the high heterogeneity due to differences in the studied populations regarding diet and/or the selenium content of the soil depending on the geographic location. (38)

A systematic review analyzed the influence of blood selenium levels on gestational diabetes mellitus. The authors included 14 observational studies in their meta-analyses and found that the selenium levels of pregnant women with

GDM were significantly decreased in the second and third trimester when compared with healthy controls, but no difference was shown for the first trimester. Subgroup analysis revealed that significant differences were found in the studies that were conducted in Asia and Africa, but not in the European ones. According to the authors, a possible explanation may be that Europeans in general consume more meat, wheat and milk which are good selenium sources. (39)

For the situation in Austria, the ANR 2012 found that 37.8% all female participants were shown to have reduced plasma selenium levels. (40) No data on the current status of pregnant women in Austria regarding the nutrient status of selenium were published at the time of writing this thesis.

Supplementation/intake:

Oxidative stress may be associated with APO such as PE, GDM or intrauterine growth restriction. A systematic review by Biswas et al. which included 22 RCT investigated if selenium supplementation during pregnancy may have positive effects on oxidative stress and subsequently on the health of mother and fetus. Studies included in the review showed that selenium supplementation positively affected several APO such as PE, GDM, pregnancy induced hypertension, miscarriage, and intrauterine growth restriction. However, as the majority of the studies exhibited methodological problems, the authors concluded that the current evidence is insufficient to recommend the supplementation of selenium during pregnancy. (41)

The authors of a review summarizing the literature on selenium supplementation also highlighted the narrow therapeutic range of selenium. The risk of overdose and subsequent negative effects on the health of mother and fetus is another important reason why recommendation of routine selenium supplementation is currently not feasible. (42)

An Algerian study researched the effects of daily selenium supplementation starting in the second trimester in addition to the standard IFA in pregnant women diagnosed with GDM. After 12 weeks, pregnant women receiving selenium supplementation had significantly lower fasting plasma glucose levels and increased activities of selenium-dependent antioxidant enzymes than pregnant women with GDM who did not receive selenium supplementation. Before the start of the supplementation, pregnant women in the intervention group were overweight, selenium deficient and had significantly decreased markers of

antioxidant defense when compared with healthy pregnant women controls. The authors concluded that selenium supplementation for deficient pregnant women may improve maternal health. (43)

An observational study conducted on a large Norwegian cohort investigated the influence of selenium intake on the incidence of pregnancy induced hypertensive disorders. No significant association between selenium intake coming from dietary sources or supplementation and pregnancy induced hypertension or PE was found. This did not support any possible beneficial effects of selenium supplementation on the risk of PE. The authors remarked that the median intake of the study population was close to the recommendations for pregnant women which also confirmed the hypothesis that selenium supplementation may only be beneficial for populations with detected selenium deficiency. (44)

2.4 Summary of the evidence for trace elements

The evidence which was reviewed for the trace elements in **chapter 2.3** will be summarized in **Table 4** and **Table 5**.

For better clarity, evidence on the outcomes PE, eclampsia or described as "hypertension in pregnancy" or "pregnancy induced hypertension" will be listed under "PIHD" (pregnancy induced hypertensive disorders).

All outcomes leading to the death of the fetus including fetal, neonatal, and perinatal mortality, stillbirth, and spontaneous abortion will be combined to "Fetal death".

"Association" denotes an association between the deficiency or the supplementation of the trace element and the respective adverse pregnancy outcome whereas "No effects" indicates that no association was found.

Table 4: Summary of the effects of deficiencies of trace elements on adverse pregnancy outcomes.

	Iron	**Iodine**	**Zinc**	**Selenium**
Anemia	Association (3)			
PIHD	Association with PE (7)	No effects (26)	Possible trends with PE (32) Association (33)	Association (38)

GDM	Trend for decreased odds (7)		No effects (32)	Association (39)
SGA	Association (7)		No effects (32)	Association (36)
LBW	Anemia leads to LBW (6, 7)	No effects (26)	Possible trends (32)	
PB	Maternal anemia associated with PB (7, 15)	No effects (26)		Association (37)
Fetal death	Association with stillbirth and neonatal mortality (7)	Association (22)		

Table 5: Summary of the effects of supplementation of trace elements on adverse pregnancy outcomes.

	Iron	**Iodine**	**Zinc**	**Selenium**
Infections			No effects (30)	
Anemia	Association (1, 2)			
PIHD	No effects (17)		No effects (30)	Association (41) No effects (44)
GDM				Association (41) Improved glucose levels and enzyme activities (43)
SGA		No effects (27)	No effects (30)	

LBW	No effects (16) Association (17)	No effects (27)	No effects (30)	
PB	No effects (16)	No effects (27)	No effects (30)	
Fetal death	No effects (16, 17)	No effects (27)	No effects (30)	Association (41)

2.5 Macro Elements

2.5.1 Sodium and Chloride

Sodium (Na) and chloride (Cl) are the main electrolytes in the extracellular area in the human body and responsible for regulating the plasma osmolality, the extracellular volume and blood pressure. Both are coupled to the water balance and regulated through the kidney by the renin-angiotensin-aldosterone system. Sodium is responsible for the maintenance of the membrane potential and the transport of substances across membranes. Chloride is essential for generation of hydrochloric acid in the stomach and is a cofactor of the peptidase angiotensin-converting enzyme which is important for the renin-angiotensin-aldosterone system.

To maintain the increased plasma volume during pregnancy, 70 mg of sodium per day are required additionally. As additional requirements are compensated through adaptations in the excretion, the recommendation for sodium for pregnant women is 1500 mg per day which is the same as for all adults. The daily recommendation for chloride is calculated based on the values of sodium and is 2300 mg per day for all adults.

High contributors to the dietary intake of sodium and chloride are sauces, processed meat and sausage products, savory snacks, cheese, and bread. The main dietary source of sodium and chloride is the addition of salt to processed foods during the manufacturing process. As the recommendations of sodium and chloride are often exceeded, unprocessed foods like fruits, vegetables and nuts which contain low amounts of sodium and chloride should be consumed more often. (45)

Nutrient status/deficiency:

Sodium and chloride levels are regulated through adjustment of the excretion by the renin-angiotensin-aldosterone system which can also adapt to decreased intakes. Symptoms of sodium or chloride deficiencies are therefore very rare and usually not due to low intake but health conditions such as excessive sweating, emesis, kidney failure, or the intake of diuretics. Losses of sodium are accompanied by chloride losses of the same magnitude except for emesis where the chloride losses are higher due to the high content of chloride in the stomach acid. (45)

Dröge et al performed an observational study on 198 women with PE or at a high risk for PE and measured sodium levels within two weeks prior to delivery. They found that lower sodium plasma levels were significantly associated with PE-associated adverse pregnancy outcomes and the reduction of the duration of pregnancy. APO associated with PE include the HELLP-syndrome (hemolysis, elevated liver enzymes, low platelet count), eclamptic seizures and renal failure on the maternal side but also fetal outcomes such as growth restriction and PB which may lead to increased neonatal mortality. The difference in the mean sodium levels between pregnant women with PE-associated adverse outcomes and those without was too small to be of clinical relevance but the authors suggest that a regular assessment of sodium levels should be taken into consideration. (46)

Intake of sodium/salt:

As no evidence on the supplementation of sodium or chloride during pregnancy could be found, this chapter will review the evidence on the effects of salt intake during pregnancy.

In the Austrian Nutrition Report 2017, it was shown for 81.2% of all participants that the intake of sodium, for which salt is the main contributor, exceeded the D-A-CH recommendations. The authors also added that the actual intake may even be higher, as the use of salt is often underreported. (12)

A Danish study measuring the sodium intake via a food frequency questionnaire (FFQ) which was conducted during the 25th week of gestation, showed that sodium intake during pregnancy has a significant positive linear association with the risk of hypertensive disorder during pregnancy. (47)

In a Japanese study which researched the effects of the sodium intake before pregnancy, the sodium intake was determined by an FFQ, and participants were healthy and normotensive women. Statistical analysis found that both the groups with lowest (1582mg of sodium per day) and highest (5085mg of sodium per day) preconceptual sodium intakes had higher risk of hypertensive disorders during pregnancy suggesting an U-shaped association. (48)

A systematic review done by Duley et al. included two RCT which compared the effects of altering dietary salt intake during pregnancy on the development of PE. As the authors did not find any significant difference between women restricting their dietary salt intake and women given advice to continue a normal diet, they concluded that there is no evidence if reducing the salt intake during pregnancy has any positive effect on the risk of PE. (49)

This review was used by the “WHO recommendations for prevention and treatment of pre-eclampsia and eclampsia” in which the WHO stated that restricting salt intake during pregnancy is not recommended. However, the evidence was only of a moderate quality according to the WHO. (5)

2.5.2 Calcium:

Calcium (Ca) is a component of the human skeleton and teeth and responsible for bone strength. Bones are on the other hand the most important calcium storage in the body. Calcium is also important for the stability of cell membranes, functions in the nerval system, for muscle contraction and blood coagulation. There is an increased demand during pregnancy due to the calcium needs of the fetus, especially in the third trimester. This is compensated through increasing the absorption rate throughout the whole pregnancy. Calcium intake exceeding the recommendation for the daily intake was not shown to exhibit additional benefits for pregnant women. Therefore, the recommendation for the daily intake is 1000 mg per day for pregnant women which is the same as the recommendation for all adults. Dairy products contain high amounts of calcium and are the main contributors to the calcium intake. Some vegetables such as broccoli and kale, mineral waters, hazelnuts, or Brazil nuts are also good sources of calcium, especially for people with lactose intolerance. (9)

Nutrient status/deficiency:

Insufficient calcium intake over a long period of time will increase bone resorption and calcium release to maintain the concentration in the serum. Demineralization of the bone is also increased during late pregnancy. However, this bone resorption process is temporary and will not result in osteoporosis. (9)

Even though populations of high-income countries show higher mean calcium intake than LMIC, there are some groups of population which are at higher risk of calcium deficiency, as for example adolescents, postmenopausal women, women with amenorrhea, humans with lactose intolerance, cow's milk allergy or who consume a vegan diet. (50)

In Austria, 75% of the women do not reach the D-A-CH recommendations for the calcium intake. (12) Rust et al., researching dietary intakes and physical activity of Austrian pregnant women via two 24h-recalls and a physical activity questionnaire in their study, concluded that the intake of calcium was unsatisfying. (31)

Calcium deficiency during pregnancy may be associated with APO. A cross-sectional study conducted in Cameroon found that pregnant women with decreased ionized calcium levels had significantly increased odds for adverse outcomes such as hypertension in pregnancy, LBW infants and macrosomia. No significant associations between total calcemia and APO were found in this study. Ionized calcium is the metabolically active and free form of calcium, not bound to proteins. The authors stated that ionized calcium is a strong predictor of fetal outcomes. (51)

In a study conducted in India, both serum calcium levels and dietary calcium intake of healthy pregnant women in their third trimester were recorded. Mothers with LBW infants were shown to have significantly lower levels of serum calcium. The other researched outcomes PE, PB and neonatal mortality were not significantly associated with serum calcium. In the study population less than one fifth reached the daily recommendations for the calcium intake but the mean serum levels were in the normal range. There was no association between dietary intake and the serum calcium level. (52)

Supplementation/intake:

A Cochrane review done by Hofmeyr et al. researched the influence of Calcium supplementation on hypertensive disorders in pregnancy and related maternal

and child outcomes. Included were only RCT which compared calcium supplementation with placebo. The authors found that a high dose of calcium (≥1g) may reduce the risk of PE, especially for women with low calcium diets. (53)

This review was used by the WHO as evidence for the WHO recommendation on calcium supplementation (1.5g to 2g) during pregnancy for the prevention of preeclampsia and its complications for populations with low dietary calcium intake. (54)

The review by Hofmeyr et al. was updated and published in 2019 with the focus on the effects of calcium supplementation on the prevention of PE before pregnancy. This updated review was used as evidence by the WHO to develop recommendation on pre-pregnancy calcium supplementation. The WHO concluded that calcium supplementation commenced before pregnancy exhibits little or no benefits regarding the prevention of hypertensive disorders during pregnancy. The evidence is insufficient to determine if calcium should be administered before pregnancy or at what gestational age during pregnancy. The pre-pregnancy supplementation should only be recommended “in the context of rigorous research”. This means that the supplementation can be implemented at a large scale if uncertainties are addressed in the research. Examples given by the WHO are including a baseline assessment prior to pregnancy to assure that the comparison groups of women taking calcium supplements and of women not taking them are a similar as possible. The WHO emphasized that the recommendation on calcium supplementation during pregnancy should still be implemented in programs and health care facilities. (55)

A systematic review done by Buppasiri et al. researched the effects of calcium supplementation on adverse pregnancy outcomes other than hypertension. The meta-analyses included 23 RCT and found no significant associations with pregnancy outcomes such as preterm birth, low birthweight, maternal mortality, maternal weight gain, mode of delivery, fetal and/or neonatal mortality and bone mineral density for mother and neonates. The authors concluded that there is not sufficient evidence regarding any other pregnancy outcomes other than pregnancy-induced hypertension and calcium supplementation should be primarily administered for prevention of preeclampsia. (56)

2.5.3 Magnesium

Most of the magnesium (Mg) in the human body is in the skeleton, muscle tissue and intestines. Magnesium is imperative for many cellular functions as it is bound to more than 600 enzymes such as kinases, nucleotidases, phosphatases and carboxypeptidases. These are dependent on the presence of ATP which requires a bound magnesium ion for it to activate. Magnesium is also important for stabilizing DNA, RNA (ribonucleic acid), and biologic membranes through formation of complexes as well as the transport of calcium, potassium and sodium through ion channels and transporters. Due to these functions, magnesium is essential for many physiological processes such as muscle contraction, cardiac rhythm, and blood pressure.

Currently, no evidence on possible changes to the magnesium needs during pregnancy is known. During pregnancy, the possibly additional needs for the fetus in the third trimester are compensated through increasing the absorption rate of magnesium. Therefore, the daily recommendation for pregnant women as well as for all female adults is 300 mg per day.

Cocoa powder, nuts, and seeds such as Brazil nuts, pine nuts, almonds, cashews, and sesame seeds contain high amounts of magnesium. Other sources and contributors to the dietary intake of magnesium are legumes, spinach, potatoes, bananas, fish and sea food, meat, and dairy products. (57)

Nutrient status/deficiency:

Magnesium deficiencies are very rare, as many plant-based and animal products contain sufficient amounts of magnesium. Another reason is that the magnesium levels in the human body are strictly regulated through adaptation of absorption mechanisms, the release of magnesium out of bones and excretion by the kidneys. Therefore, a magnesium deficiency usually only happens due to impaired gastrointestinal absorption or increased magnesium excretion caused by diseases such as diarrhea, renal diseases, alcoholism, or specific medication. (57)

A systematic review performed by Ren et al. compared the serum and plasma magnesium levels of pregnant women diagnosed with gestational diabetes with the levels of pregnant women who were healthy and included 17 observational studies in their meta-analysis. After stratification for trimesters, it was shown that magnesium levels in women with gestational diabetes were significantly lower in the third trimester. No differences in the first and second trimester were found.

However, due to the high heterogeneity among the studies the authors stated that the evidence is of low certainty. An explanation that a significant difference could only be shown for the third trimester, may have been the increase in the fetal demand of nutrients. (58)

A study conducted in Nigeria assessed the influence of the serum magnesium levels for pregnant women on the adverse pregnancy outcomes PE and PB. Hypomagnesemia, which was defined as below the mean serum magnesium level of Nigerian pregnant women measured in a previous study, was shown to be significantly associated with increased occurrence of preeclampsia and preterm delivery. The authors concluded that magnesium supplementation or a diet rich in magnesium should be recommended during pregnancy. It has to be mentioned that the prevalence of magnesium deficiency in the study population at delivery was 25%. (59)

In poor countries like Nigeria maternal malnutrition and micronutrient deficiencies are highly prevalent. (1) It cannot be ruled out that the study population in the Nigerian study was also deficient in other nutrients. Therefore, the increase in preeclampsia and preterm birth may not only be associated with low serum magnesium levels.

Supplementation/intake:

Qu et al included four RCT in their systematic review with meta-analysis investigating the effects of magnesium supplementation on glycemic control in women with GDM. The authors found significant reduction in various markers for glycemic control and positive effects on oxidative stress due to magnesium supplementation. A limitation of this study is that there were only four RCT included in the meta-analysis, all of which were conducted in Iran and information on other supplements that may have been administered was missing. As the studies were researching the effects of magnesium supplementation on women with gestational diabetes this means that the results cannot be transferred to healthy pregnant women. (60)

An RCT conducted on low-income pregnant women in Brazil investigated the effects of magnesium supplementation on PE. There was no significant difference regarding the incidence of preeclampsia when comparing the group of women receiving supplementation with the control group. The authors stated that even though around half of the study population had hypomagnesemia (defined as ≤1.8

mg/dl), magnesium supplementation was not shown to help preventing preeclampsia. (61)

Schoenaker et al. researched the dietary intakes of magnesium and calcium in women with hypertensive disorders of pregnancy such as gestational hypertension or PE. The intake of magnesium was significantly lower in pregnant women with hypertensive disorders when compared with those without but only three cohort studies could be included in the meta-analysis. The analysis for calcium including seven cohort studies found that dietary calcium intake was lower in women with hypertensive disorders, but the results were of borderline significance. The authors stated that the current evidence on the intake of dietary minerals on hypertensive disorders in pregnancy is limited. (62)

In a Cochrane review including ten RCT the effects of magnesium supplementation during pregnancy on various pregnancy outcomes was researched. No significant differences in the risk of perinatal mortality, SGA, low birth weight, PE or PIH were found. The authors concluded that magnesium supplementation during pregnancy to improve pregnancy outcomes cannot be supported due to limited current evidence. (63)

There is not enough evidence to make statements on the effects of magnesium supplementation during pregnancy or if increasing the intake of magnesium may have any benefits for pregnant women. (57)

2.6 Summary of the evidence for macro elements

The evidence for the macro elements in **chapter 2.5** will be summarized in **Table 6** and **Table 7**.

For better clarity, evidence on the outcomes PE, eclampsia or described as “hypertension in pregnancy” or “pregnancy induced hypertension” will be listed under “PIHD” (pregnancy induced hypertensive disorders).

All outcomes leading to the death of the fetus including fetal, neonatal, and perinatal mortality, stillbirth, and spontaneous abortion will be combined to “Fetal death”.

“Association” denotes an association between the deficiency or supplementation of the macro element and the respective adverse pregnancy outcome whereas “No effects” indicates that no association was found.

Table 6: Summary of the effects of nutrient status of macro elements on adverse pregnancy outcomes.

	Sodium/salt	Calcium	Magnesium
Anemia			
PIHD	Association with PE-associated APO[1] (46)	Association (51) No effects with PE (52)	Association with PE (59)
GDM			Association in 3rd trimester (58)
LBW		Association (51, 52)	
PB	Association in women with PE (46)	No effects (52)	Association (59)
Fetal death		No effects (52)	

1 Pre-eclampsia associated adverse pregnancy outcomes include the HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count), eclamptic seizures and renal failure.

Table 7: Summary of the effects of supplementation or the intake of macro elements on adverse pregnancy outcomes.

	Sodium/salt	Calcium	Magnesium
Anemia			
PIHD	Positive linear association (47) U-shaped association for pre-pregnancy intake (48) No effects for reducing salt intake (49)	Association with PE (53) Lower intake for women with PIHD (62) No effects for pre-pregnancy (55)	Lower intake for women with PIHD (62) No effects of supplements on PE (61, 63)
GDM			Improvement of glycemic control in women with GDM (60)
Mode of delivery		No effects (56)	

Maternal mortality		No effects (56)	
SGA			No effects (63)
LBW		No effects (56)	No effects (63)
PB		No effects (56)	
Fetal death		No effects (56)	No effects (63)

3. Methodology

3.1. Study design

As mentioned in **chapter 1**, all urine samples analyzed were previously taken from pregnant women in Austria during the project ESÖSM. For that study, women attending one of three hospitals in Vienna (Kaiser-Franz-Joseph-Spital, Semmelweis-Frauenklinik, Wilhelminenspital) were recruited between October 2006 and April 2008 (64). Inclusion criteria are listed in **Table 8**.

Table 8: Inclusion criteria for pregnant women in ESÖSM.

19 to 40 years of age
Pregnancy during the 2nd or 3rd trimester
No high-risk pregnancy
Singleton pregnancy
No metabolic disorders (e. g. diabetes mellitus)
No pregnancy complications
No acute infections

A high-risk pregnancy is a medical or obstetric condition potentially endangering the health of the mother or fetus during pregnancy. (65)
For pregnancy complications, the WHO states that about 15% of all pregnant women develop a "potentially life-threatening complication that calls for skilled care, and some will require a major obstetrical intervention to survive. (66)
A total of 1141 pregnant women were recruited of which 270 completed a 24h-recall for the assessment of the dietary intake of nutrients. Of those, 261 subjects

provided information on pregnancy, health, and dietary habits, as well as completed a food frequency questionnaire. Age was given by 263 and the body mass index (BMI) was known from 256 participants. (67)

To determine the nutritional status of pregnant women, blood as well as urine samples were taken from 113 women (68). Driussi and Loibl analyzed blood samples for dietary minerals and fat-soluble vitamins respectively, (64, 68) whereas Tramnitz determined the concentrations of water soluble vitamins in the urine. (69)

For this thesis, urine samples taken for ESÖSM were provided by the Department of Nutritional Sciences of the University of Vienna for analysis. After exclusion of six samples which were unlabeled and samples from 54 women which could not be analyzed due to missing sample volume, urine samples from 53 subjects were used for determination of the concentration of dietary minerals.

3.2. Methodology of Sample Analysis

All equipment, reagents and procedures for each chapter will be described in respective tables in the Annex. Reagents used for all steps in **chapter 3.2** are listed in the Annex under **Table A1**.

3.2.1. Sample Digestion

Urine samples were stored at -20°C by the Department of Nutritional Sciences at the University of Vienna until they were transported to the Austrian Agency for Health and Food Safety for analysis.

Prior to the determination of trace elements, a digestion procedure is required to release analytes from the organic matrix through oxidation and convert them into simple dissolved forms. A wet digestion method, which is the addition of acids to the samples and application of heat, was carried out at high pressure in closed systems called PTFE (polytetrafluoroethylene) bombs. This makes higher temperatures possible which increases the oxidizing potential and prevents analyte losses caused by volatilization. (70) microwave-assisted digestion employing a $KClO_3$ (potassium chlorate) solution acidified with HNO_3 (nitric acid) was chosen for this thesis. This method is especially suitable for preventing the volatility of iodine (71), reduces the duration of the digestion process and only requires small amounts of sample materials. (72)

After being completely defrosted, an aliquot of 700 to 1000 µL of each sample, depending on how much total volume was available, was withdrawn and pipetted into pressure relief vessels with PTFE liners. To these vessels 4 mL of the $KClO_3$ digestion solution was added which was prepared by dissolving 20 g of $KClO_3$ in 200 mL of ultrapure water to which 80 mL of 65% HNO_3 was added. $KClO_3$ digestion solution was shaken before withdrawing volume.

Samples of in-house reference material and certified reference material (CRM), as well as blanks containing only the $KClO_3$ digestion solution were also prepared. All samples and blanks were digested using a microwave oven following a digestion program described in the Annex in **Table A2**. Vessels were left to cool overnight and opened the next day under a fume hood. All digests were then transferred to 10 mL volumetric flasks and filled to 10.0 mL volume with ultrapure water. After shaken carefully, the volume was transferred to polystyrene multipurpose containers and tightly closed.

3.2.2. Analysis of urinary iron, zinc, sodium, calcium, magnesium via inductively coupled plasma optical emission spectroscopy.

Most elements of interest can efficiently be determined via multi-element techniques such as inductively coupled plasma optical emission spectroscopy (ICP-OES) or inductively coupled plasma mass spectrometry (ICP-MS) “within a few suitable dilutions”. (71) This is necessary due to the different concentrations of trace and macro elements in human biological samples.

For the analysis via ICP-OES, samples are introduced as aerosols into the inductively coupled plasma which consists of a partially ionized gas (argon, less than 1% ionization). Light emitted from the plasma is focused onto the entrance slit of the spectrometer to measure emission from multiple elements sequentially or simultaneously. Simultaneous reading was chosen for this thesis, as it reduces the required sample volume for the analysis. The detected signal depends on the number of atoms (element concentration of the digested sample) introduced into the plasma and the fraction of those that are excited. (73)

In preparation of the analysis, 4 mL of all digests were pipetted from the polystyrene multipurpose containers into polyethylene test tubes. This volume was used to prepare 1:10 and 1:20 dilutions with ultrapure water using a diluter/dispenser. The remaining volumes of the digests and the two dilution stages were analyzed via ICP-OES using two different methods. See **Table A3** in

the Annex for the specifications of both methods as well as the equipment used for this analysis.

Concentrations of urinary elements were calculated via calibration curves. To determine the calibration curves, four calibration solutions with specific concentrations of several elements were prepared for each method. Reagents used to make the solutions and all respective element concentrations are given in **Table A4** and **Table A5** in the Annex. Primary data were acquired via the software WinLab32™ and processed using Microsoft Excel® 2010.

3.2.3. Analysis of urinary selenium via hydride generation atomic absorption spectrometry.

Analysis of selenium in this master thesis was performed by hydride generation atomic absorption spectrometry (HGAAS). As ICP-OES was not able to achieve acceptable recovery rates for selenium and ICP-MS could only be employed for the analysis of iodine, HGAAS was selected for this element.

To determine the total concentration of urinary selenium via HGAAS all available forms of selenium have to be converted to Se(IV) / selenite to be analyzed. This is the only selenium species that is chemically suitable for hydride formation when reacting with $NaBH_4$ (74). Conversion of 99.9% can be achieved when a selenium solution is heated in HCl (6 mol/L) (75) at a temperature of 65°C. (76)

$NaBH_4$ is pumped by a mercury/hydride system into the sample solution in which Se(IV) already reacted to selenous acid (H_2SeO_3) due to previous acidification. H_2SeO_3 will then be converted to hydrogen selenide (H_2Se) through the chemical equation shown in **Figure 2**. (77)

Figure 2: Reaction of selenous acid to hydrogen selenide. Modified from source (77).

$$3H^+ + 3BH_4^- + 4H_2SeO_3 = 4SeH_2 + 3H_2O + 3H_3BO_3$$

The generated hydrogen selenide is then introduced into a quartz tube oven to be entirely atomized so the absorbance of selenium can be measured. Further information on the specific mechanisms of HGAAS can be found elsewhere. (78)

The methodology for the analysis of selenium applied in this master thesis was shown to be appropriate for the determination of total urine selenium. (79)

For the experimental procedure, a measured aliquot of 1.5 to 2 mL of the digests, depending on how much volume was available, were transferred from the polystyrene multipurpose containers to 50 mL Erlenmeyer flasks to which 10 mL of HCl (6 mol/L) was added. Twenty ng of selenium which is equal to 100 µL of a 0.2 mg/L selenium solution were added to all blanks to control for possible loss resulting from the heating step. Erlenmeyer flasks were heated at a temperature between 60 and 70 °C for one hour under a fume hood. Flasks were then covered with hourglasses and left to cool overnight.

To determine selenium via HGAAS, a $NaBH_4$ solution required for the conversion of Se(IV) to hydrogen selenide was prepared before each analysis following the procedure described in the Annex **Table A6**. The solution was filled into a plastic bottle and applied to the mercury/hydride system shortly before analysis.

Sample solutions in Erlenmeyer flasks were transferred to the sampling vessel and a volume of 10 mL of a HCl dilution (1:5) was added before analysis was started. Operating conditions of the applied method and used equipment are shown in **Table A6**. Blank values were measured by analyzing the HCl dilution (1:5) alone.

Concentrations of selenium were calculated via calibration curves. Those were determined by adding 10 mL of the HCl dilution (1:5) as well as pipetting either 50, 100 or 150 µL of the previously prepared 0.2 mg/L selenium solution to the sampling vessel which leads to amounts of 10, 20 or 30 ng of selenium respectively. All three concentration levels were measured twice, and the average was formed to determine the calibration curve. Data were acquired and processed using an in-house software.

3.2.4. Analysis of urinary iodine via inductively coupled plasma mass spectrometry.

Determination of urinary iodine via ICP-OES is not possible due to a strong spectral interference by phosphorus. Therefore, ICP-MS is the current method of choice for the analysis of iodine. (80)

For the analysis via ICP-MS, a two-step process called the 'interface' consisting of a sample and a skimmer cone, extracts the ionized analyte from the plasma. Extracted ions will then be focussed into the high vacuum area of the mass spectrometer by cylinders. The intensity of the detected signal only depends on the number of ions (element concentration of the digested sample) but not on the

extent of excitation which is the case for ICP-OES, as ions are physically transported into the MS solely based on their mass (73).
As the concentrations of iodine were determined by a standard addition method, the digest solutions in the polystyrene multipurpose containers were used to prepare three dilution stages in polystyrene test tubes via diluter/dispenser according to the procedure described in **Table 9**.

Table 9: Dilution stages prepared for the analysis of iodine.

	Sample	Ultrapure water	Iodine (0.2 mg/L)	Iodine (0.4 mg/L)	$KClO_3$ - dil.
1	500 µL	50 µL	-	-	4450 µL
2	500 µL	-	50 µL	-	4450 µL
3	500 µL	-	-	50 µL	4450 µL

“'KClO3 – dil.” was prepared by adding 10 mL of the KClO3 digestion solution to 800 mL of ultrapure water. Test tubes were placed into the autosampler rack with the three dilutions of each sample in ascending order. The ELAN® 3.4 software was also programmed to include a process of washing after all three tubes of a sample were analyzed.
This procedure was performed to largely reduce memory effects which must be considered when analyzing iodine. Operating conditions of the applied method as well as used equipment are shown in **Table A7**. Primary data were acquired via ELAN® 3.4 software installed on an IBM personal computer and processed using Microsoft Excel® 2010.

3.2.5. Analysis of urinary chloride via ion selective electrode on a pH meter
After the procedure described in **chapter 3.2.1** which introduced $KClO_3$ as the digestion solution, all digests contained a considerable amount of chlorate ions which would make determination of chloride via ICP-OES or ICP-MS redundant. Therefore, the analysis of chloride was performed via an ion selective electrode (ISE) using undigested sample urine volumes.
The mechanism of an ISE is based on membranes, that separate two phases so that the transport of ions, electrons or respective holes between those phases is modified or inhibited. An ISE is not able to directly measure the ion concentration but senses the activity of ions for which the membrane is specifically defined. This

implies that for the analysis of an element of interest, a “conversion factor” is mandatory, such as the use of calibration curves. To avoid changes in the ionic strength due to variations in sample composition, which is normal for human biological samples, the addition of an adjustor solution consisting of an inert electrolyte is recommended. (81)

For the analysis of urinary chloride via ISE on a pH meter, an aliquot of 70 to 400 µL of each urine sample, depending on how much volume was left, was pipetted into polystyrene multipurpose containers. This was diluted to a total volume of 6 to 8 mL (depending on the volume of urine used) with ultrapure water.

Concentrations of chloride were calculated via a calibration curve which was determined by preparing chloride calibration solutions with concentrations of 1, 10, 100 and 1000 mg/L of chloride using ultrapure water.

An aliquot of an ISA (ionic strength adjustment) solution was added to every sample as well as to the calibration solutions to maintain a constant ionic strength. To control for interferences, solutions containing possibly interfering ions were prepared as shown in the Annex **Table A9** and analyzed.

Before analysis was started, the reference electrolyte chamber was filled with an outer chamber filling solution. For data collection, values both in mV and in pH-units were recorded from the pH meter. Used equipment, reagents and specifics of the ISE are shown in **Table A10**. Data were acquired and processed using an in-house software.

3.3 Statistical analysis

For the statistical analysis Microsoft® Excel 365 was used for the calculation of descriptive statistics and R: A language and environment for statistical computing, version 4.2.3 for determination of quality assurance measures, non-normality, and correlations for the analyzed elements. The applied methods are mentioned in the respective paragraphs in **chapter 4**.

The α-level was selected to be at 0.05. This means, that an observation with a significance level of $p<0.05$ was considered significant.

4. Results

4.1 Measures of quality assurance

To validate the applied methodology, determination of accuracy and precision of the analyses was performed by analyzing CRM purchased from RECIPE Chemicals + Instruments GmbH and in-house reference material. For the validation of the analysis of chloride, ALVA 04/01 reference material was used obtained from participation in inter-laboratory ring trials.

The measures for accuracy and precision, the recovery in percent and the coefficient of variation respectively, are explained in the next paragraphs and listed for all analyzed elements in **Table 10**.

4.1.1 Accuracy as recovery in percent:

Recovery is determined by the amount of a specific substance in the reference material calculated as the percentage of the total value that is known. This is performed to identify a possible bias which is a systematic error that may be inherent to the applied method or due to contamination, errors with calibration curves, etc.

A recovery of 100% would be the “ultimate goal” although hardly possible. Recoveries should vary within an acceptable range and very low recovery rates should be reported and possibly corrected. (82)

4.1.2 Precision as coefficient of variation:

Precision is determined through the coefficient of variation (CV) calculated by dividing the standard deviation by the mean. This is performed to identify the magnitude of the influence of random errors.

A coefficient of variation within 10% is considered as “highly precise” for ultra-trace analyses which are concentrations below parts per million (ppm). (82) These concentration levels were the case for the analyses of iodine, iron, selenium, and zinc.

Table 10: Measures of quality assurance for all analyzed elements.

		n[1]	CV	Percent Recovery[2] (Control Range)[3]	Mean Value[4] (Control Range)[5]
Fe	ClinChek®	6	178.10%	365.67 (87.66 – 1308) %	38.6 (30.8 – 46.3) µg/L
	In-house	10	202.86%		
Ca	ClinChek®	6	5.87%		
	In-house	10	11.26%		
I	ClinChek®	6	2.92%	99.31 (82.78 – 126.45) %	120 (89.9 – 150) µg/L
	In-house	10	5.37%		
Se	ClinChek®	6	14.13%	85.73 (76.25 – 92.73) %	29.9 (24.0 – 35.9) µg/L
	In-house	10	22.87%		
Zn	ClinChek®	6	26.44%	91.41 (90.29 – 118.03) %	204 (163 – 245) µg/L
	In-house	10	14.78%		
Mg	ClinChek®	6	3.17%	85.61 (75.21 – 101.79) %	18.4 (14.7 – 22.1) mg/L
	In-house	10	7.53%		
Na	ClinChek®	6	3.66%		
	In-house	10	8.72%		
Cl	In-house	10	9.47%		
	ALVA 04/01	4		85.83 (74.89 – 90.98) %	1440 (1270 – 1650) mg/kg

1 Number of observations
2 Recovery calculated as percentage of the Mean Value
3 Range calculated as minimum - maximum in % of the minimum - maximum range of the Mean Value
4 Known value of the element concentration in the CRM
5 minimum - maximum range of the Mean Value

As for the analysis of iron, a coefficient of variation of around 200% and an average percent recovery of 365.67% was calculated, which leads to the conclusion that the applied methodology may not have been able to accurately

determine the concentrations of urinary iron. This is elaborated on in **chapter 4.2.4** in more detail.

The coefficients of variation for zinc and selenium were found to be distinctly above 10%. As mentioned before, a precision below 10% would be considered as "highly precise" for those two elements. Due to the coefficients of variation for selenium and zinc being between 10 and 30% the performance of the methodologies applied for these two elements can only be classified as "intermediate" as described elsewhere. (82)

The probability for the variance of samples, or as calculated in the scope of the quality assurance for the coefficients of variation, to fall within a certain accuracy (which for our data set could be an arbitrarily set target of 10%) is dependent on the sample size. The methodology expanding on the principles of the Cochran's theorem and further explanation is described in Schillaci et al. (83). Increasing the rather low number of repetitions of the reference material analysis (6 repetitions for ClinChek® and 10 for the in-house reference material) may have been able to bring the coefficients of variation of selenium and zinc closer to the 10% target. However, it was not possible to increase the number of repetitions as the available total volumes of all reference materials was used completely.

4.2 Analysis of spot urine samples

4.2.1 Characteristics and nutrient intakes of participants:

Data on the characteristics and the intake of nutrients of pregnant women participating in ESÖSM were compiled and provided by Ass.-Prof. Dr. Petra Rust. The data for the subsample used in this thesis are shown in **Table 11**.

Table 11: Characteristics and nutrient intake of Austrian pregnant women.

	n	Mean	SD[1]	Median	IQR[2]	Min[3]	Max[4]
Age	53	29.9	6.0	29	7	19	43
GW[5]	45	28.4	6.9	29	12	13	40
Nutrient Intake							
Fe (mg/d)	49	11.00	5.50	10.10	7.63	2.58	27.33
I (µg/d)	49	211.80	133.68	182.81	149.46	30.15	765.26

Zn (mg/d)	49	9.30	4.20	9.04	5.09	2.91	21.71
Na (mg/d)	49	3207.9	1275.0	3091.3	1544.4	629.4	7117.0
Cl (mg/d)	49	5383.3	2132.0	5027.7	2420.3	829.2	11890.8
Ca (mg/d)	49	891.9	522.7	819.4	516.5	131.8	2566.2
Mg (mg/d)	49	318.5	170.8	293.9	204.5	48.9	955.8

1 standard deviation
2 interquartile range
3 minimum
4 maximum
5 gestational week

The age of the participants of the subsample was on average 29.9 years (± 6.0 years). The youngest woman was 19 and the oldest was 43 years old. One participant (43 years) exceeding the age limitations of the inclusion criteria of ESÖSM listed in **Table 8** was also included in the analysis. The gestational age of the pregnant women was on average 28.4 weeks (± 6.9 weeks). At the time of sample collection, 20 women (44.4%) were in the 2st trimester (between the 13th and 27th gestational week (84)) and 25 (55.5%) in the 3nd trimester (between the 28th and 40th week of pregnancy (84)). Data on the intake of nutrients were recorded for 49 women and evaluated for each micronutrient in **chapters 4.2.4** to **4.2.11**. As there is currently a lack of data on the selenium content of foods, no intake of selenium for the participants was recorded.

4.2.2 Overview of the analyses of spot urine samples

The descriptive statistics of all analyzed trace and macro elements are listed in **Table 12** and **Table 13** respectively. The assessment of the mineral status is discussed in the chapters for each element.

Table 12: Mineral status of trace elements of Austrian pregnant women.

	Fe (µg/L)	I (µg/L)	Zn (µg/L)	Se (µg/L)
n	53	53	53	53
Mean	65.10	80.52	395.54	20.52
SD	150.19	61.05	306.66	12.00
Median	19.41	56.32	335.62	17.50
IQR	91.79	60.64	272.75	13.50
Min	<27	8.76	<40	1.00

Max	689.80	267.79	1717.09	56.00
DL[1]	<27	n.d.	<40	<0.58

1 detection limit

Table 13: Mineral status of macro elements of Austrian pregnant women.

	Na (mg/L)	Cl (g/L)	Ca (mg/L)	Mg (mg/L)
n	53	40	53	53
Mean	2752.3	6.56	96.41	55.92
SD	1237.3	3.14	66.73	36.31
Median	2789.8	6.12	80.59	43.85
IQR	1754.5	3.82	108.51	59.77
Min	223.2	1.89	11.38	4.93
Max	6306.1	15.58	285.60	148.45
DL	<23.5	<0.40	<0.85	<3.29

Detection limits were determined via analysis of elemental standards in aqueous solutions based on a confidence level of three standard deviations.

For the DL of iodine, the calculation yielded negative values. Therefore, it is given as “n.d.” for “not determined” in **Table 12**. According to PerkinElmer Inc, iodine can be analyzed via ICP-MS with a detection limit at or below ppt (parts per trillion) levels which for the analysis of urine samples would correspond to a detection limit around 1 ng/L (1 nanogram per liter). (85)

During the analyses for the elements iron and zinc, some values measured for the samples were found to be outside of the range of three standard deviations. Therefore, the minimum of the analyzed samples is given as the detection limit in **Table 12**. This is addressed in more detail in **chapter 4.2.4** for iron and **chapter 4.2.6** for zinc.

4.2.3 Test for normality

To test for normality is important as measures to characterize and analyze the data are decided based on the normality status so that the data can be represented correctly. For normally distributed data, the appropriate measures for central tendency and variability are the mean and the standard deviation whereas for non-parametric data, the median and IQR are used. (86) The Shapiro-Wilk test was applied to test for non-normality.

Table 14: Shapiro-Wilk test for the urinary concentration of all elements.

	n	p-Value		Results
Fe	53	5,693E-07	<0,05	Non-normality
I	53	3,346E-05	<0,05	Non-normality
Zn	53	1,863E-06	<0,05	Non-normality
Se	53	0,00121	<0,05	Non-normality
Na	53	0,550	>0,05	Normality
Cl	40	0,00503	<0,05	Non-normality
Ca	53	0,00233	<0,05	Non-normality
Mg	53	0,00270	<0,05	Non-normality

A normal distribution was only detected for the sodium data. For all the other analyzed elements non-normality was found.

Table 15: Shapiro-Wilk test for the intake of all elements.

	n	p-Value		Results
Fe	49	0.06447	>0,05	Normality
I	49	3.233E-05	<0,05	Non-normality
Zn	49	0.1619	>0,05	Normality
Na	49	0.4692	>0,05	Normality
Cl	49	0.3032	>0,05	Normality
Ca	49	0.001741	<0,05	Non-normality
Mg	49	0.007207	<0,05	Non-normality

The Shapiro-Wilk test identified normal distributions for the intake of iron, zinc, sodium, and chloride.

Statistical measures and methods appropriately adapted to the normality status for each element were applied and are described in the **chapters 4.2.4** to **4.2.11**. Results are briefly summarized in the following chapters and discussed in more detail in **chapter 5**.

4.2.4 Analysis of the nutrient status for iron

The data for the concentration of iron in spot urine samples and the intake of iron of our subsample are shown in **Table 16** and **Table 17** respectively.

Table 16: Urinary iron levels of Austrian pregnant women.

	n	Median	IQR	Min	Max	DL
Fe (µg/L)	53	19.41	91.79	<27	689.80	<27

The analyses of 27 of 53 samples (50.9%) generated values below the limit of detection. An explanation is that the concentration of iron in many urine samples was too low to be accurately determined via ICP-OES.

As more than 50% of the analyzed sample values were below the DL and could therefore not be considered as accurate measurements, and the measures of quality assurance were of inadequate accuracy as mentioned in **chapter 4.1**, the applied methodology was considered inappropriate and no conclusions on the nutrient status of iron derived from the analysis of spot urine samples were deemed feasible.

Table 17: Iron intake of Austrian pregnant women.

	n	Mean	SD	Min	Max	Reference[1]
Fe (mg/d)	49	11.0	5.5	2.6	27.3	30

1 D-A-CH Reference Values for Nutrient Intake for pregnant women. (9)

The average intake of iron for the subsample was 11.0 ± 5.5 mg/d, which is far below the D-A-CH recommendations of 30 mg/d for pregnant women. All 49 pregnant women for which data on the intake of iron were recorded did not reach the recommended intake for pregnant women.

4.2.5 Analysis of the nutrient status for iodine:

The results of the analysis for the urinary iodine concentration and the iodine intake are shown in **Table 18** and **Table 19** respectively.

Table 18: Urinary iodine levels of Austrian pregnant women.

	n	Median	IQR	Min	Max	DL	Reference[1]
I (µg/L)	53	56.32	60.64	8.76	267.79	n.d.	150-249

1 According to the WHO, iodine intake of a population is considered “adequate” if the median urinary iodine concentration is between 150 µg/L and 249 µg/L. (24)

The median urinary iodine concentration was 56.32 µg/L with an IQR of 60.64 µg/L. Following the WHO recommendations for determining the iodine status of populations, the median was distinctly below the cutoff level of 150 µg/L, which would be considered as an “insufficient” intake for the subsample. The urinary iodine concentration for 44 (83.0%) of 53 pregnant women analyzed in this master thesis fell below 150 µg/L.

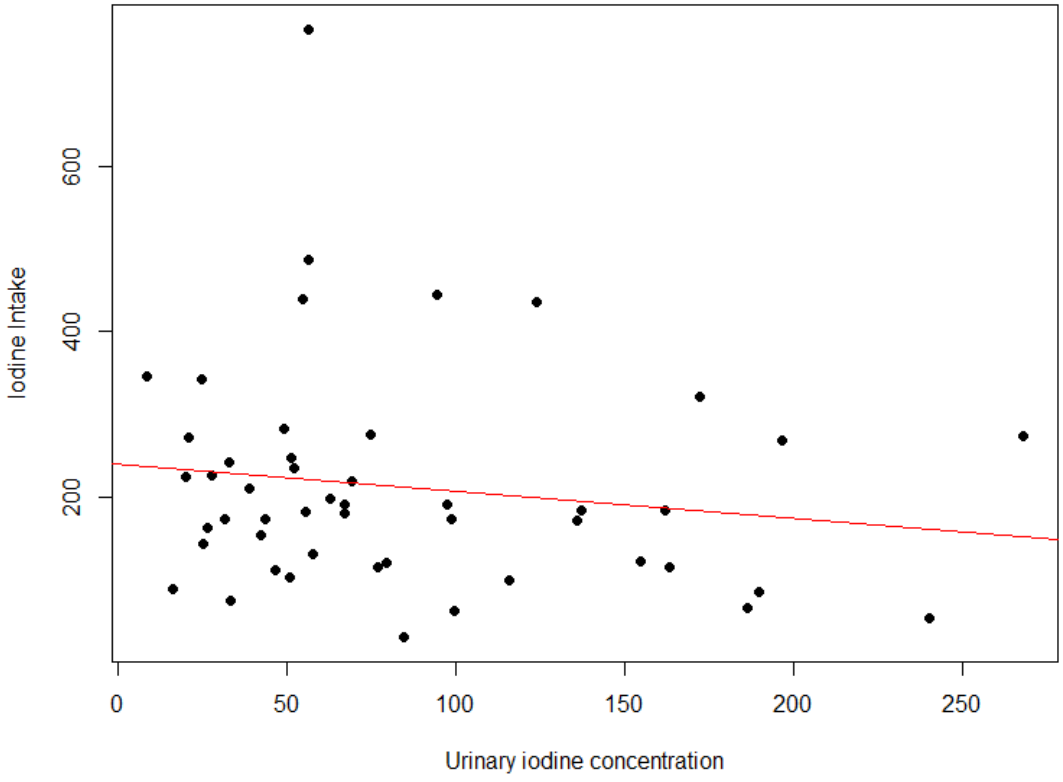
Table 19: Iodine intake of Austrian pregnant women.

	n	Median	IQR	Min	Max	Reference[1]
I (µg/d)	49	182.81	149.46	30.15	765.26	230

1 D-A-CH Reference Values for Nutrient Intake for pregnant women. (9)

The median for the iodine intake was 182.81 µg/d which is below the D-A-CH recommendations of 230 µg/d for Austrian and German pregnant women. Among the subsample, 33 participants (67.3%) did not reach the recommended daily intake. The iodine intake and urinary iodine concentration for 49 women who provided data for both measures are plotted in **Figure 3**.

Figure 3: Urinary iodine concentration and iodine intake – scatterplot (n=49).

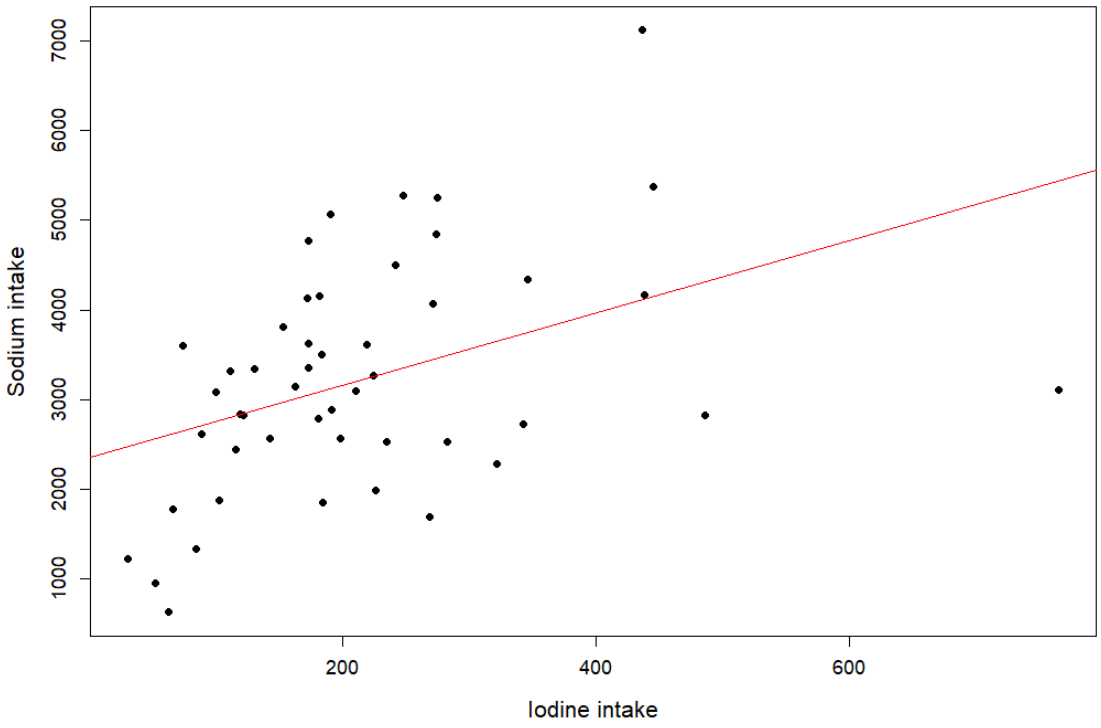


The Spearman correlation was selected as it does not rely on the data to be normally distributed. Spearman's rho was calculated to be -0.18. The test, if the true rho is not equal to 0 resulted in a p-value of 0.21. Therefore, it can be said that no correlation between the intake of iodine and the iodine concentration of spot urine was detected.

As mentioned in **chapter 2.3.2**, it is not possible to calculate the exact iodine intake from food due to missing data on the usage of iodized salt in food manufacturing or out-of-home consumption. (12)

Therefore, the iodine intake is often derived from the sodium intake, assuming that all or a proportion of consumed salt is iodized. This was verified by testing for a correlation between the intake of iodine and the intake of sodium which is shown in **Figure 4**.

Figure 4: Iodine intake and sodium intake – scatterplot (n=49).



The Spearman's correlation was selected and produced a Spearman's rho of 0.46 and a p-value of 0.0011. It can be concluded that a statistically significant correlation between the intake of sodium and iodine was detected.

4.2.6 Analysis of the nutrient status for zinc

The analysis of zinc in spot urine samples was performed for 53 participants. As non-normality was detected the median and IQR were used to describe the urinary data in **Table 20**.

Table 20: Urinary zinc levels of Austrian pregnant women.

	n	Median	IQR	Min	Max	DL
Zn (µg/L)	53	335.62	272.75	<40	1717.09	<40

The determination of one urine sample yielded a value below 0 (-4.95 µg/L). This could have been caused by a functional error during the ICP-OES analysis or other steps in the preparation of the sample. As this value may have affected further analysis, it was excluded. As there is no universally accepted cut-off level for zinc concentrations in spot urine, no assessment of the nutrient status based on this measure could be made.

However, as the EFSA (The European Food Safety Authority) estimated the mean daily urinary zinc losses to be at 0.3 mg for women (87), the corrected subsample including samples of 52 participants will be used to estimate the daily zinc excretion from spot urine.

In this regard, concentration values were multiplied by 1.75 which was also performed in the ANR 2012 to calculate daily sodium excretion based on analyzed spot urine levels. In the ANR 2012, using a small subsample of 19 participants, it was determined that the average daily urine volume was 1.75 L. (40)

As for this master thesis, no 24h urine samples to estimate the daily urine excretion were collected. Therefore, this method should only be considered as an approximation for the real value. Calculated urinary zinc concentration for an estimated 24h urine and the reference values published by the EFSA are shown in **Table 21**.

Table 21: Estimated 24h urinary zinc levels of Austrian pregnant women – corrected subsample.

	n	Median	IQR	Min	Max	Reference[1]
Zn (µg/d)	52	595.27	478.68	105.56	3004.90	300

1 Estimation of daily zinc losses for women by the EFSA converted to µg/d.

The EFSA estimation for zinc losses were used in a study by Likoswe et al. pairing serum zinc levels with spot urine zinc levels for Malawian children and women with the aim to detect threshold levels for zinc in urine for detection of zinc deficiency. The rationale behind the application of these levels is that if the dietary intake of zinc is insufficient, zinc losses through urine will be reduced via homeostatic adaptation of the organism. (88)

After the correction of the subsample and calculation of 24h urinary zinc, the median urinary zinc concentration was 595.27 µg/L with an IQR of 478.68 µg/L.

Using these calculated values, the estimated 24h zinc excretion of 10 participants (19.23%) fell below the EFSA reference levels.

Table 22: Zinc intake of Austrian pregnant women.

	n	Mean	SD	Min	Max	Reference[1]
Zn (mg/d)	49	9.3	4.2	2.9	21.7	9/11/13

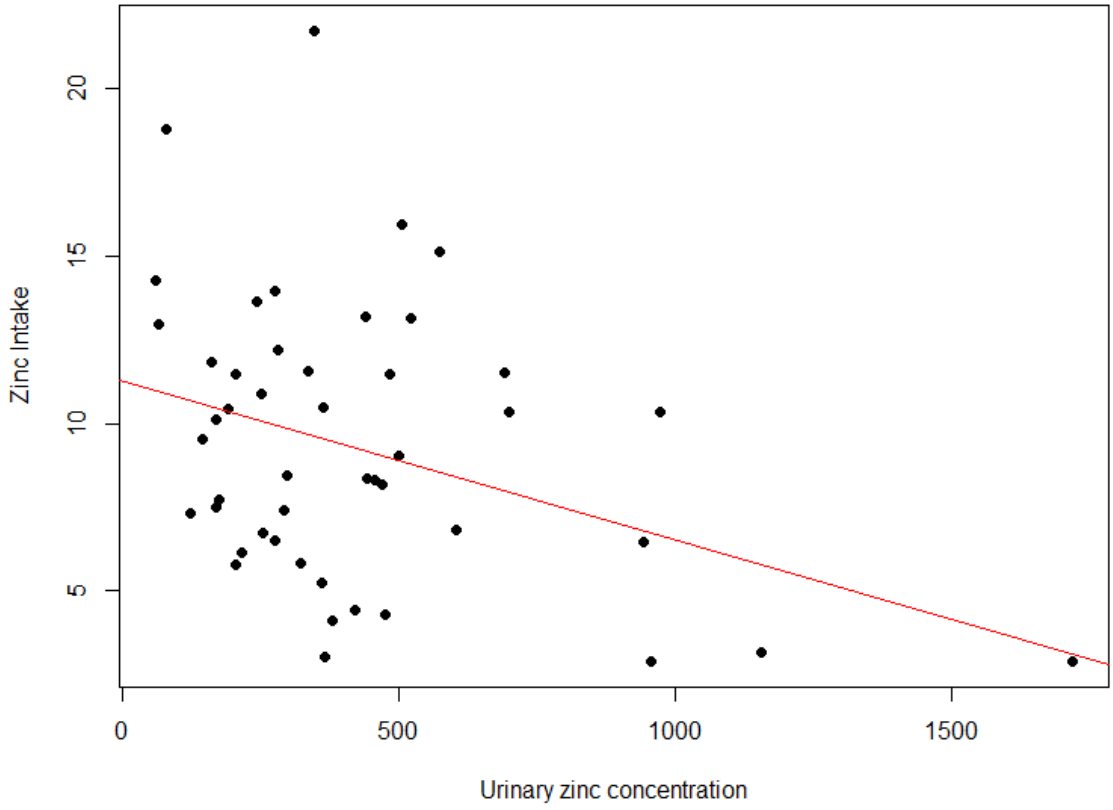
1 D-A-CH Reference Values for Nutrient Intake for pregnant women in the 2nd or 3rd trimester depending on low, moderate, or high phytate consumption. (29)

The average intake of zinc was 9.3 ± 4.2 mg/d. Considering the consumption of moderate phytate levels for all participants in the subsample, the intake of 33 (67.3%) women was below the recommended amount of 11 mg/d.

If we were to assume the consumption of low phytate levels, 24 (49.0%) participants would still fall below the D-A-CH recommendations for daily zinc intake.

The zinc intake and urinary zinc concentration of all women for whom data for both measures were recorded are plotted in **Figure 5**.

Figure 5: Urinary zinc concentration and zinc intake – scatterplot (n=48).



Even though the data for the intake of zinc were normally distributed, the Spearman's correlation was selected as the urinary zinc concentrations of the corrected subsample showed a non-normal distribution.

Statistical testing calculated a Spearman's rho of -0.26 and a p-value of 0.067 for the hypothesis: "the true rho is not equal to 0". The negative correlation and borderline significance level can be explained by the considerably high urinary zinc concentration of one participant at 1717.09 µg/L who also reported the lowest daily zinc intake of the corrected subsample with 2.91 mg/d.

Excluding this implausible value from the Spearman's correlation resulted in a p-value of 0.14 which still led to the conclusion that no correlation between the intake of zinc and the zinc concentration in spot urine was found.

4.2.7 Analysis of the nutrient status for selenium

The statistics for the selenium spot urine levels are shown in **Table 23**. Due to missing information on the amounts of selenium contained in foods, no data on

the intake of selenium were reported and subsequently no correlation could be calculated.

Table 23: Urinary selenium levels of Austrian pregnant women

	n	Median	IQR	Min	Max	DL
Se (µg/L)	53	17.50	13.50	1.00	56.00	<0.58

The median for the urinary selenium concentration was 17.50 µg/L and the IQR 13.50 µg/L. As there are no universally accepted cut-off levels for selenium concentration in spot urine, no assessment of the nutrient status based on this measure could be made.

However, reference values for selenium regarding 24h urine were published by Sauberlich. (89) Therefore, the daily selenium excretion was estimated from the spot urine following the methodology explained in **chapter 4.2.6**. Calculated urinary selenium concentration for an estimated 24h urine and the reference values converted to µg/L are shown in **Table 24**.

Table 24: Estimated 24h urinary selenium levels of Austrian pregnant women.

	n	Median	IQR	Min	Max	Reference[1]
Se (µg/d)	53	30.63	23.63	1.75	98.00	26.07–176.17

1 Calculated from reference values for Se excretion from Sauberlich (89) and the average daily urine volume determined in the ANR 2012 (40) and 1 µmol of Se being equivalent to 79 µg of Se according to Sauberlich.(89)

Using these calculated values, the estimated 24h urinary selenium excretion of 21 women (39.6%) would not reach the reference levels, whereas 32 participants (60.4%) fell inside of the reference range.

4.2.8 Analysis of the nutrient status for sodium

The results of the analysis for the urinary sodium concentration and the sodium intake are shown in **Table 25** and **Table 26** respectively.

Table 25: Urinary sodium levels of Austrian pregnant women.

	n	Mean	SD	Min	Max	DL	Reference[1]
Na (mg/L)	53	2752.3	1237.3	223.2	6306.1	<23.5	1863–4439

1 Calculated from reference values for Na excretion from Sauberlich (89) used in the ANR 2012 (40) and 1 mmol of Na being equivalent to 23.0 mg according to D-A-CH Reference Values for Nutrient Intake. (45)

The mean sodium concentration in spot urine was 2752.3 ± 1237.3 mg/L. Urinary sodium excretion of 33 women (62.3%) fell into the reference range.
For six participants (11.3%), the sodium concentration was above 4439 mg/L and for 14 (26.4%) below 1853 mg/L. The sodium excretion of 2 women (3.8%) was far below the reference range and would be considered as “distinctly reduced levels”. (40)

Table 26: Sodium intake of Austrian pregnant women.

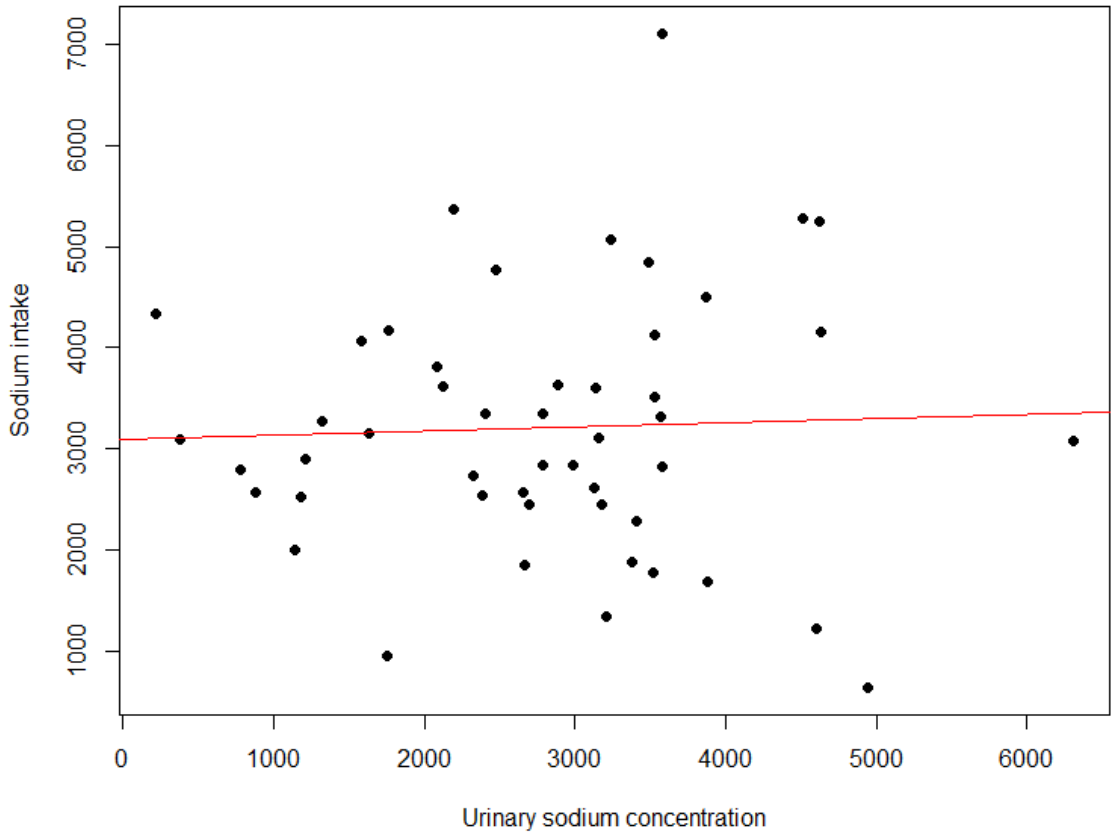
	n	Mean	SD	Min	Max	Reference[1]
Na (mg/d)	49	3207.9	1275.0	629.4	7117.0	1500 mg/d

1 D-A-CH Reference Values for Nutrient Intake for pregnant women. (45)

The mean of the sodium intake of the participants was 3207.9 ± 1275.0 mg/d. Among the subsample, 45 participants (91.8%) would exceed the estimates for adequate daily sodium intake. For 26 women (53.1%), the intake was more than twice as high as the reference value of 1500 mg/d.
The WHO recommends a daily salt intake of less than 5 g, which is approximately 2 g of Na (90). Regarding this cutoff level, the intake of 40 participants (81.6%) was above 2g of sodium per day.

Figure 6 is showing the scatterplot with the data for sodium intake and the urinary sodium concentration for 49 participants.

Figure 6: Urinary sodium concentration and sodium intake – scatterplot (n=49).



As both the data for the intake and for the spot urine concentration of sodium were normally distributed, Pearson's correlation was selected. The test resulted in a Pearson's r of 0.038 and a p-value of 0.79 for the test if the true correlation is not equal to 0. Therefore, it can be said that no correlation between the sodium intake and the concentration of sodium in the urine for this subsample was found.

4.2.9 Analysis of the nutrient status for chloride

The analysis of chloride in spot urine samples was performed for 40 participants. The data for the urinary chloride level and for the daily chloride intake, are displayed in **Table 27** and **Table 28** respectively.

Table 27: Urinary chloride levels of Austrian pregnant women.

	n	Median	IQR	Min	Max	DL	Reference[1]
Cl (g/L)	40	6.12	3.82	1.89	15.58	<0.4	3.51-3.91

1 Calculated from reference values for Cl excretion from Sauberlich (89) used in the ANR 2012 (40) and 1 mmol of Cl being equivalent to 35.5 mg according to D-A-CH Reference Values for Nutrient Intake. (45)

The median for the chloride concentrations in spot urine was 6.12 g/L and the IQR was 3.82 g/L. The number of samples analyzed for chloride was distinctly lower than for the other elements as no more volume was left for the analysis of 13 samples.

Among the 40 analyzed samples, the urinary chloride excretion for 2 women (5%) fell into the reference range. The concentrations for 6 participants (15%) were below 3.51 g/L and for 32 (80%) above 3.91 g/L. For 11 women (27.5%), the urinary chloride excretion was more than twice as high as the upper end of the reference range.

Table 28: Chloride intake of Austrian pregnant women.

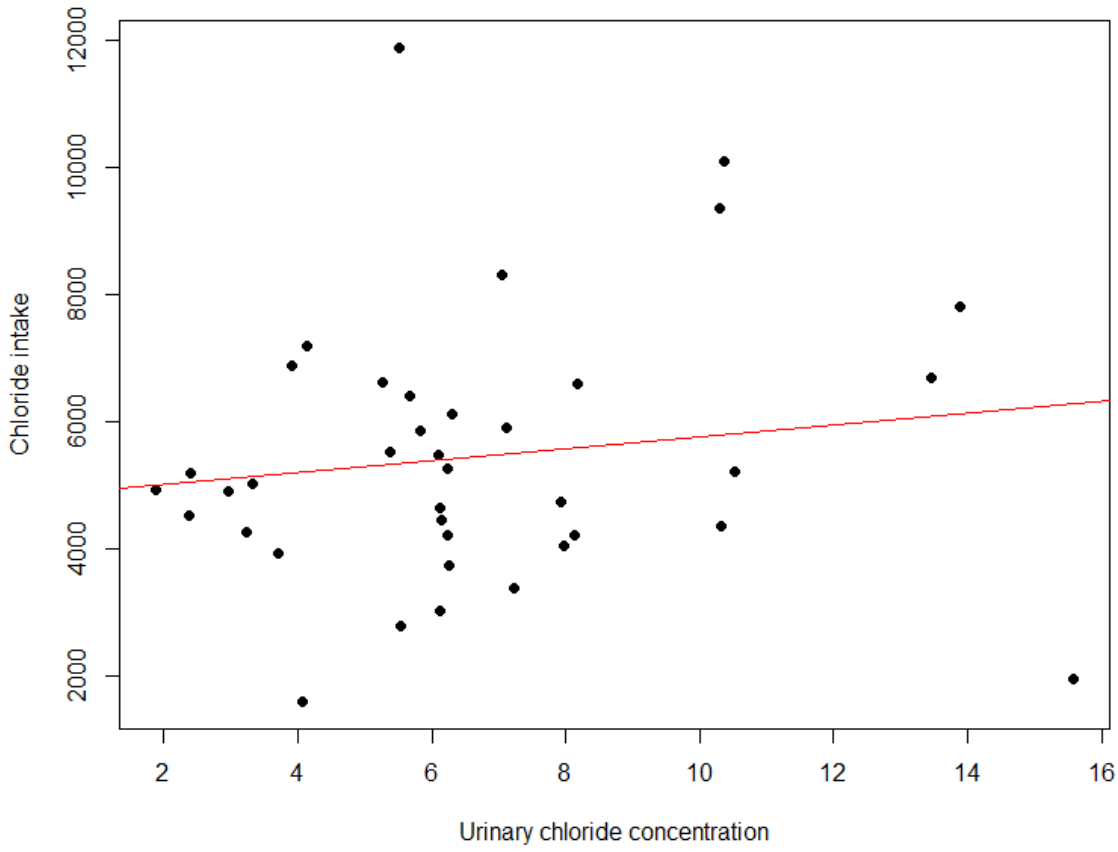
	n	Mean	SD	Min	Max	Reference[1]
Cl (mg/d)	49	5383.3	2132.0	829.2	11890.8	2300 mg/d

1 D-A-CH Reference Values for Nutrient Intake for pregnant women. (45)

Among the subsample, the mean chloride intake was 5383.3 ± 2132.0 mg/d. Of the 49 participants, 46 (93.9%) would exceed the estimates for adequate daily intake at 2300 mg/d. For 30 (61.2%) women, the intake was more twice as high as the D-A-CH references.

The chloride intake and chloride concentration in spot urine for 38 women who provided data for both measures are plotted in **Figure 7**.

Figure 7: Urinary chloride concentration and chloride intake – scatterplot (n=38).



As 11 values for the intake had to be removed when pairing the 2 datasets, the Shapiro-Wilk test for non-normality was repeated for the reduced dataset. The test detected a normal status of borderline significance (p=0.056). The Spearman's correlation was selected to determine a possible correlation as the dataset for the urinary chloride levels still showed non-normality. Statistical testing found a Spearman's rho of 0.11 with a p-value of 0.51. Therefore, no correlation was identified for this element.

4.2.10 Analysis of the nutrient status for calcium

The results of the analysis for the spot urine levels of the microelement calcium and the daily calcium intake are shown in **Table 29** and **Table 31** respectively.

Table 29: Urinary calcium levels of Austrian pregnant women.

	n	Median	IQR	Min	Max	DL
Ca (mg/L)	53	80.59	108.51	11.38	285.60	<0.85

The median for the urinary calcium concentration was 80.59 µg/L and the IQR 108.51 µg/L. Publicly accepted reference levels for the calcium concentration in spot urine do not exist currently. Therefore, no assessment of the nutrient status for calcium derived from the measured urine levels could be made.

However, as there are reference values for the calcium excretion in 24 hours published by Sauberlich (89) which were also used to evaluate the calcium status in the ANR 2012 (40), the same methodology as in **chapter 4.2.6** was applied to the spot urine calcium concentrations.

The calculated values for calcium excretion over 24 hours are shown in **Table 30**.

Table 30: Estimated 24h urinary calcium levels of Austrian pregnant women.

	n	Median	IQR	Min	Max	Reference[1]
Ca (mg/d)	53	141.03	189.89	19.91	499.81	50–400

1 Calculated from reference values for daily Ca excretion from Sauberlich (89) and the average daily urine volume determined in the ANR 2012 (40) and 1 mmol of Ca being equivalent to 40 mg of Ca according to Sauberlich.(89)

After calculating the daily excretion from the spot urine calcium values, concentrations for 44 women (83.0%) fell inside of the reference range. The 24h calcium excretion for 8 participants (15.1%) was below the reference range and for 1 woman (1.9%) above the upper end.

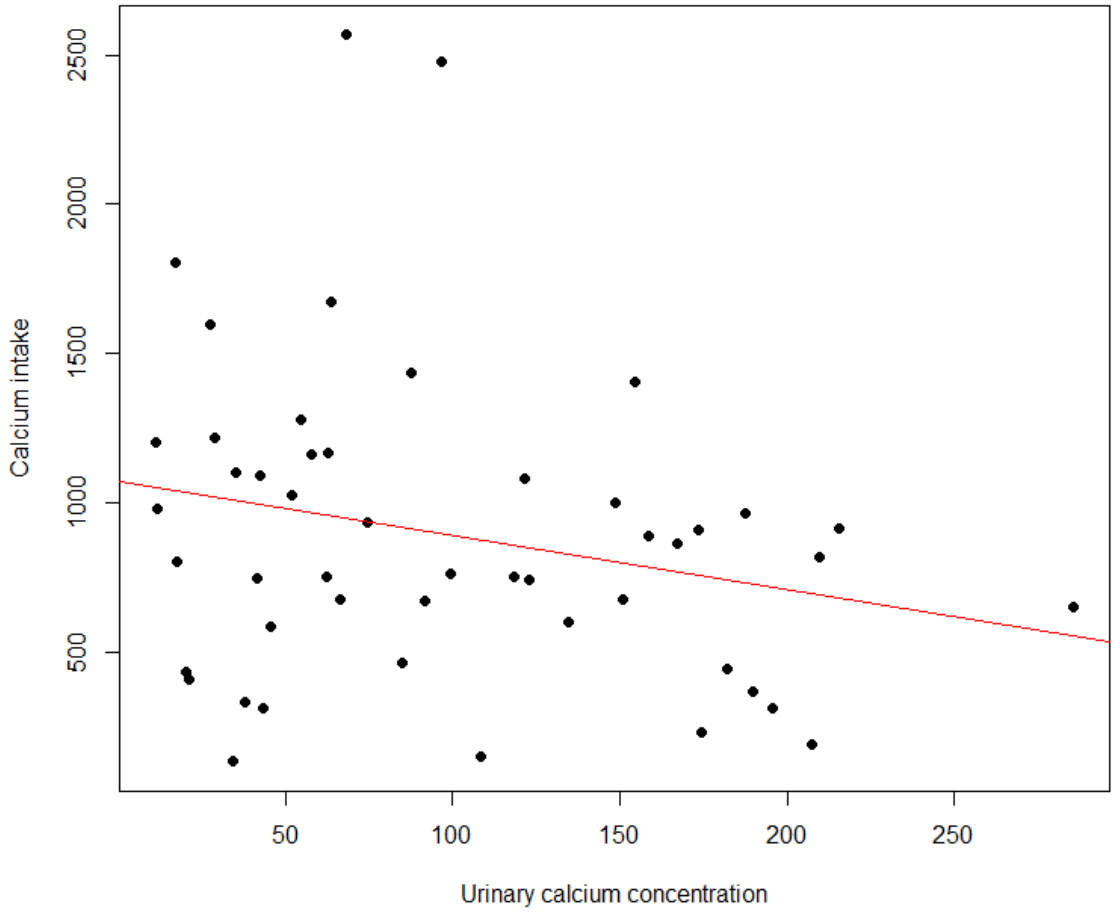
Table 31: Calcium intake of Austrian pregnant women.

	n	Median	IQR	Min	Max	Reference[1]
Ca (mg/d)	49	819.4	516.5	131.8	2566.2	1000 mg/d

1 D-A-CH Reference Values for Nutrient Intake for pregnant women. (9)

The median for the calcium intake was 819.4 mg/d (IQR 516.5 mg/d) which is slightly below the recommended 1000 mg per day. For the calcium intake, 32 women (65.3%) fell below the D-A-CH recommendations and 17 (34.7%) were above. Data for the intake of calcium and the spot urine levels for 49 participants are plotted in **Figure 8**.

Figure 8: Urinary calcium concentration and calcium intake – scatterplot (n=49).



As both datasets for the intake and the urinary calcium concentration were non-normally distributed, possible correlation was determined via Spearman's correlation. The test found a Spearman's rho of -0.25 and a p-value of 0.090 which led to the conclusion that the hypothesis "the true rho is not equal to 0" can be rejected.

4.2.11 Analysis of the nutrient status for magnesium

The analysis of magnesium in spot urine samples was performed for 53 participants whereas the data on the intake is available for 49 participants. As non-normality was detected for both datasets the median and IQR will be used to describe the data shown in **Table 32** for urinary concentration and in **Table 33** for the intake.

Table 32: Urinary magnesium levels of Austrian pregnant women

	n	Median	IQR	Min	Max	DL
Mg (mg/L)	53	43.85	59.77	4.93	148.45	<3.29

The median for the magnesium concentration in the urine was 43.85 mg/L with an IQR of 59.77 mg/L. As there are no publicly accepted cutoff levels for the magnesium concentration in the urine, no assessment of the nutrient status of this element based on this measure could be made.

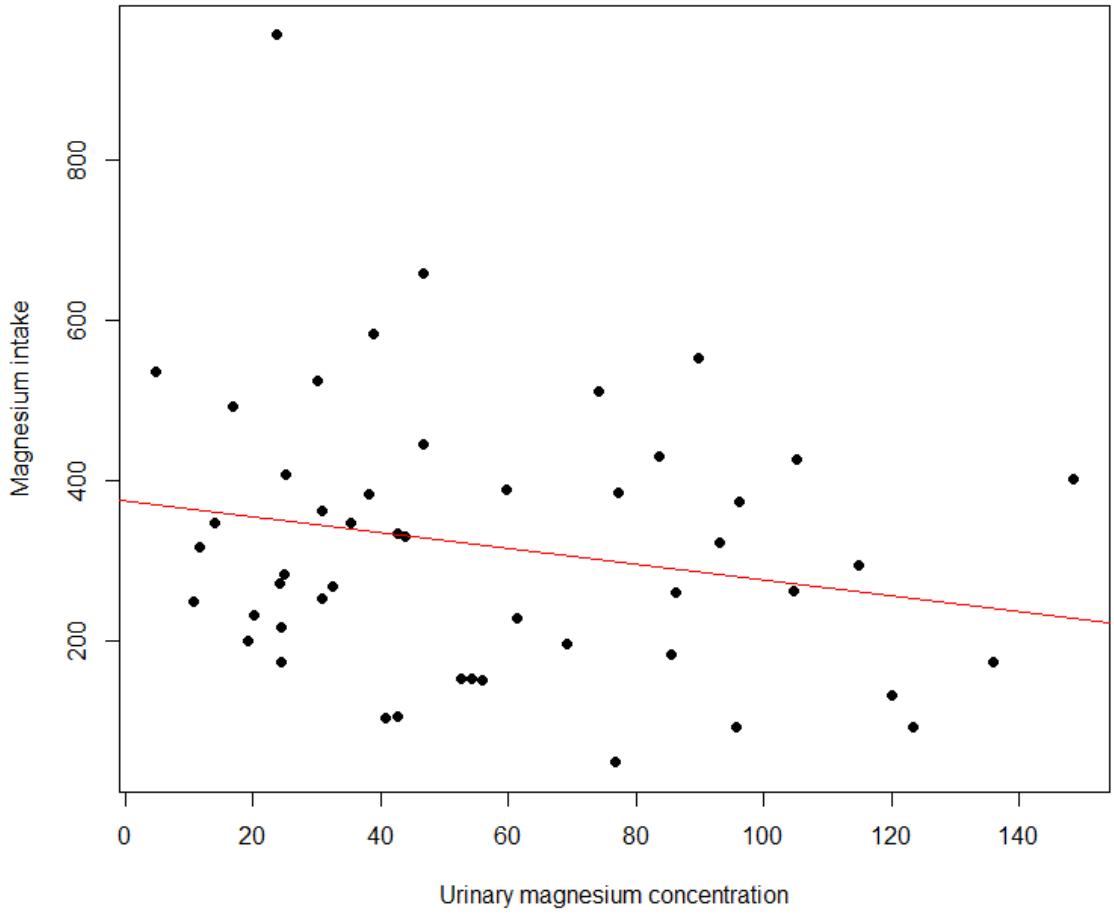
Table 33: Magnesium intake of Austrian pregnant women.

	n	Median	IQR	Min	Max	Reference[1]
Mg (mg/d)	49	293.9	204.5	48.9	955.8	300 mg/d

1 D-A-CH Reference Values for Nutrient Intake for pregnant women. (57)

The median of the magnesium intake was 293.9 mg/d and the IQR 204.5 mg/d. Among the subsample, the intake for 24 (49.0%) women was below the estimates for adequate intake of 300 mg per day. For 25 (51.0%) participants, the intake surpassed the D-A-CH references. The intake of magnesium and the urinary magnesium concentration are plotted in **Figure 9** for 49 participants.

Figure 9: Urinary magnesium concentration and magnesium intake – scatterplot (n=49).



Spearman's correlation was selected as the data for the intake and the magnesium concentration in spot urine were non-normally distributed. The test found a Spearman's rho of -0.18 and a p-value of 0.212. No correlation for the urinary concentration and the intake of magnesium was detected.

4.2.12 Summary of results

The results from **chapter 4.2.3** to **4.2.11** are summarized in **Table 34**. The nutrient status for each element was assessed as "inadequate" or "adequate" and the number of participants for both assessments is given in percentages for every analyzed element.

Regarding the intake for an element, inadequate denotes the number of participants not reaching the D-A-CH reference values. The intake of sodium and chloride were deemed inadequate if the intake was higher than the recommendations. Deficiencies of sodium or chloride are not associated with low intake and very rare and consuming more sodium and chloride than recommended is more prevalent which was previously discussed in detail in **chapter 2.5.1**.

Regarding the assessment of urine levels, a nutrient status was considered inadequate if the reference levels were not reached. For zinc, selenium, sodium, chloride, and calcium, for which reference ranges were calculated, inadequate means that the urine concentrations of participants did not fall inside of the reference range.

Table 34: Nutrient status for all analyzed elements.

		adequate	inadequate	Correlation
Fe	Intake	0%	100%	Not detected
I	Spot urine	17.0%	83.0%	Not detected
	Intake	32.7%	67.3%	
Zn	24h urine	80.8%	19.2%	Not detected
	Intake	32.6%	67.3%	
Se	24h urine	60.4%	39.6%	
Na	Spot urine	62.3%	37.7%	Not detected
	Intake	8.2%	91.8%	

Cl	Spot urine	5.0%	95.0%	Not detected
	Intake	6.1%	93.9%	
Ca	24h urine	83.0%	17.0%	Not detected
	Intake	34.7%	65.3%	
Mg	Intake	49.0%	51%	Not detected

5. Discussion:

5.1 Iron:

As 50.9% of the iron concentrations of the analyzed spot urine samples fell below the limit of detection, the methodology was deemed inappropriate as the iron concentrations in the samples were too low to be accurately determined. Compared to the trace element zinc, which was also analyzed using the same methodology, the urinary median concentration was about 17 times higher than the median concentration of iron.

Procedures to increase the sample concentration e.g., by reducing the volume through evaporation and performing the process of analysis again, could have been a solution for this problem. However, due to the low total sample volume available, it was not possible to perform a 2nd run of the complete analysis using a concentrated sample to attain reliable results for iron.

As the daily excretion of iron through skin, stool and urine is very low (9) no reference values for iron regarding urinary concentration exist. When comparing to the analysis of serum ferritin which is currently the most suitable method (40) the assessment of the iron status via determination of iron levels in urine samples is not an effective procedure.

The determination of urinary iron was performed in this master thesis as analyzing multiple elements using methods such as ICP-OES is efficient considering the amount of time and resources and does not require additional work.

Regarding the intake of iron for the subsample, all 49 participants did not reach the recommended intake of 30 mg/d (9) for pregnant women.

This is similar to the evidence generated by the ANR of 2017, mentioned in **chapter 2.3.1**, which showed that more than 80% of participating women did not reach the D-A-CH recommendations for iron intake. (12)

As it was shown by Zeisler et al., the prevalence of iron deficiency increased with increasing weeks of gestation. (11) Among the women included in this subsample, 55.5% were already in their 3rd trimester at the time of sample collection. This leads to the conclusion that low iron concentrations may have already been considerably prevalent among participants in this subsample. The assessment of the iron status for pregnant women is imperative and multiple measurements throughout pregnancy should be considered as iron deficiency is associated with several APOs as described in detail in **chapter 2.3.1**.

5.2 Iodine:

The urinary iodine concentration of 83.0% of pregnant women analyzed in this master thesis was below 150 µg/L which is considered as an insufficient iodine status according to the WHO. Similar results were found in the study by Lindorfer et al., which was discussed in **chapter 2.3.2**, where the UIC for 81.2% of 246 pregnant women was shown to be below 150 µg/L. (23) As the chosen methodology exhibited considerably high accuracy and precision as described in **chapter 4.1**, these results can be considered reliable. However, a difference was found between the proportions of participants with an inadequate nutrient status derived from the urine levels (83.0%) and from the intake (67.3%).

As described earlier, deriving the iodine intake from the salt intake can only be an estimation of the true iodine intake at best. This may be an explanation for the difference in the percentages between the two approaches. A strong statistically significant correlation between the intake of iodine and the intake of sodium was found, which confirms that the iodine intake was based on the sodium/salt intake in this dataset. Additionally, the dietary intakes of iodine were not correlated with the iodine levels in spot urine. It can be concluded that the determination of the iodine status from the intake may not be a reliable method but possibly yield inaccurate results.

As stated by the WHO, analysis of UIC is a cost-efficient and very accurate measure to assess iodine status of populations. (24) In this subsample of 53 Austrian pregnant women, it was shown that the iodine status for more than two thirds, depending on the approach, was inadequate.

Even though salt iodization may have improved the iodine status of the Austrian population, it cannot be said that this is also the case for pregnant women for whom requirements are greatly increased. To ensure the well-being of mother and

child during pregnancy, as was already stated by Lindorfer et al., a possible iodine supplementation starting before pregnancy should be considered. (23)

5.3 Zinc:

The analysis of zinc resulted in the applied methodologies only being of intermediate performance. Urinary zinc concentrations were considerably lower when compared to elements for which the methodology was of high quality such as calcium or sodium. As described in **chapter 5.1** for iron, procedures to increase the sample concentration may have also been able to improve the performance for zinc.

Some values among the subsample may be impossible or implausible, such as the analysis resulting in a value below 0 for one participant or one participant with the highest urinary zinc concentration reporting the lowest daily zinc intake. It is unclear if these can be attributed to false reporting, errors during the process of analysis and data recording, or the methodology. As the subsample is small, a few potentially wrong values may have affected the data set significantly.

In this master thesis the analysis of estimated 24h zinc urine concentrations found that 19.2% of all participants did not reach the EFSA cutoff values and would thus be at a zinc deficiency. This is in stark contrast to the intake of zinc which showed that 67.3% of the pregnant women, considering moderate phytate consumption, did not reach the D-A-CH recommendations.

In the ANR 2017, it was shown that 31.5% of all participants, which included both men and women, did not reach the recommendations for the intake. (12)

Determination of plasma zinc levels for all 113 pregnant women in the ESÖSM study of whom blood and urine samples were collected, was performed by Driussi. It was shown that plasma zinc concentrations for 28.3% of participants were below the reference value of 53 mg/l published by Sauberlich. (64) There is a considerable difference to the 19.2% of pregnant women in the analyzed subsample which did not reach the EFSA reference values for 24h urinary zinc excretion.

According to Sauberlich measurements of zinc concentration in the urine cannot be considered as a reliable indicator (89). The study by Likowske et al., described in **chapter 4.2.6**, which researched the association between zinc levels in serum and spot urine came to a similar conclusion. The authors found only a poor correlation and stated that urine has limited utility as a biomarker for zinc.

Analyses of plasma or serum zinc levels are more commonly used to assess the zinc status of populations. However, medical professionals to withdraw venous blood and intensive work is required for the analysis which leads to a difficult applicability for large scale surveys. Urine zinc concentration could be a biomarker with much easier sample collection. (88)

Additionally, serum/plasma concentrations of zinc are affected by many factors such as food intake, time of day, inflammatory processes, pregnancy, age, etc. and vary strongly. (29)

Zinc status will often be estimated via the intake of zinc which is also only partially suitable due to its absorption rate not only being influenced by the consumed amount of zinc but also by other factors such as the uptake of phytates, dietary intake of protein (29) and supplementation of iron which competes with zinc as mentioned in **chapter 2.3.3**. Routine supplementation for pregnancy includes iron which can make reaching the increased requirements even more difficult. (30) It cannot be ruled out that the zinc nutrient status for pregnant women in this subsample, may have been worse than what was shown by the assessment of the dietary intake. However, this was not confirmed by the results of this master thesis, as the analysis of the urine samples produced a distinctly lower percentage of participants at an inadequate nutrient status (19.2%) than when derived from the dietary intake (67.3%).

If there are any negative health consequences for zinc deficient pregnant women in this subsample is difficult to determine as the evidence on the effects of zinc deficiency on APO is weak. Most of the studies were conducted in LMIC and can hardly be carried over to Austria which was discussed in **chapter 2.3.3**. The WHO also does not recommend zinc supplementation during pregnancy due to the lack of positive effects on APO. (30)

Both the analysis of serum zinc levels and dietary zinc intake have their weaknesses regarding the assessment of the nutrient status of zinc for populations. As spot urine samples were not identified as a reliable alternative in the scope of this master thesis, future studies researching urinary zinc excretion as a possible biomarker and possible correlation with dietary intake and serum zinc levels should be conducted with 24h samples and higher sample volume which was one of the major weaknesses of the applied methodology.

5.4 Selenium:

For selenium, 24h urine excretion was estimated to compare the analyzed data with reference values published by Sauberlich as described in **chapter 4.2.7**. (89) Following this procedure, the nutrient status for 39.6% of the participants was deemed inadequate.

As it is not possible to record the consumption of selenium from dietary sources, no comparison between the assessments derived from urinary levels and dietary intake could be made. Studies finding a significant correlation between 24h urine selenium concentration and dietary intake (91) must be scrutinized as to whether the authors have access to accurate data on the selenium content of food and if these data could be applied to other populations.

The analysis of urinary selenium levels in this master thesis showed that more than one third of pregnant women in the subsample did not reach the reference values. This assessment is similar to the evidence generated by the ANR 2012 which found that 37.8% of female non-pregnant participants showed deficient plasma selenium levels. (40) Determination of the concentration of selenium in plasma is currently the most convincing marker for the assessment of selenium nutrient status. (9)

According to a statement by the EFSA, 24h urinary selenium concentrations may also be used to determine the selenium status. However, as selenium concentrations in the urine are influenced by recent intake and exhibit high intra-day variability, only the status of populations and not of individuals can be assessed. (92)

The analysis of plasma selenium concentrations for 113 participants in the total sample performed by Driussi found that 31.6% of all pregnant women did not reach the reference area for selenium plasma of 40-80 g/l. (64) These results are comparable to the 39.6% of the analyzed subsample which did not fall into the reference area for urinary selenium published by Sauberlich.

Future studies should be assessing both plasma and 24h urinary selenium concentrations to identify possible correlations between both parameters and confirm if analysis of selenium in 24h urine samples could be a suitable biomarker for determination of the selenium nutrient status of populations which was implied by the results of this master thesis.

Selenium analysis was performed by HGAAS which is not a multi-element technique and distinctly less efficient. Analyses described in **chapter 4.1** showed

that this methodology was of intermediate performance but as mentioned in **chapter 3.2.3** ICP-OES or ICP-MS could not be used for this determination.

5.5 Sodium and chloride:

For sodium and chloride, the measured urinary concentrations were compared to calculated reference ranges published by Sauberlich. (89) Regarding the sodium excretion in the urine, concentrations for 62.3% of participants fell into the reference range. In the ANR 2012, where the sodium status was also evaluated via the reference values from Sauberlich, it was shown that concentrations for over 90% of all adult women fell into the reference range. (40)

The intake of sodium for 91.8% of all pregnant women in this subsample was higher than the D-A-CH estimates for adequate daily sodium. This is slightly worse than what was found in the ANR of 2017 which showed that the sodium intake for 81.2% of all participants exceeded the estimates for an appropriate intake. (12) The values for the intake of this subsample were not compared with the assessment in the ANR 2012 as the D-A-CH reference values for the daily intake of sodium were different before the year 2016. It can be concluded that the sodium status for pregnant women in this subsample was more inadequate than what was shown for adult non-pregnant women in the ANR 2012 and ANR 2017.

The analyses of the subsample showed a considerable difference between the percentage of participants exceeding the estimates for adequate daily intake (91.8%) and the percentage of urinary sodium concentrations above the upper end of the reference range (11.3%). A possible explanation for this discrepancy is that the reference values by Sauberlich may be slightly outdated and too lenient as the evidence on the intake of sodium or salt may have changed over the last decades.

About 86% to 93% of ingested sodium will be excreted via the urine, which means that the sodium excretion is about as high as the sodium intake. (45)

From a rational point of view the assessment of the sodium status should thus also be comparable for both biomarkers. This was not the case for the applied methodology for sodium in this master thesis.

For chloride, the urinary concentrations for 95,0% of all pregnant women was not in the reference range. Analysis of the dietary data showed that the daily intake of chloride exceeded the estimates for adequate daily intake for 93.9% of

participants. It can be concluded that the assessment of the nutrient status for chloride was similar for both measures. This gives the impression that the reference values published by Sauberlich may still be suitable for chloride but not for sodium as mentioned before.

The ANR 2012 also compared urinary chloride excretion to the reference values from Sauberlich. They found that the excretion of chloride for 83.2% of all participants was inside of the reference range. (40) This is very different to the results of the researched subsample which showed that only 5,0% of urinary chloride concentrations fell into the reference range.

If this is due to the different methodology or other reasons cannot be stated at this point. As the reference range for chloride published by Sauberlich is very narrow, it also seems questionable how most chloride concentrations were found to be inside of the reference range in the ANR 2012 when virtually opposite results were produced by the analysis performed for this master thesis.

For the analysis of sodium and chloride in plasma samples by Driussi, all participants showed sodium and chloride plasma concentrations inside or above the reference ranges. (64) This is comparable to what was shown by the urine sample analyses in this master thesis.

In conclusion, as consumption of salt is the major source for both sodium and chloride, the nutrient statuses derived from the intake were inadequate for more than 90% of all pregnant women. These are alarming results, considering that the intake of salt is often underreported, and therefore the true intake could be higher. (12)

5.6 Calcium

For urinary calcium excretion, the comparison between the 24h excretion levels calculated from the spot urine and the reference values by Sauberlich found that 83,0% of pregnant women fell into the reference range and 15.1% were below lower end of the range at 50 g/d. (89)

The calcium analysis of plasma samples performed by Driussi for the total sample consisting of 113 pregnant women showed that calcium concentrations for only 2.65% of all participants fell below the calcium reference levels of 8.76 mg/dl plasma published by Sauberlich. Driussi concluded that the nutrient status of calcium for this sample is very adequate. (64) This can also be stated based on the results derived from the urine samples in this master thesis.

In comparison, the ANR 2012 which used the same methodology, showed that 24h urinary calcium concentrations for 53.1% of female non-pregnant participants fell below the lower end of the reference range (40) which is distinctly higher than the 15.1% found in the current subsample.

Regarding the calcium intake of pregnant women, 65.3% of participants in the subsample did not reach the daily recommendations. Comparable results were found in the ANR 2017 and ANR 2012 which assessed the calcium status for female non-pregnant women and showed that 75% and 70.5% respectively did not reach the D-A-CH recommendations of 1000 mg per day. (12, 40)

As 83% of the subsample fell into the reference range for 24h urinary calcium excretion but almost two thirds did not reach the recommendations for the daily intake, a distinct difference between both methodologies was found in the present analysis. This may partially be explained by the increased calcium absorption during pregnancy (9). Other factors that may have added to this divergence are that calcium concentrations in the urine were compared to a reference range and not a cut-off level and the fact that the 24h urine concentrations were calculated and not measured.

Judging only the assessment of the dietary intake, it can be concluded that Austrian pregnant women may be insufficiently supplied with calcium, which was also stated by Rust et al. in 2020. (31)

The WHO also recommends a calcium supplementation during pregnancy for the prevention of preeclampsia and complications for populations with low intake of calcium. (55) However, taking the aforementioned results of plasma and urine samples into consideration, it is questionable if this recommendation can be given based on data of dietary calcium intake alone or if it needs a confirmation through assessment of the calcium status through plasma or urine analysis.

5.7 Magnesium

As there are no reference values for the excretion of magnesium in the urine, the nutrient status could only be evaluated based on the magnesium intake.

To determine magnesium status in clinical practice, serum concentrations are often used even though they are not truly representative of the real magnesium content. As less than 1% of total body magnesium is contained in the serum and little correlation between serum magnesium and the concentration of magnesium

in specific tissues or the whole body were found, serum magnesium levels are not unequivocally a reliable biomarker for the assessment of the nutrient status. (93)
No publicly accepted reference values for urinary magnesium exist. In order to identify those, balance studies researching the association of serum and urine levels need to be conducted. (94) As no statistically significant correlation between the intake of magnesium and spot urine concentrations were found in this master thesis, no cutoff-levels for urinary magnesium could be derived from the intake.
Serum magnesium levels of participants in this subsample were analyzed by Driussi (64) but data used in Driussi's thesis could not be matched with the participants of the subsample. Analyzing serum in addition to the spot urine samples would also have exceeded the scope of this master thesis.
Analyzing the daily intake of Austrian pregnant women in the researched subsample showed that 49% of all participants did not reach the recommendations for the daily intake. Very similar results were published in the ANR 2017 which found that the magnesium daily intake for 51.4% of all participants was below the D-A-CH references. (12)
Magnesium deficiencies are rare and the evidence of possible influence on APOs in healthy pregnant women is weak. Additionally, supplementation of magnesium during pregnancy can also not be recommended due the lack of evidence on positive effects on health. (57) Therefore, no conclusions on possible negative effects derived from the presented magnesium intake data on the health of participants in the subsample could be made.
The determination of magnesium in large scale studies through multi-element techniques is very easy and cost-efficient. If reference values for urinary magnesium were known, this methodology could be applied in studies on LMIC populations. Pronounced micronutrient deficiencies are highly prevalent in these populations and negative effects on the health of pregnant women are common as was described in the evidence presented in **chapter 2.5.3**. Balance studies researching the correlation of serum and urinary magnesium concentrations to identify reference values for magnesium in urine samples should be conducted in the future.

6. Conclusion

One weakness of the methodology that was applied in this master thesis was that all samples were analyzed in one run. This was showcased in the methodology only being of intermediate performance for zinc and inappropriate for iron. For accurate results, multiple runs with different concentrations of the sample solution should be performed as not all elements can be analyzed in one run. However, when choosing the process to increase the concentration of an element in a sample solution it must be ensured that no noticeable losses occur, particularly for elements present in ppm concentrations such as iodine, iron, selenium, and zinc. Another solution for this problem would be to use methods with very low detection limits such as ICP-MS for ultra-trace analyses.

It can be concluded that the analysis of iodine in spot urine samples which was performed via ICP-MS was of high performance and produced very reliable results. As ICP-MS was only calibrated to be used for the determination of iodine, future studies should be conducted employing ICP-MS for the analysis of iron, zinc, and selenium in urine samples.

The assessment of the nutrient status for zinc and calcium produced conflicting results. Determination of urinary concentrations found that most of the pregnant women in this subsample were at an adequate nutrient status for both elements whereas dietary intake showed that about two thirds of the participants was below the DACH-recommendations which is virtually the opposite. However, this does not necessarily imply that two thirds were at a deficiency as the DACH reference values include an added safety margin.

For calcium, comparing the results of the analysis of plasma samples performed by Driussi with the present results for urinary calcium, it can be stated that as both assessments were comparable, the analysis of calcium in urine samples may be a reliable biomarker to determine the nutrient status.

As considerable differences were found between the results of the analyses of plasma levels, urinary concentrations and dietary intake of zinc, this thesis was unable to show if determination of zinc in spot urine can be used to confidently assess the nutrient status or not.

The assessment of the nutrient status for selenium, sodium, chloride produced comparable results across all analysed parameters with one exception being the comparison of urinary sodium concentrations with the reference range published

by Sauberlich leading to a partially implausible assessment for the nutrient status of sodium. Considering a possible explanation that the reference range need to be updated, the analyses of urine samples for all three elements can be considered as reliable biomarkers for the assessment of the nutrient status.

For magnesium, future studies need to be conducted regarding the identification of reference values for urinary magnesium before statements regarding the applied methodology can be made.

In conclusion, the results of this master thesis indicate that the nutrient status for five out of eight of the analysed elements can be determined via analysis of urine samples and reliable assessments in this regard can be made.

7. Zusammenfassung

Ausreichende Versorgung mit Mineralstoffen ist besonders in der Schwangerschaft essenziell, da die Gesundheit der Mutter als auch die des Fötus durch Mangelerscheinungen negativ beeinflusst werden. Um möglichen negativen Schwangerschaftsverläufen vorzubeugen, ist es wichtig den Versorgungsstatus mit Mineralstoffen zu überprüfen.

Das Ziel der Masterarbeit ist den Mineralstoffstatus von Eisen, Jod, Zink, Selen, Natrium, Chlorid, Calcium und Magnesium bei Schwangeren durch Analysen von Spontanurinproben zu bestimmen und zu evaluieren, ob diese Auswertung zuverlässige Ergebnisse zur Ermittlung des Nährstoffstatus erzielen kann.

Diesbezüglich wurden die Konzentrationen der zuvor genannten Mineralstoffe in Harnproben von 53 schwangeren österreichischen Frauen bestimmt. Die Ergebnisse wurden mit den ernährungsbedingten Aufnahmemengen und den Daten der Plasmaanalysen, welche im Vorfeld der Arbeit erfasst bzw. durchgeführt wurden, verglichen, um eine Beurteilung des Mineralstoffstatus durchzuführen. Ebenfalls wurden Korrelationen zwischen der Aufnahme und den Harnkonzentrationen der Mineralstoffe untersucht.

Die Harnanalysen von Jod, Selen, Natrium und Chlorid erwiesen sich im Vergleich zur Nährstoffaufnahme und Plasmakonzentrationen als zuverlässige Indikatoren hinsichtlich der Bestimmung des Nährstoffstatus. Da die Analyse von Eisen ergab, dass Harnkonzentrationen zu niedrig waren um korrekt bestimmt zu werden, ist die angewandte Methodik für Eisen ungeeignet. Bei Zink gab es Unterschiede zwischen den Ergebnissen der Harnanalysen, Plasmakonzentrationen und Aufnahmedaten wodurch die Analyse von Spontanurinproben zur Bestimmung des Versorgungsstatus als nicht ausreichend gesehen werden kann. Die Beurteilungen des Ernährungsstatus von Calcium im Harn und Plasma ergaben vergleichbare Ergebnisse, die sich von der Nährstoffaufnahme stark unterschieden. Dies kann teilweise durch gesteigerte Absorption während der Schwangerschaft begründet werden. Aufgrund fehlender Referenzwerte für Harnkonzentrationen konnte keine Beurteilung für Magnesium durchgeführt werden.

Basierend auf den Ergebnissen der Harnanalysen waren die Probandinnen mit Zink, Selen und Calcium ausreichend versorgt. Die Jodversorgung war unzureichend und die für Natrium und Chlorid zu hoch.

8. Summary

Adequate supply of minerals is particularly essential during pregnancy, as deficiencies can negatively impact the health of both the mother and the fetus. To prevent potential adverse pregnancy outcomes, it is important to assess the mineral status.

The aim of the master's thesis is to determine and evaluate the mineral status of iron, iodine, zinc, selenium, sodium, chloride, calcium, and magnesium in pregnant women through analyses of spontaneous urine samples, aiming to ascertain whether this evaluation can yield reliable results for determining the nutrient status.

In this regard, concentrations of the aforementioned minerals were determined in urine samples from 53 pregnant Austrian women. The results were compared with dietary intake levels and data from plasma analyses conducted or collected prior to the study to assess the mineral status. Additionally, correlations between intake and urine concentrations of the minerals were examined.

The urine analyses for iodine, selenium, sodium, and chloride proved to be reliable indicators compared to nutrient intake and plasma concentrations for determining the nutrient status. However, the analysis for iron indicated that urine concentrations were too low to be accurately determined, making the methodology unsuitable for assessing iron status. In the case of zinc, there were discrepancies among the results of urine analyses, plasma concentrations, and intake data, indicating that the analysis of spontaneous urine samples may not be sufficient to determine the supply status. Assessments of the nutritional status of calcium in urine and plasma yielded comparable results that significantly differed from nutrient intake, partly attributable to increased absorption during pregnancy. Due to the lack of reference values for urine concentrations, no assessment could be made for magnesium.

Based on the results of the urine analyses, the subjects were adequately supplied with zinc, selenium, and calcium. However, iodine supply was inadequate, while sodium and chloride levels were too high.

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ANNEX

Table A1: Main reagents used in the sample analysis:

HNO_3 - Nitric acid 65%, Suprapur®, Art. 100441 (Merck KGaA, Darmstadt, Germany)
HCl - Hydrochloric acid 37%, AnalaR NORMAPUR®, Art. 20252.335 (VWR Chemicals International S.A.S, Fontenay-sous-Bois, France)
$KClO_3$ - Potassium chlorate, AnalaR NORMAPUR®, Art. 26746.396 (VWR Chemicals International BVBA, Leuven, Belgium)
NaOH - Sodium hydroxide pellets, pro analysi, Art. 106498 (Merck KGaA, Darmstadt, Germany)
$NaBH_4$ - Sodium borohydride 98%, to prepare solutions in chapter 3.2.3. Art. 13432 (Alfa Aesar GmbH, Karlsruhe, Germany)
KIO_3 - Potassium iodate, pro analysi, to prepare solutions in chapter 3.2.4. Art. 5053 (Merck KGaA, Darmstadt, Germany)
Selenium standard 1000 mg Se, (SeO_2 in HNO_3), Titrisol®, Art. 109915 (Merck KGaA, Darmstadt, Germany)
Chloride standard solution 1000mg/L Cl^-, traceable to SRM from NIST, NaCl in H_2O, CertiPUR®, Art. 1.19897 (Merck KGaA, Darmstadt, Germany)
$NaNO_3$ - Sodium nitrate, pro analysi, used as ISA in chapter 3.2.5. Art. 6537 (Merck KGaA, Darmstadt, Germany)
$KClO_3$ digestion solution: dissolve 20g of $KClO_3$ in 200 mL ultrapure water, add 80 mL 65% HNO_3, fill into plastic bottle, shake before withdrawing volume
Ultrapure water, from: Millipore Milli-Q® Plus, Water Purification System (Millipore Corp) 18.2 MΩcm resistivity
ClinChek® - Urine Control lyophilised Art. 8922 (RECIPE Chemicals + Instruments GmbH, Munich, Germany)
ALVA 04/01 Pflanzenprobe für Ringversuche

Table A2: Equipment used in chapter 3.2.1.

Microwave oven:
MLS 1200 mega + HPR-1000/6 rotor system (MLS Microwave Laboratory Systems GmbH, Leutkirch, Germany)
Digestion program: 3 min at 250 W 2 min at 0 W 5 min at 250 W 5 min at 400 W 6 min at 500 W 20 min at 0 W for ventilation
Polystyrene multipurpose containers with snap lid (Greiner Bio-One GmbH, Frickenhausen, Germany)

Table A3: Equipment used in chapter 3.2.2. Analysis of urinary iron, zinc, sodium, calcium, magnesium via ICP-OES

ICP-OES	
Perkin Elmer Optima™ 3000 XL (PerkinElmer Inc)	
GemTip™ cross-flow pneumatic nebulizer	
Ryton® double-pass Scott-type spray chamber	
Axial view plasma	
Simultaneous multi-element detection	
Autosampler: AS-90 plus (PerkinElmer Inc)	
Software: WinLab32™ (PerkinElmer Inc)	
Method #1 for pure digests:	
RF (Radio Frequency) power	1450 W
Sample flow rate	1.2 mL/min
Read delay	50 sec
Integration time	10 sec for each of 2 replicates
Wash time	60 sec at 1.35 mL/min
Argon flow rates:	
Plasma gas flow	15 L/min
Auxiliary gas flow	0.5 L/min
Nebulizer gas flow	0.7 L/min
Method #2 for 1:10 and 1:20 dilution stages:	
RF (Radio Frequency) Power	1350 W
Sample flow rate	1.3 mL/min
Read delay	50 sec
Integration time	10 sec for each of 2 replicates
Wash time	40 sec at 1.3 mL/min
Argon flow rates	
Plasma gas flow	15 L/min
Auxiliary gas flow	0.5 L/min
Nebulizer gas flow	0.75 L/min
Diluter / dispenser Microlab® 1000 (Hamilton, Bonaduz, Switzerland)	

Table A4: Reagents used to prepare four standard solutions for each of both methods applied in chapter 3.2.2.

Al, Aluminium standard 1000 mg Al, ($AlCl_3$ in H_2O) Titrisol®, Art. 109967 (Merck KGaA, Darmstadt, Germany)
Ca, Calcium standard 1000 mg Ca, ($CaCl_2$ in 6.5% HCl) Titrisol®, Art. 109943 (Merck KGaA, Darmstadt, Germany)
Cd, Cadmium standard 1000 mg Cd, ($CdCl_2$ in H_2O) Titrisol®, Art. 109960 (Merck KGaA, Darmstadt, Germany)
Co, Copper standard 1000 mg Cu, ($CuCl_2$ in H2O) Titrisol®, Art. 109987 (Merck KGaA, Darmstadt, Germany)
Cr, Chromium standard 1000 mg Cr, ($CrCl_3$ in 4.2% HCl) Titrisol®, Art. 109948 (Merck KGaA, Darmstadt, Germany)
Fe, Iron standard 1000 mg Fe, ($FeCl_3$ in 15% HCl) Titrisol®, Art. 109972 (Merck KGaA, Darmstadt, Germany)
Mg, Magnesium standard 1000 mg Mg, ($MgCl_2$ in 6% HCl) Titrisol®, Art. 109949 (Merck KGaA, Darmstadt, Germany)
Mn, Manganese standard 1000 mg Mn, (MnCl2 in H2O) Titrisol®, Art. 109988 (Merck KGaA, Darmstadt, Germany)
Mo, Molybdenum standard 1000 mg Mo, [$(NH_4)6Mo_7O_{24}$. $4H_2O$ in 0.7% NH4OH] Titrisol®, Art. 109926 (Merck KGaA, Darmstadt, Germany)
Na, Sodium standard 1000 mg Na, (NaCl in H_2O) Titrisol®, Art. 109927 (Merck KGaA, Darmstadt, Germany)
P, Phosphate standard 1000 mg PO_4 (H_3PO_4 in H_2O) Titrisol®, Art. 109870 (Merck KGaA, Darmstadt, Germany)
Pb, Lead standard 1000 mg Pb, ($Pb(NO_3)_2$ in H_2O) Titrisol®, Art. 109969 (Merck KGaA, Darmstadt, Germany)
S, K2SO4, pro analysi, Art. 5153 (Merck KGaA, Darmstadt, Germany)
Sb, Antimony standard 1.000 g/L ($SbCl_3$ in HCl 5 mol/L) Art. 19771 (Merck KGaA, Darmstadt, Germany)
Sn, Tin ICP standard 1000 mg/L Sn, traceable to SRM from NIST, $SnCl_4$ in HCl 7%, CertiPUR®, Art. 170362 (Merck KGaA, Darmstadt, Germany)

Table A4: Reagents used to prepare four standard solutions for each of both methods applied in chapter 3.2.2 – continuation.

Sr, Strontium standard 1000 mg Sr, (SrCl2 in 7% HCl) Titrisol®, Art. 109993 (Merck KGaA, Darmstadt, Germany)
V, Vanadium standard 1000 mg V, (VOSO4 in 8.6% H2SO4) Titrisol®, Art. 109994 (Merck KGaA, Darmstadt, Germany)
Zn, Zinc standard 1000 mg Zn, (ZnCl2 in 0.06% HCl) Titrisol®, Art. 109953 (Merck KGaA, Darmstadt, Germany)
ICP multi-element standard solution I (19 elements in dilute nitric acid) CertiPUR®, Art. 115474 (Merck KGaA, Darmstadt, Germany)
ICP multi-element standard solution III (4 elements in dilute nitric acid) 1000 mg/L: Ba, Ca, Mg, Sr, CertiPUR®, Art. 115626 (Merck KGaA, Darmstadt, Germany)

Table A5: Element concentrations of standard solutions in mg/L used in 3.2.2. Standards for method #1 - prepared with $KClO_3$ digestion solution.

	Standard 1	Standard 2	Standard 3	Standard 4
Al 396.150	2,00	4,00	10,00	2,00
Ba 455.405	4,10	0,20	0,00	2,00
Be 313.109	0,02	0,04	0,00	0,00
Ca 315.892	4,00	10,00	40,00	62,00
Cd 228.803	0,40	0,80	2,00	0,00
Cd 214.439	0,40	0,80	2,00	0,00
Co 238.893	0,40	0,80	0,00	0,00
Co 228.617	0,40	0,80	0,00	0,00
Cr 267.713	0,50	1,00	2,00	0,00
Cr 205.562	0,50	1,00	2,00	0,00
Cr 357.874	0,50	1,00	2,00	0,00
Cu 324.758	0,40	0,80	2,00	0,00
Fe 234.350	0,30	0,60	2,00	10,00
Fe 259.942	0,30	0,60	2,00	10,00
Mn 257.614	0,10	0,20	1,00	2,00
Mn 294.924	0,10	0,20	1,00	2,00
Mo 202.035	0,00	1,00	2,00	4,00
Ni 232.006	1,00	2,00	0,00	0,00
Ni 231.606	1,00	2,00	0,00	0,00
P 213.621	0,00	0,00	10,00	20,00
Pb 220.357	4,00	8,00	2,00	0,00
Sb 206.837	0,00	2,00	4,00	8,00
Sb 231.148	0,00	2,00	4,00	8,00
Sn 189.930	0,00	2,00	4,00	8,00
Sr 421.538	4,02	0,04	0,00	2,00
V 292.404	0,00	1,00	2,00	4,00
Zn 213.859	0,40	0,80	2,00	4,00

Table A5: Element concentrations of standard solutions in mg/L used in 3.2.2. – continuation. Standards for method #2 - prepared with diluted HNO_3.

	Standard 1	Standard 2	Standard 3	Standard 4
Ca 315.892	1,00	2,00	3,00	0,00
Mg 280.274	5,00	10,00	20,00	0,00
Mg 285.213	5,00	10,00	20,00	0,00
Na 589.577	5,00	10,00	20,00	40,00
Na 330.243	5,00	10,00	20,00	40,00
P 214.917	5,00	10,00	20,00	40,00
P 213.621	5,00	10,00	20,00	40,00
S 180.674	3,33	6,66	10,00	0,00
S 182.562	3,33	6,66	10,00	0,00
Sr 421.538	0,50	1,00	2,00	0,00

Table A6: Equipment and reagents used in chapter 3.2.3.

$NaBH_4$ solution:	
5 g of NaOH dissolved in 500 ml ultrapure water to create alkaline solution. 15 g of $NaBH_4$ added	
HGAAS	
Perkin Elmer 3030 Atomic Absorption Spectrophotometer (PerkinElmer Inc)	
equipped with Mercury/Hydride System, MHS-20 (PerkinElmer Inc)	
System 2 Selenium Electrodeless Discharge Lamp, N3050672 (PerkinElmer Inc)	
Operating conditions	
Lamp current	280 mA
Wavelength	196.0 nm
Spectral slit width	0.7 nm
Purge #1 time	30 sec
Reaction time	13 sec
Purge #2 time	20 sec
Cell temperature	950 °C
Batch hydride generation method	

Table A7: Equipment used in 3.2.4 Analysis of urinary iodine via ICP-MS

ICP-MS	
Perkin Elmer SCIEX® ICP mass spectrometer ELAN® DRC II (PerkinElmer Inc)	
Meinhard® nebulizer	
Cyclonic spray chamber	
Autosampler: AS-93 plus (PerkinElmer Inc)	
Software: ELAN® 3.4 (PerkinElmer Inc)	
Operating conditions	
RF (Radio Frequency) power	1350 W
Autolens voltage	7 V
Argon flow rates	
Plasma gas flow	15 L/min
Auxiliary gas flow	1.2 L/min
Nebulizer gas flow	0.9 L/min
Sample flow rates	
Peristaltic pump speed	20 rpm
Sample flush	45 sec
Read delay	40 sec
Integration time	1450 msec
Wash time	70 sec
Detection	
Dual-stage discrete dynode detector:	
Analog stage - for high intensity signals	
Pulse stage (digital) - for low intensity signals	
Both signals are measured simultaneously	
Peak hopping method	
4 replicates	
Diluter / dispenser Microlab® 530B (Hamilton, Bonaduz, Switzerland)	

Table A8: Solutions used to control for possible interferences in chapter 3.2.5.

100 mg/L Cl - 4 mL	Na_2SO_4 - 4 mL	$NaNO_3$ (5 mol/L) - 160 µL
100 mg/L Cl - 2 mL	Na_2SO_4 - 6 mL	$NaNO_3$ (5 mol/L) - 160 µL
100 mg/L Cl - 4 mL	Na_2HPO_4 - 4 mL	$NaNO_3$ (5 mol/L) - 160 µL
100 mg/L Cl - 2 mL	Na_2HPO_4 - 6 mL	$NaNO_3$ (5 mol/L) - 160 µL
100 mg/L Cl - 4 mL	ultrapure water - 4 mL	$NaNO_3$ (5 mol/L) - 160 µL
100 mg/L Cl - 2 mL	ultrapure water - 6 mL	$NaNO_3$ (5 mol/L) - 160 µL

Table A9: Equipment used in chapter 3.2.5. Analysis of urinary chloride via ISE on a pH meter

SCHOTT® Instruments ISE - Combination Electrode Cl 60 (SI Analytics GmbH, Mainz, Germany)	
Combination electrode → no separate reference electrode required	
Measuring range	2 - 35000 mg/L Cl^-
Reproducibility	±2%
pH range	2 - 12
Temperature range	0 - 80 °C
Membrane material	Pressed piece of ion crystal
Outer chamber filling solution, 1 mol/l KNO_3, ELY/BR/503, Art. 106575 (WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany)	
Metrohm 691 pH Meter, Type 1.691.0020 (Metrohm AG, Herisau, Switzerland)	