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„Maternal Iron Intake: A Pilot Cohort Study on Maternal Iron Status, Neonatal Iron Levels, and Placental Expression of Transferrin Receptor 1 and Ferritin“

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Abbreviations

AKH Wien	Allgemeines Krankenhaus Wien, General Hospital Vienna
BLS	Bundeslebensmittelschlüssel
BMI	Body Mass Index
BP	Basal plate
CP	Chorionic plate
CRP	C-reactive protein
Ct	Cycle Threshold
CTB	Cytotrophoblast
D-A-CH	Germany, Austria and Switzerland
DIOS	Dysmetabolic iron overload syndrome
DMT1	Divalent metal ion transporter 1
Dyctb	Duodenal cytochrome b
EPO	Erythropoietin
Fe^{+2}	Ferrous iron; heme iron
Fe^{+3}	Ferric iron; non-heme iron
FEC	Fetal endothelial cells
FFQ	Food Frequency Questionnaire
FPN1	Ferroportin 1
Ft	Ferritin
FTH1	Ferritin heavy chain
FTL1	Ferritin Light Chain
GW	Gestational Week
Hb	Hemoglobin
HBC	Hofbauer cells
HCP1	Heme-carrier protein 1
ID	Iron deficiency
IDA	Iron deficiency anemia
IgG	Immunoglobulin G
IRE/IRP	Iron-Responsive Element/Iron-Regulatory Protein
IV	Intravenous
IVF	In-Vitro-Fertilisation
n.a.	Not available
NAFLD	Non-alcoholic fatty liver disease
NIR	Near-infrared
NTBI	Non-transferrin bound iron
ÖNWT	Österreichische Nährwerttabelle
p.c.	Post conception
PHT	Primary human trophoblast
RBCT	Red blood cell transfusion
RDA	Recommended Dietary Allowance
ROS	Reactive oxygen species
qRT-PCR	Quantitative Real-Time Polymerase Chain Reaction
SGA	Small for gestational age
sFe	Serum Iron
SNPs	Single-nucleotide polymorphisms
STB	Syncytiotrophoblast
STEAP	Six-transmembrane epithelial antigen of the prostate
sTfR	Soluble transferrin receptor

TBI	Transferrin bound iron
Tf	Transferrin
TFR1	Transferrin Receptor 1
Tfsat	Transferrin saturation
UBC	Ubiquitin C
UC	Umbilical cord
WHO	World Health Organization
ZIP	ZRT/IRT-like protein

Zusammenfassung

Die Gewährleistung einer ausreichenden Eisenversorgung für Schwangere und ihre ungeborenen Kinder bleibt weiterhin eine Herausforderung. Es ist noch nicht abschließend geklärt, ob bzw. inwiefern eine verringerte Eisenzufuhr bei der Mutter zu Eisenmangelanämie (iron deficiency anemia: IDA) führt und sich diese auf das ungeborene Kind übertragen kann. Insbesondere die genaue Rolle der Plazenta und ihre Fähigkeit zur Regulation bei maternaler IDA sind bisher nicht vollständig verstanden. Ziel dieser Arbeit war es, zu zeigen, 1) ob eine unzureichende Eisenzufuhr mit maternaler IDA assoziiert ist, 2) ob sich eine maternale IDA in einem verringerten Eisenstatus des Neugeborenen wiederfindet und 3) ob in den Plazenten von Frauen mit IDA eine veränderte Gen- und Proteinexpression des Transferrinrezeptor 1 (TFR1) sowie der leichten Kette von Ferritin (FTL1) auftritt. Dafür wurden 97 Mutter-Neugeborenen-Paare im Allgemeinen Krankenhaus Wien rekrutiert und ihr Eisenstatus erhoben (Hämoglobin, Eisen, Ferritin, Transferrin, Transferrinsättigung, löslicher Transferrinrezeptor sowie C-reaktives Protein). Zudem wurde die plazentare Expression von TFR1 und FTL1 untersucht. Mittels Fragebogendaten (Food Frequency Questionnaire) wurde die maternale Eisenversorgung über die Ernährung berechnet. IDA wurde definiert als Hämoglobin <11 g/dl und Ferritin <30 µg/l. In dieser Studienpopulation zeigte sich kein Zusammenhang zwischen der Eisenzufuhr und der maternalen IDA. Es konnte keine wesentliche Beeinflussung des Eisenstatus von Schwangeren, sowohl mit als auch ohne IDA, auf den Eisenstatus ihrer Neugeborenen im Nabelschnurblut festgestellt werden. In Plazenten von Frauen mit IDA zeigte sich eine Hochregulation von *TFR1*, wie auch eine Veränderung auf Proteinebene von TFR1 und von FTL1. Als Nebenergebnisse schienen auf, dass die Frauen in der IDA-Gruppe eine höhere Vitamin C Zufuhr sowie einen höheren Body Mass Index (BMI) hatten als jene in der nicht IDA-Gruppe. Zusammenfassend lässt sich sagen, dass die Ergebnisse dieser Arbeit gut mit ähnlichen Studien übereinstimmen. Es ist noch ungeklärt, ob sich eine maternale IDA aufgrund von bisher unbekannten (plazentaren) Kompensationsmechanismen nicht auf das Kind überträgt, oder ob es zur Beschreibung des Neugeborenen-Eisenmangels möglichweise andere Biomarker braucht.

Abstract

Ensuring an adequate supply of iron for pregnant women and their unborn children remains a challenge. It is not conclusively established whether or to what extent insufficient iron intake in the mother is associated with iron deficiency anemia (IDA) and whether this condition can be transmitted to the unborn child. Specifically, the precise role of the placenta and its regulatory capacity in maternal IDA has not been fully understood yet. The objectives of this study were to demonstrate 1) whether reduced iron intake leads to maternal IDA, 2) whether maternal IDA is reflected in a decreased iron level in the newborn, and 3) whether there is altered gene and protein expression of Transferrin Receptor 1 (TFR1) and Ferritin Light Chain (FTL1) in placentas of women with IDA. For this purpose, 97 mother-infant pairs were recruited at the General Hospital Vienna, and their iron status (hemoglobin, iron, ferritin, transferrin, transferrin saturation, soluble transferrin receptor, and C-reactive protein) was assessed. Additionally, the placental expression of TFR1 and FTL1 was investigated. Maternal iron intake through diet was calculated using questionnaire data (Food Frequency Questionnaire). IDA was defined as hemoglobin <11 g/dl and ferritin <30 µg/l. In this study population, no correlation between iron intake and maternal IDA was observed. The iron status of pregnant women, both with and without IDA, could not be found to have a substantial impact on the iron status of their newborns in umbilical cord blood. Placentas from women with IDA showed upregulation of *TFR1* and changes at the protein level of both TFR1 and FTL1. As additional findings, it appeared that women in the IDA group had a higher vitamin C intake and had a higher Body Mass Index (BMI) than those in the non-IDA group. In conclusion, the results of this study align well with similar studies. It remains unclear whether maternal IDA does not transfer to the child due to as-yet-unknown (placental) compensation mechanisms or whether additional biomarkers to describe neonatal iron deficiency are required.

1 Introduction

1.1 Human iron metabolism

The trace element iron has a remarkable range of redox states (Fe^{-2} to Fe^{6+}) with ferrous iron (Fe^{+2}) and ferric iron (Fe^{3+}) as the biologically most important ions. Iron complexes are essential in life and play an important role in the biosynthesis of RNA, DNA and vitamins as well as in the transport of oxygen and as part of the oxidative phosphorylation in mitochondria (Frey & Reed, 2012). In cells iron is bound to proteins, although there are small unbound proportions. This so-called labile iron pool is redox-active and can form reactive oxygen species (ROS), that are consequently damaging lipids and proteins (Nakamura et al., 2019). As iron overload causes harm and many mechanisms in the body are iron dependent, iron metabolism must be regulated tightly. Part of the regulation is the balanced uptake of dietary iron in form of heme (Fe^{+2}) and non-heme (Fe^{3+}) iron allocated in the duodenum (Figure 1) (Wallace, 2016).

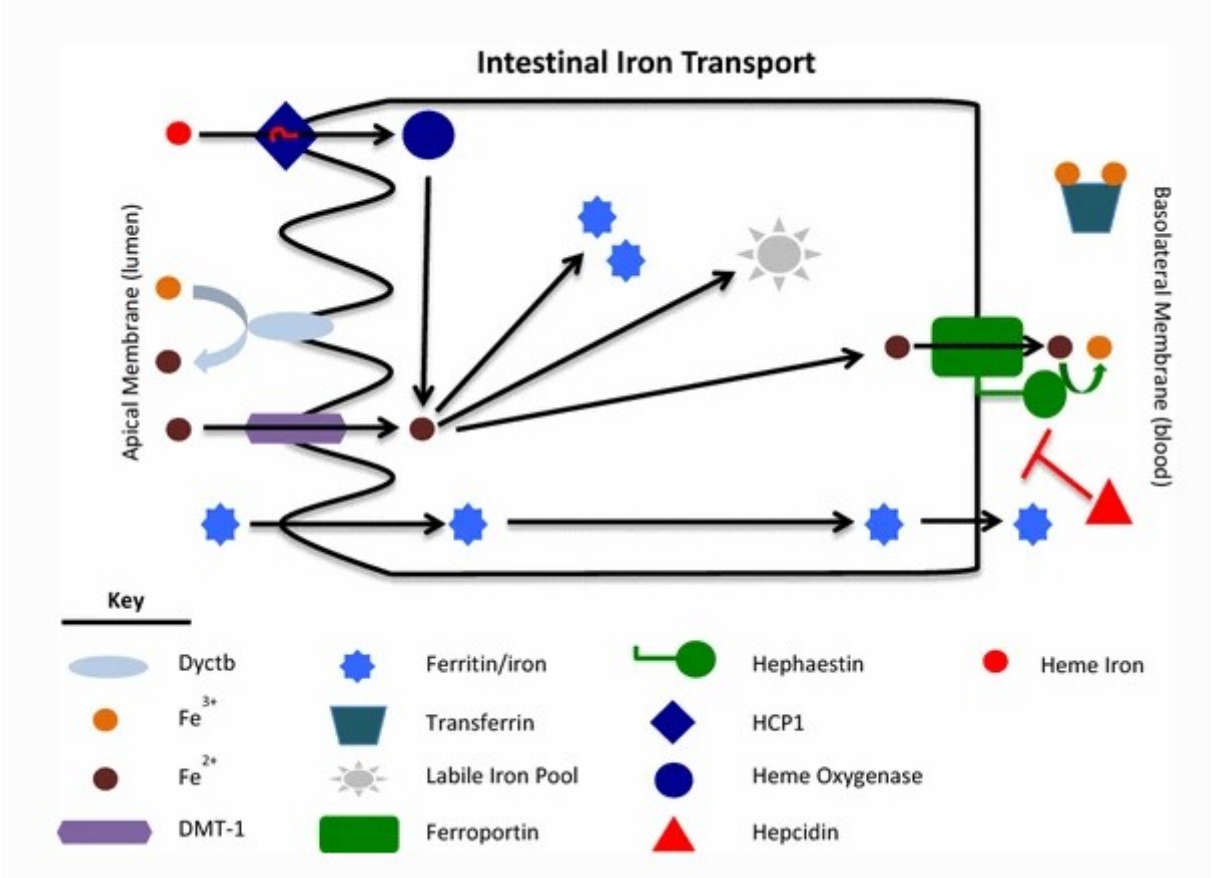

Figure 1. Intestinal iron transport. Dietary non-heme iron is reduced via duodenal cytochrome b (Dyctb) to Fe^{+2}, then all Fe^{+2} is transported into the enterocyte via divalent metal ion transporter (DMT1). Fe^{+2} is either stored in Ferritin as Fe^{3+} or directly exported at the basal membrane via ferroportin. To reach circulation, ferrioxidase hephaestin oxidises Fe^{+2} to Fe^{+3}, which consequently is bound to transferrin. Ferroportin is a target of hepcidin and therefore a main control point for iron homeostasis. Abbreviations: Duodenal cytochrome b (Dyctb), ferrous iron, (Fe^{+2}), ferric iron (Fe^{+3}), heme-carrier protein 1 (HCP1), divalent metal ion transporter 1 (DMT1). From: Duck & Connor (2016).

In countries with substantial consumption of meat, two third of dietary iron is non-heme iron (Conrad & Umbreit, 2000). Prior to absorption it needs to be reduced to Fe^{+2} by the ferrireductase duodenal cytochrome b (Dyctb), located at the apical site of enterocytes and is then imported by divalent metal ion transporter 1 (DMT1) ibidem (Wallace, 2016). Meanwhile the underlying mechanisms of heme uptake remain incomplete. The heme-carrier protein 1 (HCP1) has been identified in enterocytes, but has low affinity to heme and its primary role seems to be the uptake of folate (Duck & Connor, 2016). In rodents DMT1-mutants with no functional DMT1 can survive and therefore other transport mechanisms for iron species (e.g., ferritin [Ft]) are likely to exist (Mackenzie & Garrick, 2005). Supporting that, a study in human epithelial cells (CaCo-2 cells) confirmed that Ft crosses the cell membrane via clathrin-dependent endocytosis (San Martin et al., 2008). In addition, members of the ZRT/IRT-like protein (ZIP) family are able to transport non-transferrin bound iron (NTBI) across the cell membrane (Bogdan et al., 2016).

Upon entering the enterocyte, iron is stored in Ft, when iron demand is low. Ft is a highly conserved protein consisting of total 24 subunits, with different ratios of heavy chains (FTH1) to light chains (FTL1). Ft is capable of storing up to 4500 Fe^{3+} ions (Harrison & Arosio, 1996). FTH1 has a weight of 21 kDa and FTL1 19 kDa. The proportion of these within a Ft protein and therefore the weight, differs from tissue to tissue and from the iron load (Dörner et al., 1985). As iron in the cytosol is present as Fe^{2+}, it must be oxidised by the ferrioxidase activity of Ft (its heavy chain) before storage (Duck & Connor, 2016). At this state, if iron demand remains low, stored iron will get shedded at the end of an enterocyte's life span. Otherwise, in demand of iron, Ft proteins release their storage iron so that iron can be exported via ferroportin 1 (FPN1) located at the basolateral side of enterocytes. In case of increased demand, newly absorbed iron will be directly exported via FPN1 to reach the circulation (Anderson & Frazer, 2017). FPN1 is an important regulator in iron homeostasis as it is a target of the liver-derived peptide hormone hepcidin. By binding of hepcidin to FPN1, FPN1 degradation is induced, stopping iron efflux (Nemeth et al., 2004). Empty and full body iron storages as well as hypoxia and inflammation alter hepcidin secretion (Anderson et al., 2009).

After export, ferrous iron has to be oxidised by the ferroxidases hephaestin or ceruloplasmin before it is bound to the iron transport protein transferrin (Tf) (Eid et al., 2014).Tf has two lobes, each capable of loading up with one ferric iron, resulting in three possible states: apo Tf (no Fe^{3+} bound), mono Tf (one Fe^{3+} ion bound) and holo/diferric Tf (two Fe^{3+} ions bound) (Leverence et al., 2010). The most common states in serum are apo and mono Tf expressed in reference values for transferrin saturation (Tfsat) of >16% to <45% (Camaschella, 2015b). Depending on the number of iron atoms bound to Tf, conformational changes take place in the molecule (Thakurta et al., 2004). The main path for iron entering cells is via Transferrin Receptor 1 (TFR1), a homomeric transmembrane protein (Kawabata, 2019). TFR1 is ubiquitously present and in humans TFR1 has high expression in placenta and bone marrow alongside 23 other tissues (Fagerberg et al., 2014). The homodimeric subunits consists of 760 amino acids each and have about 95 kDa. The N-terminal is the transmembrane domain, whereas the C-terminal comprises the binding site for Tf (Al-Refaei et al., 2020; Lawrence et al., 1999). Holo Tf has high affinity to TFR1, whereas apo Tf does effectively not bind to TFR1, both depending on pH value of 7.4. The Tf-TFR1 complex is internalised by clathrin-mediated endocytosis. After the vesicles merge with early endosomes, iron is released because of the acidic environment and consequently Fe^{3+} is reduced to Fe^{2+} by a ferrireductase like six-transmembrane epithelial antigen of the prostate (STEAP) 3. Ferrous iron is transported to the cytosol via DMT1 whereas Tf and TFR1 are going to be recycled (Figure 2) (Kawabata, 2019). The Tf-TFR1 complex returns to the cell surface via early or recycling endosomes (Johnson et

al., 2007). In addition to Tf transport, TFR1 is a receptor for circulating Ft composed of only FTH1, especially in the absence of diferric Tf. The Ft-TFR1 complex gets incorporated by endocytosis and is then degraded in lysosomes. The role and importance of this pathway remains unclear and takes place only in cells with high TFR1 expression (Li et al., 2010). TFR1 can be proteolytically truncated from cell membranes. This 'shedding' releases soluble transferrin receptor (sTfR) into the circulation, which is proportional to cell-bound TFR1 and reflects the degree of tissue iron demands. Like TFR1, sTfR has two iron binding sites and gets incorporated by clathrin-mediated endocytosis (Speeckaert et al., 2010).

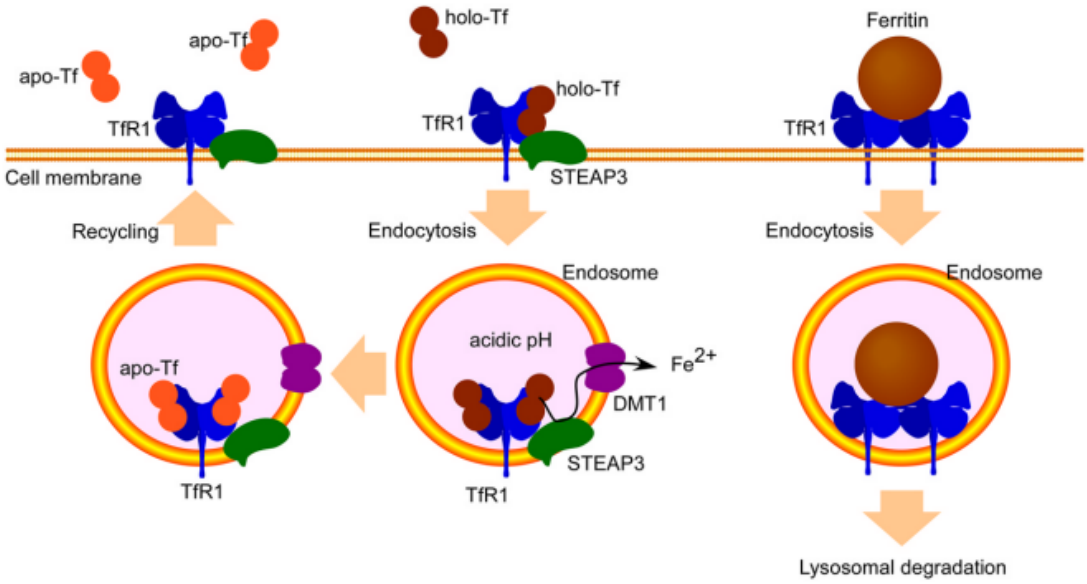

Figure 2. Iron uptake into cells. (Middle) TFR1 binds iron-loaded transferrin, then the transferrin-TFR1 complex is internalised and emerges with an endosome. Fe^{3+} dissociates at low pH and is reduced to Fe^{2+} by STEAP3 and released into the cytosol by DMT1. (Left) Apo transferrin and TFR1 return to the cell membrane. (Right) Hypothetical outline of ferritin uptake. Ferritin is an additional ligand for TFR1. Cells with high TFR1 expression are capable of internalising ferritin complexes, probably more than one TFR1 is required in this process. Abbreviations: Transferrin (Tf), Transferrin Receptor 1 (TFR1), ferrous iron, (Fe^{+2}), ferric iron (Fe^{+3}), divalent metal ion transporter 1 (DMT1), six-transmembrane epithelial antigen of the prostate (STEAP). From: Kawabata (2019).

1.2 Iron nutrition, iron deficiency and iron deficiency anemia in pregnancy

Germany, Austria and Switzerland (D-A-CH) have set the dietary iron requirement for pregnant women at 30 mg/d, which is twice that of non-pregnant women of reproductive age (Deutsche Gesellschaft für Ernährung, 2000). The average absorption rate of iron in industrialised countries is between 10 to 15%, mainly depending on the amount of individual meat and vitamin C intake (Gassmann, 1991). If the recommendations are followed, an iron intake of 15 mg/d is equivalent to 1.5 to 2.2 mg/d of absorbed iron and 30 mg/d of dietary iron is equivalent 3 to 4.5 mg/d absorbed iron.

In the first trimester of pregnancy, as menstruation stops, iron requirements drop to 0.8 mg/d of absorbed iron and therefore even below the needs of non-pregnant women of childbearing age. During the second trimester the iron needs raise to 4 to 6 mg/d of absorbed iron and reach its climax in late pregnancy with up to 10 mg/d. Hand in hand the iron absorption rate decreases in the first trimester and from second trimester onward it raises steadily (Bothwell,

2000). In total, approximately 1 gram of iron is required during pregnancy for the development of the placenta and the fetus as well as for sufficient erythrocyte expansion (Fisher & Nemeth, 2017). Irrespective of dietary recommendations and individual requirements, the average iron absorption in the general population following a Western diet ranges from 1 to 5 mg per day. (Lee & Okam, 2011). Many pregnant women meet the requirements during early pregnancy, but especially in the third trimester, they are hardly accomplished by only average nutrition, which often leads to anemia (Bothwell, 2000).

By definition anemia is a serum hemoglobin (Hb) level that is two standard deviations below average in the reference group. The World Health Organization (WHO) defines the threshold for pregnant women in third trimester at <11 g/dl (World Health Organization, 2008). Severe anemia is defined as <8 g/dl (Cei et al., 2017). Anemia and iron deficiency anemia (IDA) are sometimes used synonymously, and although IDA is the most common form of anemia, low Hb also can be a consequence of chronic or acute blood loss (e.g., through menstruation, surgery, or injury), malabsorption, cancer, hemoglobinopathies, HIV, malaria, hookworm infections and other reasons (Camaschella, 2015a; World Health Organization, 2008). In addition to iron deficiency (ID), there are other micronutrient deficiencies that may increase the risk of anemia including deficiencies of vitamin B12 and folate, as well as vitamin A, riboflavin and copper (World Health Organization, 2008).

As mentioned earlier, iron is essential during pregnancy for the production of erythrocytes. The expansion of plasma volume surpasses the expansion of red blood cells, resulting in hemodilution characterised by reduced hematocrit, Hb, and erythrocyte levels (Soma-Pillay et al., 2016). To define pregnancy ID, low serum Ft levels (<10-30 µg/l) are widely used as cut off levels. Most authors propose to add serum Tfsat <15% to diagnose inadequate iron supply (Camaschella, 2015a; Means, 2020). It is important to note that in individuals with inflammation, some iron biomarkers are altered (e.g., serum iron, hepcidin, serum Ft, sTfR, transferrin bound iron) (Suchdev et al., 2017). In such cases, rarely used biomarkers like erythropoietin (EPO), sTfR and the ratio of sTfR to log Ft are suggested for the diagnosis of ID (Lee et al., 2014; Weiss & Goodnough, 2005).

Children and young women are predisposed for ID amongst all demographic groups, particularly pregnant women due to their higher demands of iron (Camaschella, 2015a). Especially pregnant women with a vegan/vegetarian diet are at risk of developing ID (Piccoli et al., 2015). Low iron stores at the beginning of pregnancy can lead to adverse health outcomes for the pregnant women and the fetus, whether in developed countries or in poorer ones (World Health Organization, 2008). In 2011, 30% of non-pregnant women of reproductive age were estimated to suffer from anemia worldwide, and as mentioned before the most common form is IDA (Stevens et al., 2013). Consequently, many pregnancies already start with ID, although it is crucial to enter pregnancy with normal Hb and plenished iron stores to prevent ID/IDA in pregnant women mitigate possible negative consequences (Bothwell, 2000).

Maternal ID and IDA are risk factors of placental hypertrophy, premature delivery, small for gestational age (SGA), hypertensive disorders/preeclampsia and increased perinatal/neonatal mortality (Chen et al., 2018; Finkelstein et al., 2020; Georgieff, 2020; Huang et al., 2001; Kozuki et al., 2012; Lin et al., 2018; Martí et al., 2001; Rahman et al., 2016; Rahmati et al., 2020; Rao & Georgieff, 2007).

As described above, the fetal iron demand increases during pregnancy, and in the third trimester, approximately 80% of total fetal iron needs are delivered through the placenta to the fetal blood (Widdowson & Spray, 1951). During the third trimester, iron-dependent

neurogenesis and cognitive development in the fetus reaches its climax (Radlowski & Johnson, 2013). Adverse outcomes were observed between maternal ID and behaviour and cognition of infants, although aftereffects might prolong into childhood or even further (Janbek et al., 2019).

A low-threshold method to combating IDA is a nutrition-based approach. The systematic review of randomised controlled trials by Skolmoswka et al. suggests that increasing iron-rich food (e.g., meat, fish and green leafy vegetables) and greater intakes of vitamin C to facilitate iron bioavailability are the only effective approaches. Vitamin D might also have positive effects on iron uptake, but more studies are needed. Interestingly, inhibitors of iron absorption like phenols, phytates and calcium are probably not playing an important role (Skolmowska et al., 2022). The recommendation for vitamin C intake in women from month 4 of pregnancy onwards is 105mg/d (Deutsche Gesellschaft für Ernährung, 2015).

As a public health intervention in prenatal care, the WHO recommends supplying all pregnant women with 30 to 60 mg/d of elementary iron. The higher recommendation of 60 mg/d refers to areas, where anemia represents a severe public health issue (World Health Organization, 2012). Many European countries like Germany (Deutsche Gesellschaft für Ernährung, 2018), UK (National Institute for Health Reseach, 2017) or France (Haute Autorité de Santé, 2005), explicitly do not recommend a prophylactic iron supplementation, as excessive intake of iron supplements can be associated with low birth weight, premature birth and oxidative stress in the newborn (Casanueva & Viteri, 2003; Hwang et al., 2013). The Austrian Society for Nutrition has no official recommendation for or against prophylactic iron supplementation (Österreichische Gesellschaft für Ernährung, 2023). The Austrian Society for Gynaecology and Obstetrics recommends supplementation and/or treatment only in the case of ID (Fischer et al., 2022). A study in Austria reported that in 89% of pregnancies the treating physician endorsed iron supplementation for prophylaxis. Ultimately, 67% of women who were not previously diagnosed with anemia before becoming pregnant reported the use of any kind of oral iron in pregnancy (Spary-Kainz et al., 2019).

Apart from prevention measures, the current gold standard for treatment of ID/IDA is oral medication in form of iron salts, such as ferrous sulfate, fumerate or gluconate (i.e., elementary iron doses of 100 to 200 mg/d). Newer studies suggest that iron supplements should not be taken daily but alternating (every second day) to overcome the problem, that after administration, hepcidin is increased for up to 48h, harming the absorption of subsequent iron doses. However, gastrointestinal side effects occur often under oral therapy (Camaschella, 2019).

A next step in the treatment of severe ID/IDA is intravenous (IV) administration of iron containing agents. In addition to failure of oral therapy (due to intolerance, defective absorption, no improvement), the indications for IV iron therapy are severe anemia (Hb <7-8 g/dl) and the need of rapid Hb increase (i.e., in second and third trimester of pregnancy). A common concern on IV iron is hypersensitivity reactions. Fortunately the risk was lowered significantly after switching from iron dextran to the superior ferric carboyxymaltose (Camaschella, 2019). When iron dextran is avoided, severe adverse events appear in under 1 of 200.000 cases (Avni et al., 2015). In case of severe anemia with rapid need of bumping up the Hb, there is the possibility to use red blood cell transfusion (RBCT). As a blood product from a foreign donor, this therapy option bears the risk of infections as well as an incorrect usage and application (patient mix-up etc.), leading to a possible immunological reaction consequently. RBCT is a limited resource, hence the indication should be meticulously assessed (Cortés Buelvas, 2013). Another therapeutic option for IDA in pregnancy is the use of adjuvant

recombinant human EPO (subcutaneous) along with iron sucrose (IV). The reported side effects include flu-like symptoms, allergic responses, risk of thrombosis as well as hypertensive reactions (John et al., 2012).

1.3 Impact of maternal overweight and obesity on iron status in pregnancy

Overweight and obesity are rising in Austria over the past years, and especially obesity is a risk factor for many non-communicable diseases as well as for several mental disorders (Dorner et al., 2023). One of these impairments is found in the association between obesity and iron deficiency, with the mechanisms for this not yet fully understood. Probably the low-grade inflammation status in obesity triggers the upregulation of hepcidin, leading to a subsequent decrease in intestinal iron absorption (Alshwaiyat et al., 2021). While a definitive experimental study is still pending to validate the observations made in studies linking obesity and iron deficiency, recent evidence has uncovered a causal relationship. Employing Mendelian Randomisation analysis, researchers examined the heterogeneity and potential causality of single-nucleotide polymorphisms (SNPs) associated with diverse anthropometric indicators of obesity (used as instrumental variables) in comparison to genetic variants linked to IDA. Their results suggest a causality of obesity for IDA (Wang et al., 2023). Interestingly, a phenomenon called “dysmetabolic iron overload syndrome (DIOS)” a status with high Ft and normal to elevated Tfsat has been described in patients with non-alcoholic fatty liver disease (NAFLD) or metabolic syndrome and other insulin-resistant conditions. In severe and progressed forms of obesity, the obesity-related iron deficiency as described above is more prevalent than DIOS (Datz et al., 2013). The pathophysiological theory for DIOS posits that in individuals with excessive diet, dietary iron shifts from the liver to overload in adipocytes as a consequence of multifactorial processes that are not fully known yet. Hepcidin is upregulated in DIOS, which is difficult to explain in a state of iron overload. It is suggested that there is a hepcidin resistance due to higher blood sugar levels and insulin resistance (Deugnier et al., 2017).

Pregnancy weight gain is guided by established recommendations, varying according to the weight at the beginning of pregnancy, with a lower recommended weight gain for those starting with a higher pre-pregnancy weight (Institute of Medicine (US), 2009). Many adverse outcomes in pregnancy are associated with pre-pregnant obesity like growth restriction or macrosomic fetuses (Higgins et al., 2011). In pregnancy, placentas of overweight and obese women are more likely to be hypertrophic and therefore the ratio of fetal to placental weight, which is a proxy for placental efficiency, is lower in these placentas compared to both normal and underweight women. Low placenta efficiency itself is associated with an increased risk of pre-eclampsia and spontaneous preterm delivery (Wallace et al., 2012). The higher rate of stillbirth in obese women is probably explainable by the inefficient functioning of the placenta as well (Nohr et al., 2005). Other complications that are associated with obesity in pregnancy are gestational hypertensive disorders, gestational diabetes and a programming of their babies for obesity and metabolic disease in adult life (Higgins et al., 2011). There are very limited studies addressing the iron status in overweight and obese pregnant women. The comparative analysis of these studies is complicated due to variations in the timing of blood collection throughout pregnancy and the use of diverse markers to assess iron status. Nevertheless, the iron status in maternal blood and/or cord blood was impaired in obese women across these studies. Additionally, higher hepcidin and C-reactive protein (CRP) levels can be seen in all obese pregnant women when compared to normal weight. These findings contribute to the ongoing hypothesis, supported by all authors, that the compromised maternal and fetal iron

status in obese women results from chronic inflammation, including elevated hepcidin levels (Dao et al., 2013; Garcia-Valdes et al., 2015; Jones et al., 2016).

1.4 The human placenta

In embryogenesis, on day 5 post conception (p.c.) the blastocyst differentiates out of the morula. The blastocyst is two-layered, with an outer layer termed trophoblast and the inner embryoblast. Cells from the trophoblast are the origin of extraembryonic tissues (main parts of placenta and fetal membranes), whereas the embryoblast becomes the embryo, the umbilical cord (UC), and the stromal parts of the placenta. This differentiation takes place before nidation, which occurs on day 6 to 7 p.c. (Huppertz, 2018). During nidation, the blastocyst hatches from the zona pellucida, a glycoprotein layer, and attaches to the differentiated uterine epithelium, termed decidua (Sathananthan et al., 2003; Vinketova et al., 2016). At this time, the trophoblast cells proliferate to a multilayer and furthermore to a syncytium termed syncytiotrophoblast (STB). The part of the early STB that is in direct contact with the uterine epithelium, penetrates it to embed the conceptus. On the basal side of the STB, former mono-nuclear trophoblast cells remain in a monolayer and are termed cytotrophoblasts (CTB). In contrast to the STB, the CTB can proliferate further (as stem cells) to fuse into and expand the STB. Starting on day 8 p.c., small vacuoles occur within the STB. These fuse to lacunae, whilst the material in between are termed trabeculae. At this stage, the placenta can be categorized into three segments: the chorionic plate (CP) on the embryonic pole, the basal plate (BP) on the abembryonic pole (in contact with the decidua) and in between the lacunae-trabeculae system (Figure 3a) (Huppertz, 2018).

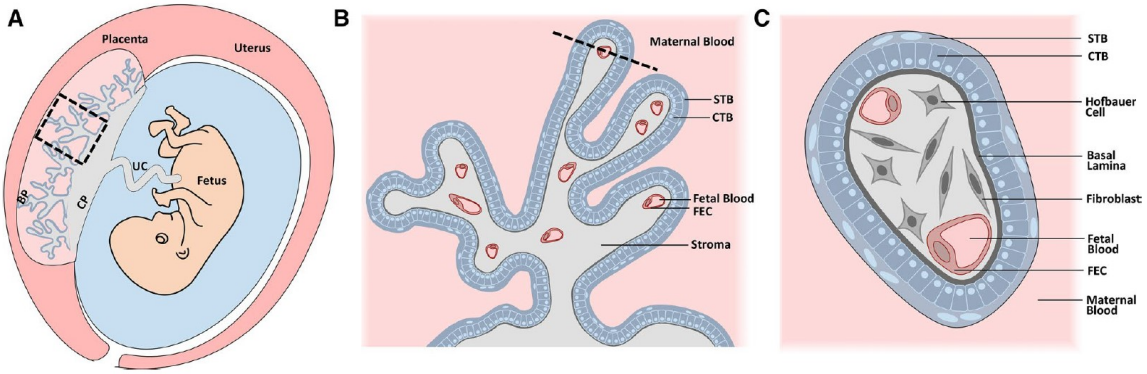

Figure 3. Concept of the human placenta. (A) Scheme of position and structure of the human placenta and a tertiary chorionic villus in longitudinal section (B) and in cross-section (C). Abbreviations: Basal plate (BP), chorionic plate (CP), umbilical cord (UC), syncytiotrophoblast (STB), cytotrophoblast (CTB), fetal endothelial cells (FEC). From: Gundacker et al. (2016)**.**

Around day 13 p.c. and onward, trabeculae are developing into primary villi, structures with a core of CTB surrounded by STB (Figure 4a), which are protruding into the intervillous space (former lacunae). Emanating from the extraembryonic mesoderm, mesenchymal cells migrate into the primary villi and displace the CTB from within, giving rise to secondary villi (Figure 4b). Differentiating from these mesodermal cells, fetal endothelial cells (FECs) and blood cells (alongside fibroblasts and macrophages) occur, marking the tertiary villi (Figure 3b&c; Figure 4c). Throughout pregnancy the villi enduringly grow and sprout to enlarge the surface for exchange of substances to meet the extended needs of the fetus (Huppertz, 2018).

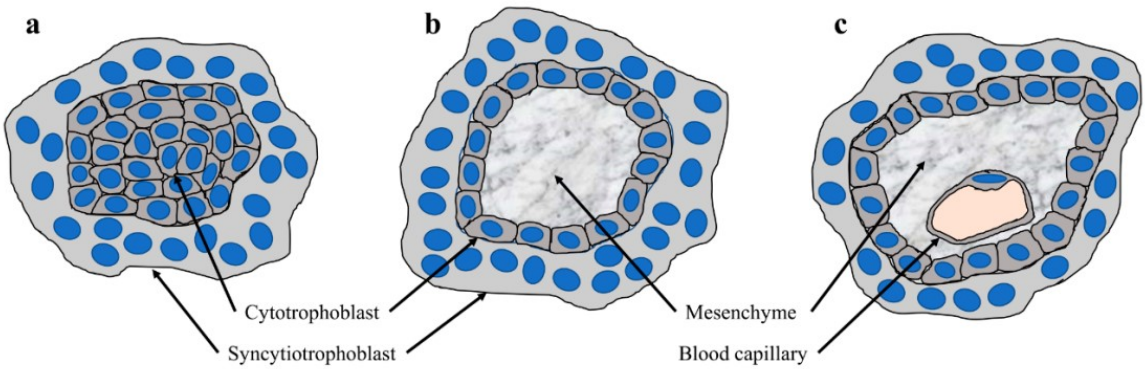

Figure 4. Cross-section of villi. (a) primary, (b) secondary (c) and tertiary villi.
From: Moser et al. (2019).

The connection between the maternal blood and the intervillous space (and thus indirectly with the fetus) is established around the 12th week of gestation (GW), when, in addition, the UC is fully developed (Huppertz, 2018; Spurway et al., 2012). Maternal nutrients are required to cross the placental barrier, a multi-cell layer between maternal and fetal circulation, consisting of STB, CTB, basal lamina and FECs (Figure 3c). During the first trimester, the layer of CTB cells is complete, then gets thinner and discontinuous as pregnancy progresses to ensure facilitated diffusion for the growing needs of the offspring (Huppertz, 2018).

1.5 Correlation of maternal and fetal iron status

A major focus of this study of iron metabolism in the human placenta, within which this master's thesis was conducted, is the transfer of iron from the mother to the newborn, especially in term IDA pregnancies of otherwise healthy women. When fetal iron status is mentioned, it refers to the parameters of iron status that are analysed in umbilical cord blood. This is the case both in this master's thesis and in all the mentioned studies.

Observational studies that do not primarily focus on mothers with severe IDA face the challenge of identifying biomarkers in newborns. A systemic review including 65 studies examined the correlation of iron biomarkers (e.g., Hb, Ft, serum iron [sFe], hepcidin, and Tfsat) of pregnant women and newborns. Maternal iron status and neonatal iron status were not found to be highly correlated. This highlights the need for alternatives for the assessment of fetal iron status (Sanni et al., 2020). Although study designs and cut-off values differ in studies on maternal to neonatal iron transfer, there are different robust correlations for severe maternal IDA. Hb, Ft, sFe and the number of reticulocytes reflect the poor iron status of pregnant women in their newborns (Basu et al., 2016; Choi et al., 2000; El-Farrash et al., 2012; Kumar et al., 2008; Shao et al., 2012; Singla et al., 1996). As mentioned above, maternal IDA is a risk factor for various unintended outcomes. Additionally, maternal IDA is a risk factor for the development of IDA in the infant itself, beside the primarily risk factor prolonged exclusive breast-feeding without complementary feeding (Sundararajan & Rabe, 2021).

1.6 Iron metabolism of the human placenta

Although the general knowledge about iron metabolism is becoming more complete, there are still many uncertainties regarding iron metabolism in the placenta (Figure 5) (Cao & Fleming, 2016). The major source of maternal iron entering the placenta is internalised Tf bound iron (TBI) via TFR1, which is mainly localised on the apical side of the STB (Berkowitz et al., 1985; Cao & Fleming, 2016). The uptake of TBI by endocytosis is almost certain, whereas molecular mechanisms are not conclusive yet, and the fate of TBI-clathrin-complexes is not fully

deciphered (Cao & Fleming, 2016). It is believed that the acidic milieu in vesicles supports the release of ferric iron, which is then reduced probably by ferrireductase STEAP3 or STEAP4 and exported into the cytoplasm by DMT1 and/or ZIP 8 and ZIP 14 (Cao & Fleming, 2016; Sangkhae & Nemeth, 2019). As it has been shown, that FPN1 is localised on the basal side of the STB, it is the postulated main iron exporter (Bastin et al., 2006). Interestingly to note, TFR1 is also found on the basal side of the STB, but in much less quantity than on the microvillous membrane (Bastin et al., 2006; Vanderpuye et al., 1986; Verrijt et al., 1997). After iron enters the placental stroma, its further transfer remains unknown. Due to the inevitability that ferrous iron must be oxidised before binding to fetal Tf, at least three ferroxidases (zyklopen, ceruloplasmin, hephaestin) can fulfil this role as they are known to be expressed in the human placenta (Chen et al., 2010; Guller et al., 2008; Li et al., 2012). Iron, possibly as TBI, traverses the fetal endothelium in a yet undescribed manner. Based on the undetectable expression of FPN1 and the weak staining of TFR1 in placental endothelial cells, it has been assumed that a different mechanism than in the STB is responsible for iron transfer across endothelial cells (Bastin et al., 2006; Cao & Fleming, 2016; Mandò et al., 2011).

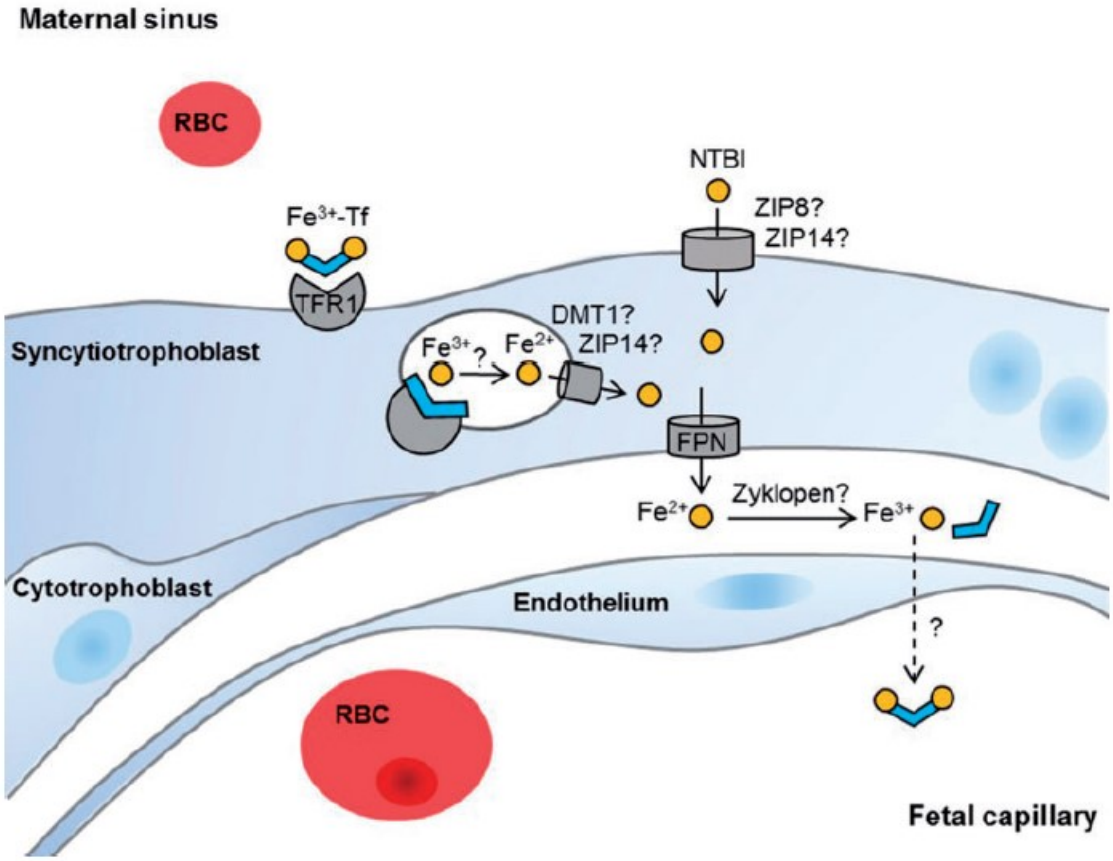

Figure 5. Iron transport across the human placenta. Transferrin bound ferric iron is taken up in the STB by TFR1 via endocytosis. Upon release in acidic endosomes, iron is reduced and consequently exported probably via DMT1 or ZIP14 into the cytosol. On basal STB, iron is exported by FPN1, before it is transported into FECs. NTBI might also enter the STB via ZIP8 or ZIP14 by yet unknown mechanisms. Abbreviations: Ferrous iron; (Fe^{+2}), ferric iron (Fe^{+3}), red blood cells (RBC), transferrin (Tf), Transferrin Receptor 1 (TFR1), ferroportin (FPN), non-transferrin bound iron (NTBI), divalent metal ion transporter 1 (DMT1), ZRT/IRT-like protein (ZIP). From: Cao & Fleming (2016).

In addition to TBI, there might be another maternal iron source imported by the placenta. The candidate transporters of NTBI into the STB include ZIP8 or its homolog ZIP14 as shown in placental mouse tissue (C57BL/6J), in cell lines (BeWo, HEK 293T and *Xenopus* oocytes) and in

animal models (hypomorphic Slc39a8 *(neo/neo)* mice) (Girijashanker et al., 2008; Gálvez-Peralta et al., 2012; Wang et al., 2012). Furthermore, transporters for heme species were found to be upregulated in human placentas in case of a suboptimal maternal iron status (Cao et al., 2014). Similarly, a Ft receptor is expressed on the microvillous membrane of the STB and its expression increases in moderate IDA (Liao et al., 2001). These findings are to some degree in line with previous animal studies, where Ft radioisotopes were present in placenta and fetus after Ft injection (Lamparelli et al., 1989).

1.7 Placental expression of TFR1 and FTL1 in IDA

During late pregnancy, in healthy women, expression patterns of iron metabolism related genes in the placenta are changing due to the increased iron requirement of the fetus (Cao & Fleming, 2016). *TFR1* and *FPN1* are upregulated in human placenta with gestational age (Inoue et al., 2021). Others found no significant upregulation of TFR1 but of Ft and FPN1 in human placenta from GW 24 to 40 (Bradley et al., 2004). In rats, expression of TFR1 in the liver, as well as duodenal DMT1 was increased, indicating the extended iron needs (Millard et al., 2004).

There are differences in placental gene and protein expression in anemic versus non-anemic mothers. In general, it is shown that in humans and animals (mice, rats) and cell models, iron depletion leads to increased placental TFR1 on mRNA and protein level (Cao et al., 2021; Gambling et al., 2001; Garcia-Valdes et al., 2015; Sangkhae et al., 2020; Young et al., 2010). In a study examining protein expression of iron transport proteins in IDA, they found significant higher protein expression of FPN1, DMT1 and zyklopen in anemic women, although on mRNA level only *DMT1* was also increased (Venkata Surekha et al., 2021). Knowledge about Ft expression in anaemia is less available. In a mouse model, placental Ft gene expression did not increase with gestational age and no significant difference was found between the iron-deficient and iron-replete group (Sangkhae et al., 2020). Another group found that Ft protein in ID mice was downregulated, and heavy and light chain were downregulated as well on mRNA level in these mice (Cao et al., 2021). In humans, a study that included three groups, i.e. no anemia (Hb >11 & hematocrit > 33% & Ft> 15), mild anemia (Hb between 9-11 g/dl) and moderate anemia (Hb <9 g/dl), found Ft protein expression in the placenta was lower in moderate anemia, compared to the other two, but only tendencies have been found on mRNA level for *FTL1* and *FTH1* (Li et al., 2008). Overall, expression patterns of Ft or FTL1 in placenta are far less investigated than expression for TFR1, and therefore was an inducement to be further examined within this thesis.

1.8 Aims of the thesis

Based on the current knowledge gaps, this study aimed to investigate

1) if reduced dietary iron uptake leads to maternal IDA
2) whether maternal IDA leads to lower iron status in the newborn
3) whether placentas from IDA pregnancies show altered expression of iron regulated TFR1 and FTL1

2 Materials and Methods

Protocols of the stated laboratory methods and details on used chemical and antibodies are provided in the appendix.

2.1 Study population and Sampling

124 mother-child pairs were recruited in the General Hospital Vienna (AKH Wien) in the period between 2020 and 2022. The study was approved by the ethics committee of the Medical University of Vienna (Vote no. EK 1404-2015) and was part of the "Placental Iron Metabolism" Project (GFF NÖ Project No. LS-18-008). The inclusion criteria were healthy single term pregnancy delivered between GW 37 and 42, maternal age of 18 to 45 years, and a signed informed consent. Participants with iron supplementation and allergies (e. g., pollen, nickel or penicillin) as well as In-Vitro-Fertilisation (IVF) pregnancies were also included. Cases with COVID-19 infection during pregnancy, bariatric surgery, hypo- and hyperthyroidism, and medications that may affect iron metabolism (e. g., heparins), however, were excluded. Finally, 97 mother-child-pairs took part in the study. An identification number was given to each participant for pseudonymisation. Eligible participants were interviewed by trained staff one day before birth or at the day of birth. All study participants gave birth via Cesarean section. Collection of venous blood samples occurred before the operation. After birth, venous umbilical cord blood was drawn. Blood values and iron parameters from umbilical cord blood were not available for all newborns as blood was lost from the umbilical cord during the detachment and/or delivery of the placenta intraoperatively. Blood samples were processed by the clinical laboratory of the AKH Wien for determining blood counts and iron status. An approximately 1 cm^3 piece of the abembryonic pole of the placenta was stored in RNAlater® Solution (Thermo Fisher Scientific) at 4°C or -20°C for further usage. 4°C storage took place when early procession of tissue was possible. Medical records were used to survey birth outcome data (birth weight, birth length and head circumference).

The study participants were grouped into women with IDA and women without iron deficiency anaemia (non-IDA). The IDA group was defined by Hb <11 g/dl and serum Ft <30 µg/l according to the diagnostic criteria for pregnancy IDA in the 3rd trimester. ID cases defined as Ft <30 µg/l but Hb >11 g/dl were added to the non-IDA group as they were too small as a subgroup for further statistical analysis (Achebe & Gafter-Gvili, 2017; Api et al., 2015; World Health Organization, 2008). Weight categorisation of women according to their pre-pregnancy body mass index (BMI) was as following: Individuals with a BMI ranging from 18.5 to less than 25 kg/m^2 were categorised as normal weight, those with a BMI between 25 and less than 30 kg/m^2 overweight, and individuals with a BMI of 30 kg/m^2 or higher were classified as obese. Due to the limited number of obese participants, they were combined with those categorised as overweight for further statistical analysis.

2.2 Questionnaire

In the questionnaire, which can be found in the appendix, the items can be categorized into the following groups: Course of pregnancy, sociodemographic factors, pregnancy health, and Food/Food-Frequency-Questionnaire (FFQ). An overview of this categorisation is provided in Figure 6. Although all medications and supplements were inquired, there was a focus on medications for treating iron deficiencies, as well as supplements specifically for pregnant women, and iron supplements. Regarding weight, pre-pregnancy weight and weight at the end of pregnancy were queried together with height, but all were self-reported. The FFQ commenced by inquiring about the individual's dietary form (mixed diet, vegetarian, vegan, or other), and then proceeded with a series of single-choice questions centred around iron-rich foods, vitamin C rich foods and foods that prevent intestinal iron uptake. Bread, meat, sausages and vegetables are the most important sources for dietary iron in middle Europe duo to consumption frequency (Arab-Kohlmeier et al., 1989). Therefore, these foods were primarily queried in the questionnaire, either as individual or grouped items (Figure 6). Furthermore,

specific food groups that inhibit iron absorption were queried, such as dairy products (calcium) or black and green tea and coffee (polyphenols). However, the aim of the study was not to measure these substances as a whole. In order to avoid recall bias, study participants were asked about their diet during the last month of pregnancy, and not the entire duration of pregnancy. During the interview, portion sizes of foods were explained using hand sizes. For each item the participants were asked how many portions they had eaten (i.e., "never", "less than one per week" or "x per week"). If "x per week" was chosen, the women were asked to quantify the portions. For tabulation the category "less than one per month" was converted to 0.5 portions per week. To calculate the individual estimated dietary iron intake, each item got assigned an iron content (as provided in the *Bundeslebensmittelschlüssel (BLS) version 3.0.2* and *Österreichische Nährwerttabelle (ÖNWT) version 2.0* both via https://www.oenwt.at/) and then normalised to portion size. The results were multiplied with consumed portions per week, then summed up to generate the weekly estimated dietary iron intake. The sum was divided by 7 for the daily estimated dietary iron intake. The same procedure was done for dietary vitamin C intake. All foods, along with their corresponding nutritional values as surveyed in the questionnaire, can be found in the appendix (Supplementary Table 1, in german). Additionally, all food variables and portion sizes are listed (Supplementary Table 2, in german). The expanded questionnaire (with more specific questions about maternal diet) was used to calculate dietary iron and vitamin C intake and was completed by a subset of 82 women.

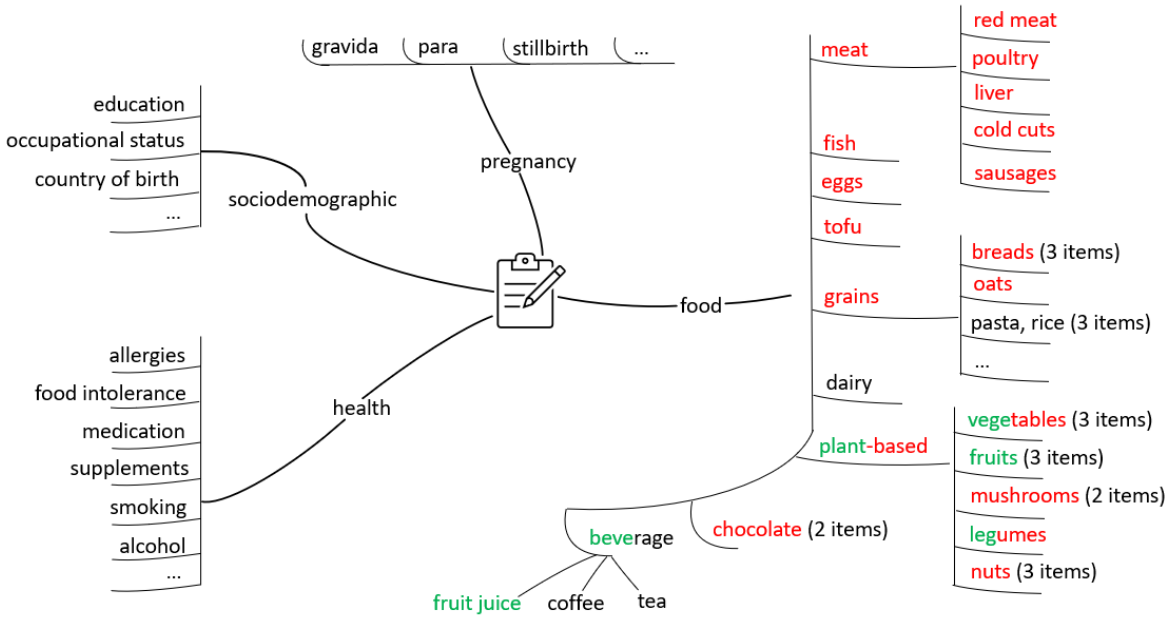

Figure 6. Questionnaire and compilations of items. Red labeled items specify foods contributing pertinent to iron intake. Green coloured items designate vitamin C-rich foods.

2.3 RNA Isolation and Reverse Transcription

RNA isolation was performed from whole placenta tissue according to the modified manufacturer's protocol using the PARIS™ Kit (Thermo Fisher Scientific). RNA concentrations were measured with a Nanodrop-1000 (Thermo Fisher Scientific). Using the GoScript™ Reverse Transcription System (Promega) up to 1000 ng of RNA were diluted in RNAs-free water to a final volume of 10 µl. Then, for synthesis of cDNA, the RNA was reversed transcribed with

random hexamers (Promega) in a thermocycler (Peqlab) following the manufacturer's protocol.

2.4 qRT-PCR

2 µl of cDNA was used as a template in 15 µl total reaction volume performing the TaqMan® Gene Expression Assay (applied biosystems) in a StepOnePlus™ Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) System using *StepOne Software 2.3* (applied biosystems) following the manufacturer's instructions. For relative mRNA quantification, the comparative Ct method was used, where ubiquitin C (UBC, Thermo Fisher Scientific, Hs00824723_m1) was the reference gene and Transferrin Receptor (TRFC, Thermo Fisher Scientific, Hs00174609_m1) the target gene.

2.5 Protein Isolation

Tissues were lysed with Cell disruption buffer using the PARIS™ Kit (Thermo Fisher Scientific). After centrifugation for 10 min at 15 000 x g at 4°C, supernatants were collected, and protein concentration was measured with a spectrometer (Bio-Rad) using the Quick Start™ Bradford Protein Assay (Bio-Rad) and then stored at -20°C.

2.6 Immunoblotting

20 µg Protein, mixed with 4X Protein Loading Buffer (LI-COR) supplemented with 10% β-mercaptoethanol (Merck) were incubated 10 min at 72°C. Separation of proteins took place on 12.5% polyacrylamide gels followed by transformation on a nitrocellulose membrane. PageRuler™ Prestained Protein Ladder (Thermo Fisher Scientific) was used as marker. After blotting, the membrane was treated with a total protein stain (Revert™ 700 Total Protein Stain Kit, LI-COR) to assess sample loading. Intercept® (TBS) Blocking Buffer (LI-COR) was used for blocking. Overnight, primary antibodies were incubated at 4°C. On the following day, blots were washed and employed with secondary antibodies for 1 h at 4°C. For detection of total protein and antibodies, near-infrared (NIR) fluorescence was measured with the Odyssey CLx imager (LI-COR) using *Image Studio Lite 5.2* software. Primary antibodies, FTL1 (Abcam) and TFR1 (Cell Signaling Technology) were diluted 1:1000. Employed secondary antibodies, IRDyes 800CW Goat anti-Mouse immunoglobulin G (IgG)1-Specific (LI-COR) and IRDyes 800CW Goat anti-Rabbit IgG Secondary Antibody (LI-COR) were diluted 1:20 000. Densitometric analysis of immunoblot signals was performed using *ImageJ.*

2.7 Statistics

Data were processed using *MS 365 Excel version 2207.* Statistics were computed with *R* 4.0.5 and 4.2.3, *IBM SPSS 27.0.0.0*. and *GraphPad Prism 9.4.0.* SPSS and GraphPad were used for designing graphs. All tests were performed as two-sided with a critical significance level of $\alpha = 0.05$. To compare group differences (non-IDA vs IDA and vegetarian vs omnivore) Mann-Whitney-U test was performed. In general, non-parametric statistical tests were performed as non-normal data distribution was assumed. In case of categorical variables, hypotheses were tested using Chi-Square test. Bivariate correlations were conducted on several continuous variables of interest (e.g., anthropometric data, blood parameters, food items), and they were represented using the Spearman's correlation coefficient (R_S). For binary logistic regression, eligible variables significantly associated ($P<0.1$) with outcome variable (non-IDA and IDA cases) in Chi-Square test or Mann-Whitney-U test were chosen. Other plausible variables (i.e., iron intake and birthweight) were also included into the regression model. In case of multicollinearity (tested by Spearman's correlation) the best fitting variable was included. Based on the crude model (n=97), insignificant variables ($P>0.05$) were stepwise excluded (six iterations) to eliminate factors with low explanatory power. In qRT-PCR, the relative gene

expression level was calculated by the comparative cycle threshold (Ct) method, also known as the 2^-ΔΔCt method. Gene expression analyses were conducted in technical duplicates using samples from 47 individuals and were subsequently summarized using the arithmetic mean. The presented values for blood counts, iron parameters, gene expression data, and densitometry data represent mean values ± standard deviation.

3 Results

3.1 Dietary iron, iron status and BMI in maternal IDA

The characteristics of the study population are shown in Table1. When comparing non-IDA and IDA groups, no significant difference was observed in nutritional iron uptake. Additionally, between the two groups, there were no differences in the intake of iron-absorption-inhibiting food groups such as dairy products or green and black tea or coffee. However, there was a notable disparity in the nutritional uptake of vitamin C; specifically, uptake was significantly higher in the IDA group ($P<0.05$). Pre-pregnancy BMI was significantly higher in the IDA group compared to non-IDA and same tendencies ($P<0.1$) appeared for end-pregnancy BMI (Table 1). The characteristics of the newborns are presented in Table 2. A comparison between newborns of non-IDA and IDA women revealed no statistically significant differences. None of the surveyed women in the dataset reached the Recommended Dietary Allowance (RDA) for iron intake of 30 mg/d day. For vitamin C intake, it was 59.3% in the non-IDA group and 70.6% in the IDA group that met the RDA. In the IDA group, 10 women were classified as normal weight, 7 as overweight, and 3 as obese. In contrast, the non-IDA group consisted of 49 normal weight women, 14 overweight, and 4 obese individuals. When splitting the pre-pregnancy BMI into the categories as described in the methods (normal and overweight-obese), 50% of women in the IDA group were categorised as overweight-obese, in contrast to 36.4% in the non-IDA group, which was significantly different ($P<0.05$). The only significant difference between overweight-obese and normal weight was cord sTfR ($P<0.05$), and maternal Hb and CRP as well as cord Tf had tendencies ($P<0.1$). In the overall group, pre-pregnancy BMI and end-pregnancy BMI correlated positively with maternal CRP and Tf and negatively with Hb. For cord blood, both BMIs correlated negatively with Ft, and only end-pregnancy correlated positively with Tf and sTfR and negatively with Tfsat. All correlations had low R_s values, with end-pregnancy BMI to maternal CRP having the highest ($P<0.001$, $R_s=0.36$). No correlation was found between one of these BMIs and *TFR1* expression. To confirm differences between various iron status parameters, respectively, between the non-IDA and IDA group, both maternal and fetal blood samples, were tested. Interestingly, not a single women had severe anemia (Hb < 8 g/dl). By definition, in mothers, hemoglobin and ferritin differed significantly, as did serum iron, Transferrin saturation, and soluble transferrin receptor, whereas in cord blood, no significant differences were found (Figure 7, Supplementary Table 3). The entire dataset was divided into omnivores (N=87) and vegetarians (N=9), with none of the women indicating that they are vegan or follow any other dietary form. Within the omnivore group, the levels of Tf in maternal and in cord blood were significantly higher compared to vegetarians ($P<0.05$; $P=<0.01$) as depicted in Figure 8.

Table 1. Characteristics of the maternal study population.

	Total			*Non-IDA*			*IDA*		
	N (%)	**Mean (SD)**	**MED (Min-Max)**	**N (%)**	**Mean (SD)**	**MED (Min-Max)**	**N (%)**	**Mean (SD)**	**MED (Min-Max)**
Mother									
Maternal age (y)	97	33.8 (5.7)	35 (20-45)	77	34.3 (5.9)	35 (20-45)	20	32.1 (4.9)	33.5 (25-39)
Education									
less than high school	28 (28.9)			19 (25.0)			9 (47.3)		
high school	28 (28.9)			26 (34.2)			2 (10.5)		
academic	39 (40.2)			31 (40.8)			8 (42.1)		
Occupational status									
in education	2 (2.1)			2 (2.9)			0 (0.0)		
employed *	56 (57.7)			41 (58.6)			15 (78.9)		
unemployed	31 (32.0)			27 (38.6)			4 (21.1)		
Pre-pregnancy BMI (kg/m²)	97	23.6 (4.3)	22.7 (16.4-40.0)	77	23.0 (3.5) [a]	22.5 (16.4-34.6)	20	26.0 (6.0) [a]	24.8 (19.2-40.0)
End-pregnancy BMI (kg/m²)	92	28.7 (4.8)	27.8 (19.8-43.6)	73	28.0 (4.0)	27.4 (19.8-40.4)	19	31.2 (6.5)	30.9 (22.2-43.6)
Gravida	97	2.9 (2.0)	2 (1-16)	77	2.9 (2.0)	2 (1-16)	20	3.1 (1.8)	2 (1-7)
Para	97	1.3 (1.5)	1 (0-11)	77	1.3 (1.5)	1 (0-11)	20	1.3 (1.5)	1 (0-6)
Pregnancy length (d)	97	270.1 (5.5)	270 (259-288)	77	270 (5.8)	270 (259-288)	20	270 (4.3)	271 (264-280)
Smoking									
never	58 (59.8)			45 (58.4)			13 (65.0)		
former	25 (25.8)			21 (27.3)			4 (20.0)		
current	14 (14.4)			11 (14.3)			3 (15.0)		
Iron therapy during pregnancy									
neither suppl. nor therapy	9 (9.3)			7 (9.2)			2 (10.0)		
suppl. only	48 (49.5)			36 (47.4)			12 (60.0)		
therapy with or without suppl.	39 (40.2)			33 (43.4)			6 (30.0)		
Diet									
omnivore	87 (89.7)			70 (92.1)			17 (85.0)		
vegetarian	9 (10.3)			6 (7.9)			3 (15.0)		
Iron intake (mg/d)	81	9.0 (4.0)	7.9 (3.4-25.7)	64	8.6 (3.5)	7.8 (3.44-18.3)	17	10.3 (5.3)	9.5 (4.7-25.8)
Vitamin C intake (mg/d)	81	155 (101)	132 (21-568)	64	140 (86) [a]	122 (21-383)	17	208 (134) [a]	172 (41-568)

Abbreviations: Standard deviation (SD), median (MED), minimum (Min), maximum (Max), iron supplements (suppl.), non-iron deficiency anemia (non-IDA), iron deficiency anemia (IDA), Body Mass Index (BMI). [a]P<0.05 from Mann-Whitney-U test and Pearson's chi-squared test. *incl. maternity leave.

Table 2. Characteristics of the newborn study population.

	Total			*Non-IDA*			*IDA*		
	N (%)	**Mean (SD)**	**MED (Min-Max)**	**N (%)**	**Mean (SD)**	**MED (Min-Max)**	**N (%)**	**Mean (SD)**	**MED (Min-Max)**
Newborn									
Sex, female	44 (45.4)			36 (48.0)			8 (40.0)		
Birth weight (g)	95	3340 (408)	3320 (2480-4830)	75	3309 (395)	3314 (2480-4530)	20	3460 (445)	3350 (2910-4830)
small for gestational age (g)	7	2644 (157)	2560 (2480-2860)	7	2644 (157)	2560 (2480-2860)	0		
average for gestational age (g)	84	3355 (311)	3325 (2520-4050)	65	3346 (312)	3320 (2520-4050)	19	3387 (314)	3350 (2910-3880)
large for gestational age (g)	4	4245 (517)	4180 (3790-4830)	3	4050 (416)	3830 (3790-4530)	1	4830	
Birth length (cm)	94	51.2 (2.1)	51 (46-57)	75	51.2 (1.9)	51 (47-57)	19	51.1 (2.8)	52 (46-55)
Head circumference (cm)	94	35.0 (1.3)	35 (32-38)	75	34.9 (1.3)	35 (32-38)	19	35.3 (1.2)	35 (33-37)

Abbreviations: Standard deviation (SD), median (MED), minimum (Min), maximum (Max), non-iron deficiency anemia (non-IDA), iron deficiency anemia (IDA). No significances from Mann-Whitney-U test and Pearson's chi-squared test. *Birth weight split up in 3 groups: small for gestational age (<10. percentile), average for gestational age (≥10. p. & ≤ 90 p.) and large for gestational age (>90. p) calculated on data from Voigt. (Voigt et al., 2006) via https://www.pedz.de/de/neo.html.

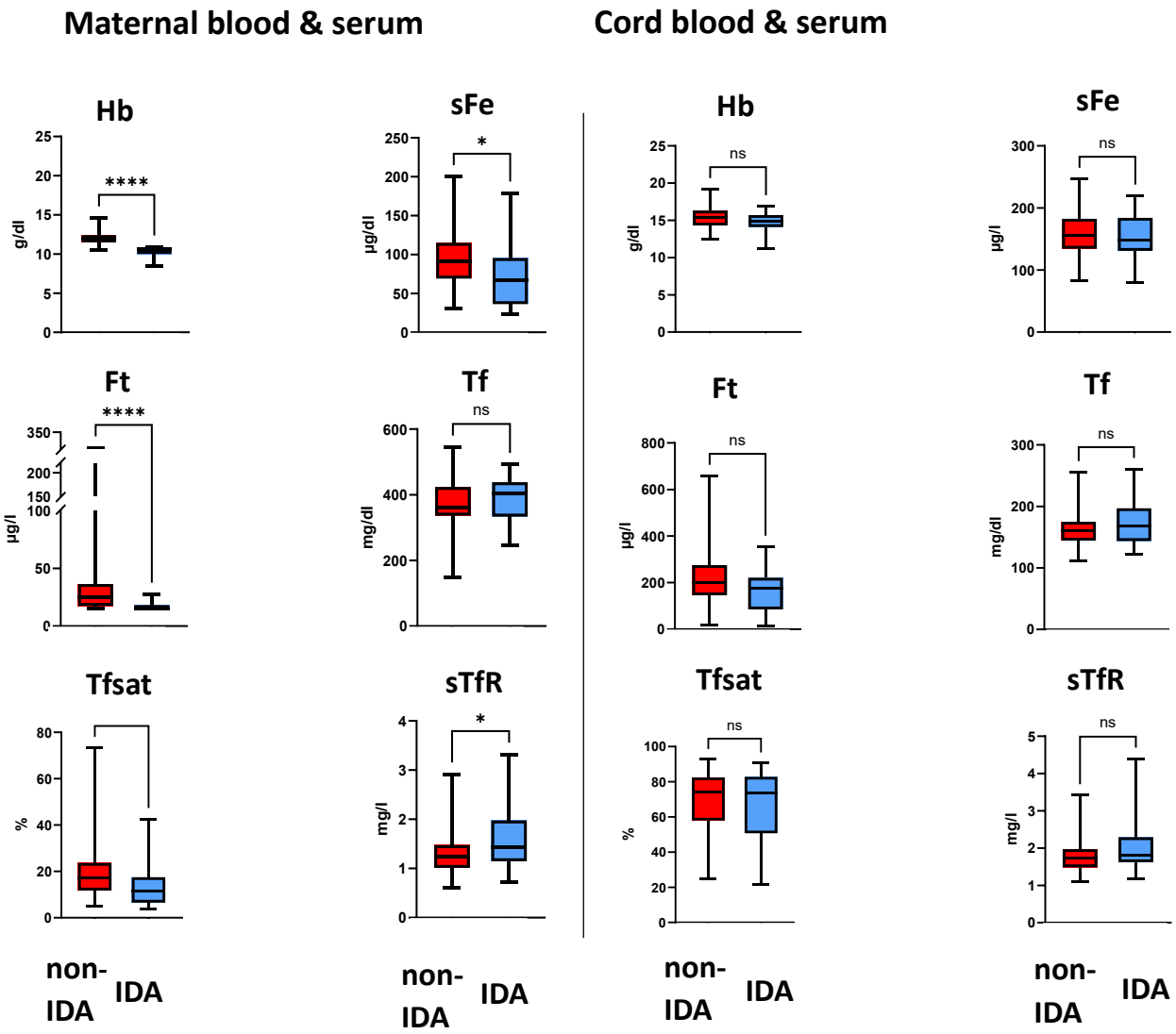

Figure 7. Comparison of iron status parameters in non-IDA and IDA cases. Left: Parameters in maternal blood & serum (N=96-97). Right: Parameters in cord blood & serum (N=86-92). Hemoglobin (Hb), serum iron (sFe), serum Ferritin (Ft), transferrin (Tf), transferrin saturation (Tfsat), soluble transferrin receptor (sTfR), non-iron deficiency anemia (non-IDA), iron deficiency anemia (IDA). Mann-Whitney-U test. ns =P>0.05; *= P<0.05; **= P<0.01; ****=P<0.0001.

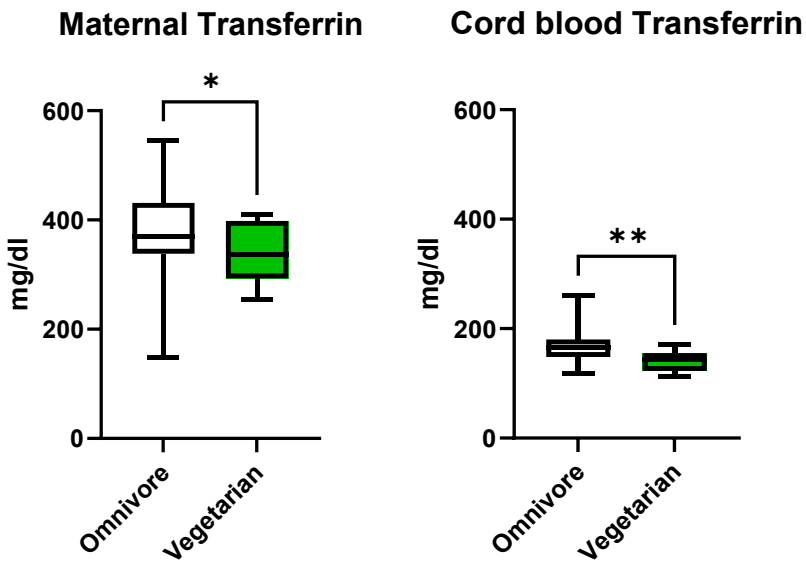

Figure 8. Transferrin status in omnivore and vegetarian mothers. Left: Transferrin in mothers. N=96. Right: Transferrin in cord blood. N=91. Mann-Whitney-U test. *= P<0.05; **= P<0.01.

3.2 Maternal IDA in relation to iron status in the newborn

Regarding the hypothesis whether maternal iron deficiency transfers to the child, various correlations were computed. Some correlations were significant (P<0.05), but overall had low R_s values (Figure 9). Some correlations exhibited trends (P<0.1), although R_s values were equally low and are not shown.

3.3 Expression of iron related genes TFR1 and FLT1 in placentas from IDA pregnancies

To validate the anticipated alteration in placental *TFR1* gene expression in IDA, mRNA expression was compared within a subgroup of the population, consisting of individuals with detectable *TFR1* gene expression in placentas (N=47). The *TFR1* levels were found to be significantly elevated in the IDA group (Figure 10A). These results were confirmed by immunoblotting, where TFR1 gave a stronger signal in the IDA group (Figure 10B). To confirm iron deficient status in the placenta FTL1 was blotted, and when compared to the non-IDA group, FTL1 bands appeared noticeably fainter in IDA placentas when comparing to the non-IDA group (Figure 10C).

Within the same subgroup with detectable *TFR1* gene expression, binary logistic regression was conducted to determine factors potentially predictive of the outcome variable, IDA. After pre-pregnancy BMI, expression of *TFR1* emerged as the factor with the second highest estimate (Table 3).

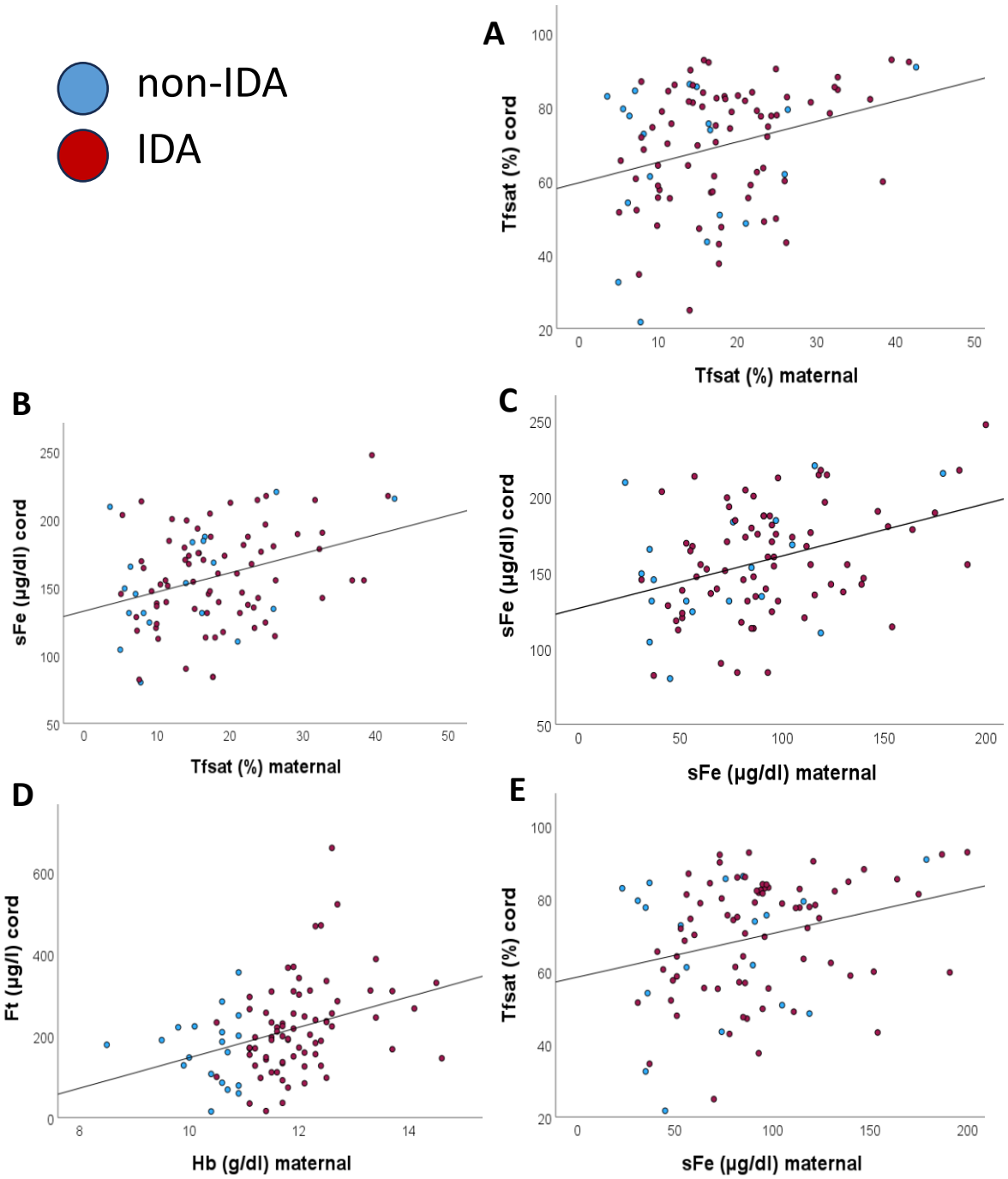

Figure 9. Correlations between different iron parameters in maternal blood and cord blood. (A) Correlation between maternal Tfsat and cord Tfsat (P<0.05, Rs=0.24). (B) Correlation between maternal Tfsat and cord sFe (P<0.01, Rs=0.28). (C) Correlation between maternal sFe and cord sFe (P<0.001. Rs=0.35). (D) Correlation between maternal Hb and cord Ft (P<0.001, Rs=0.40). (E) Correlation between maternal sFe and cord Tfsat (P<0.05, Rs=0.27). (A-E) N=92. Abbrevations: Hemoglobin (Hb), serum Iron (sFe), ferritin (Ft), transferrin saturation (Tfsat), non-iron deficiency anemia (non-IDA), iron deficiency anemia (IDA). Spearman's correlation.

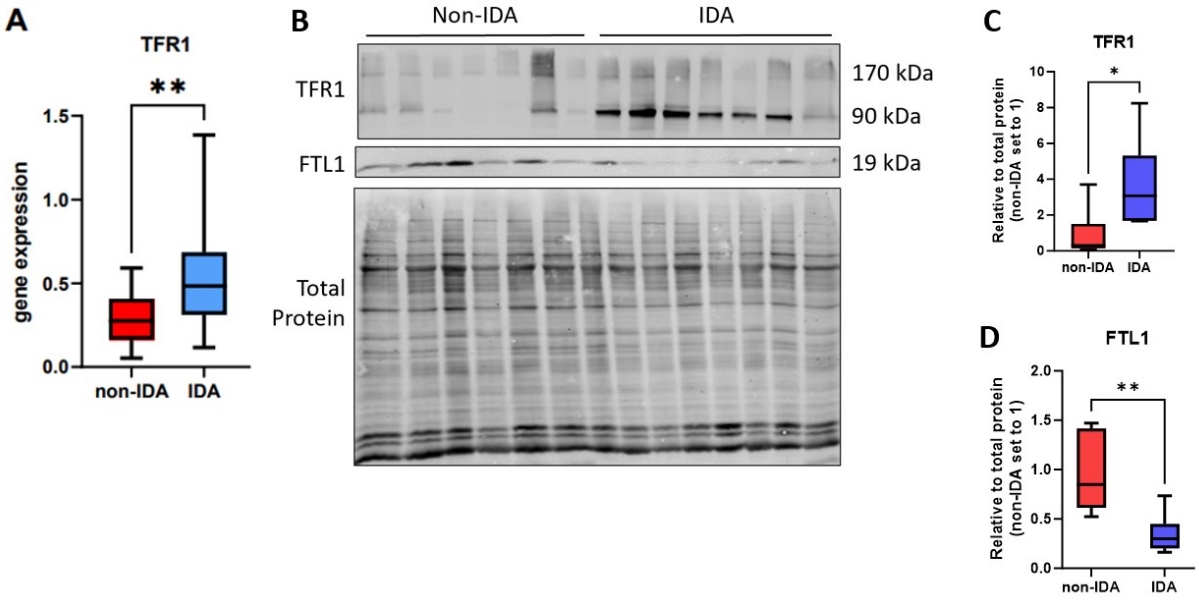

Figure 10. Placental TFR1 and FTL1 expression comparing non-IDA and IDA cases. (A) Gene expression of *TFR1* based on technical duplicates, N=47. (B) Western blot of TRF1 and of FTL1 (data from Lab Gundacker). (C) Relative expression of TFR1. (D) Relative expression of FTL1. (B-D) N=14. Abbrevations: Transferrin Receptor 1 (TFR1), ferritin light chain (FTL1), non-iron deficiency anemia (non-IDA), iron deficiency anemia (IDA). Mann-Whitney-U-test. *= P<0.05; **= P<0.01.

Table 3. Factors associated with maternal IDA in binary logistic regression analyses.

	Estimate	Std. error	z-value	Sig.	Odds Ratio
Intercept	-9.044	3.001	-3.013	0.003	0.000
Gene expression *TFR1*	0.068	0.027	2.508	0.012	0.070
Pre-pregnancy BMI (kg/m²)	0.232	0.099	2.347	0.019	1.261

IDA coded = 1, non-IDA coded= 0. The seven factors included in the crude model were age (years); pre-pregnancy BMI (kg/m²); gene expression *TFR1*; iron therapy during pregnancy (cat) coded 1: neither supplements nor therapy, 2: supplements only, 3: therapy with or without supplements; education (cat) coded 1: less than high school, 2: high school, 3: academic; iron intake (mg/d) and birthweight (cat) coded 1: small for gestational age, 2: average for gestational age, 3: large for gestational age. Iron intake and birthweight were chosen as plausible factors associated with maternal iron status. N=47. Abbreviations: Body mass index (BMI), iron deficiency anemia (IDA), Transferrin Receptor 1 (TFR1), standard error (Std. error), significance (sig.). Pearson's chi-squared test p-value = $1.4*10^{-4}$.

4 Discussion

4.1 Dietary iron uptake and maternal IDA

At date, there is no representative data collection of iron and vitamin C intake in pregnant women in Austria. The calculated consumed dietary iron and vitamin C in this study are similar to other studies that use FFQ for assessing the diet (Iglesias-Vázquez et al., 2023; Milman, 2020) although others found higher mean intake in their populations (Brito et al., 2023; Vioque et al., 2013). The FFQ, as one tool for assessing food consumption, is susceptible, like all nutrition assessment tools, to random errors but is also prone to various biases and altogether

may have influenced the results (De Moor et al., 2003). Compiling the questionnaire, especially the FFQ, presents a challenge as it involves inquiring about all pertinent iron-rich foods and their quantities. However, considering the significance of time as a valuable asset in the clinic, it is essential to strike a balance between these poles, avoiding excessive length. In hindsight, adjustments, such as splitting the item "red meat" into various animal groups, would have enhanced the calculation of meat as a crucial source of iron, acknowledging the diverse iron content across different animal species (Kongkachuichai et al., 2002). Regarding biases, a notable observation was the high level of education among the participants in this study, suggesting a potential selection bias that may not accurately represent the Vienna population. Any women who met the inclusion and exclusion criteria were attempted to be enrolled in the study. However, women who neither spoke German nor English, and for whom no translator was available, could not be included in the study. These women were mostly of migrant background, which is associated with lower educational status and thus, the educational level of the study group may not be representative (Eurostat, 2022). Another potential error may manifest as social desirability bias. In this case, the actual amount of certain foods consumed differs from the quantity reported in the questionnaire. Participants tend to report consumption levels that are more socially acceptable for foods associated with health and, conversely, less for those perceived as less healthy (Kipnis et al., 2002). This bias of so-called overreporting and underreporting is linked with the recall or reporting bias, where the psychosocial influences mentioned before may account for the gaps in recalling the foods or the quantities consumed (Lissner et al., 2007). Although 'finger as a ruler' and hand sizes, which were mainly used during the interviews are superior to kitchen measures, the actual food quantities are still often underestimated and overestimated (Gibson et al., 2016). After collecting data, another challenge arises when utilising food composition databases, the centre piece for calculating iron and vitamin C intake in this context. Natural variations in food exist, and, for processed foods, there may be limited information available regarding their ingredients and composition (Leclercq et al., 2001). However, the FFQ primarily focused on iron, with vitamin C considered only secondarily, resulting in the omission of various vitamin C-rich foods within the questionnaire. Moreover, differing recommendations for RDA values exist among nutrition organisations. For instance, in the U.S., the RDA is 27 mg/d for iron and 85 mg/d for vitamin C, while the D-A-CH guidelines are at the higher end of the spectrum, specifying 30 mg/d and, respectively, 110 mg/d (Deutsche Gesellschaft für Ernährung, 2000; Deutsche Gesellschaft für Ernährung, 2015; Institute of Medicine (US), 2000, 2001). These variations may account for the relatively low percentages of women in this study who met the RDAs for iron and vitamin C. Needless to say, the majority of women, nearly 90% took supplements and/or iron medications. Unfortunately, and considered as a limitation in this study, it was hardly possible to determine the exact dosage as well as the products consumed and therefore, they were not considered in the calculation of the daily intake. Nevertheless, the percentages of intake in general align with a survey in Austria, where 10% reported taking iron medications, and 73% (without distinguishing whether they also had iron medication) reported taking iron supplements or iron-containing multivitamin preparations (Spary-Kainz et al., 2019). Conclusive, while the results appear to be accurate, it is crucial to acknowledge the relatively small number of participants and potential inherent biases when drawing further insights from this data.

No difference was found between the non-IDA and IDA group regarding their intake of iron. The same is true when comparing omnivores and vegetarians. The literature provides a controversial picture. A review about risk factors for IDA in pregnancy found that there is an elevated risk when meat is consumed in a frequency of ≤1/week (Zhang et al., 2022). A British

study found higher iron intake within the vegetarian group, noting the potential influence of a confounding factor related to higher socioeconomic status in the vegetarian group (Alwan et al., 2011). Vitamin C is known to enhance intestinal iron absorption, and some clinical recommendations suggest combining it with oral iron treatment in cases of IDA (Cook & Reddy, 2001; Loganathan et al., 2023; Skolmowska et al., 2022). Interestingly, the IDA group had a higher dietary intake of Vitamin C compared to the non-IDA group. A study conducted in China identified an association within their study population, indicating that the consumption of vitamin C-rich foods ≥2/day was linked to an increased risk of IDA (Zhang et al., 2021). A recent meta-analysis showed that combining oral iron medication with vitamin C did not result in a significant change in Hb and Ft levels among patients with IDA when compared to iron medication alone. Notably, two of the nine included studies in this meta-analysis focused exclusively on pregnant women (Loganathan et al., 2023). Despite the overlap between all these findings, further research is required to question the significance of vitamin C in IDA patients before definite conclusions can be drawn.

Vegetarianism was not identified to be a risk factor for IDA in this data set and vegetarian women had no lower iron status compared to the omnivores. The only notable distinction was in the elevated Tf levels in omnivore mothers and in cord blood of their offspring. Information on iron status of pregnant vegetarian women is limited. In a review that did not specify the inclusion or exclusion of pregnant women, it was determined that Hb and Ft levels are significantly lower in vegetarian women compared to omnivores (Pawlak et al., 2018). A meta-analysis indicated that Ft levels are lower in vegetarians than in omnivores; however, pregnant and lactating women were excluded in this study (Haider et al., 2018). Within present data, the higher Tf levels observed in omnivore mothers are notably atypical and deviate from the expected pattern. Typically, vegetarians, rather than omnivores, exhibit higher Tf levels (Śliwińska et al., 2018).

4.2 Elevated BMI in pregnancy and iron status

As other studies have shown, pre-pregnancy and pregnancy obesity are associated with impaired maternal iron status (Dao et al., 2013; Garcia-Valdes et al., 2015; Jones et al., 2016; Tussing-Humphreys et al., 2021). In the present study population, a significant difference in pre-pregnancy BMI was observed between the non-IDA and IDA groups, with trends also noted in end-pregnancy BMI. Maternal Hb showed a negative correlation, while Tf had a positive correlation with both BMIs. Although not all aforementioned studies found robust correlation data between BMI and changes in cord blood, Jones et al. (2016) reported a negative association between pre-pregnancy BMI and neonatal iron status through their bivariate linear regression models. Infants born to women with higher BMIs had lower cord Ft and sFe concentrations and higher sTfR concentrations. This matches with the present findings where Ft in cord blood was negatively correlated, both with pre-pregnancy and end-pregnancy BMI. sTfR showed a positive correlation with BMI, but only in end-pregnancy. In addition, sTfR was positively correlated with BMI, but only in end-pregnancy. This aligns with, Dao et al. (2013) and Garcia-Valdes et al. (2015) as they reported, that Tfsat in cord was lower in obese women within their study populations. Similar to the studies by Tussing-Humphreys et al. (2021) and Garcia-Valdes et al. (2015) (see Table 4), this study found no correlation between TFR1 expression on mRNA and protein levels and weight. In the present study, maternal CRP was also associated with BMI. However, there was no difference in CRP between the non-IDA and IDA groups. Although hepcidin was not measured in the present study, all other findings support the hypothesis described by these authors regarding the pro-inflammatory status in obesity and, consequently, the dysregulation of hepcidin and the associated iron deficiency.

4.3 Maternal IDA in relation to iron status in the newborn

While certain correlations were statistically significant, it does not appear that any single cord blood parameter in this study stood out as a prominent biomarker for IDA. This could be attributed to the moderately large sample size. In a larger study population, these trends may potentially become more pronounced. Many studies have found an association between maternal iron status and fetal iron status, but the correlations were insignificant, probably because of the small sample sizes (N=95-103) (Emery & Barry, 2004; Harthoorn-Lasthuizen et al., 2001; Paiva Ade et al., 2007). In larger studies (N=527 or N=3702) the correlations were significant, and even more significant in cases of severe maternal IDA. This was true for serum iron, Ft and reticulocytes, but like in this thesis, the correlations were of low order (Choi et al., 2000; Shao et al., 2012). In addition, studies of small sample size (N=45-85) actually found significant differences in iron status of newborns delivered by non-anemic and anemic women, especially when severe IDA was the case (Basu et al., 2016; El-Farrash et al., 2012; Kumar et al., 2008; Singla et al., 1996). In a study, where iron supplementation during pregnancy versus placebo was examined, iron supplementation did not affect fetal iron status. In the verum group they formed a subgroup with all women with complete adherence data and found a significant increase of serum Ft in cord blood with higher intake of iron capsules (Zhao et al., 2015). Needless to say, these studies are heterogenous regarding their cut-off values, groupings and their examined outcomes and that there are also studies showing no tendency for correlating maternal-fetal iron status (Ervasti et al., 2009; Preziosi et al., 1997). Nevertheless, Ft might be a reasonable biomarker for IDA in neonates, but probably only in mothers with severe IDA.

4.4 Expression of iron related genes TFR1 and FLT1 in placentas from IDA pregnancies

The present result that the expression of TFR1 is upregulated at both mRNA and protein level is reflected in the current study landscape, as indicated in Table 4. Although content of this table was created carefully, it is not based on a systematic literature review, and there may be additional studies on this topic. Most of these studies are difficult to compare as they involve different species, group classifications, and overall protocols. Nevertheless, TFR1 consistently appears to be upregulated in women with depleted iron status, especially when iron stores are severely deficient. In the logistic regression model (Table 3) *TFR1* was one of the explanatory factors for IDA, reciprocal to the findings of Best et al. (2016), who observed that low maternal Ft levels and high maternal sTfR levels during midgestation predict elevated TFR1 expression. A recent study that did not examine TFR1 but regulatory hormones of iron homeostasis found that maternal EPO measured midgestation and at delivery was best able of identifying women with anemia, and maternal hepcidin-to-EPO ratio was best able to identify IDA (Delaney et al., 2021). Hepcidin plays a pivotal role in maintaining iron homeostasis, and interestingly, it may directly impact placental iron transporters. In an in vitro trophoblast cell model using Jeg-3 trophoblast cells, both TFR1 and FPN1 were observed to be downregulated upon treatment with hepcidin. The authors discussed that initially, FPN1 undergoes degradation, resulting in lower iron efflux in hepcidin-treated cells. Consequently, due to the retention of iron within the cells, reflecting higher intracellular iron stores, TFR1 expression decreases. In their research on primary human tissues with corresponding blood samples from mothers and cords, there was an inverse association between maternal hepcidin and placental TFR1 at both mRNA and protein levels, and for placental *FPN1* at the mRNA level. Notably, there were no associations with fetal hepcidin and these transporters. However, it is worth noting that cord blood was collected postnatally, following a proinflammatory event (birth), and may therefore not necessarily reflect the status during pregnancy (McDonald et al., 2022).

Ft expression in the human placenta is well understood. Given that the majority of iron derived from transferrin-iron is incorporated into ferritin iron, FTL1 was utilized as a functional indicator of TFR1 in this specific study. As expected, FTL1 was upregulated in the status iron repletion (non-IDA), to store iron and give protection from “free” reactive ferrous iron, that is toxic for the cell as is generates hydroxyl or lipid radicals. This homeostasis is regulated through the Iron-Responsive Element/Iron-Regulatory Protein (IRE/IRP) regulatory network, which functions at the posttranscriptional level by de-repressing mRNA translation when iron is abundant (Muckenthaler et al., 2008). Reports on the localisation of the iron storage protein are controversial (Cao & Fleming, 2016). Consistent results suggest that Ft is localised in fetal villous stroma and in Hofbauer cells (HBC), which are macrophages of fetal origin and located in the placental stroma (Bastin et al., 2006; Drachenberg & Papadimitriou, 1994). Iron storage in fetal villous stroma including HBCs are hypothesised to be important as (temporary) iron repository when maternal iron supply exceeds the demand of the fetus (Cao & Fleming, 2016). This hypothesis is supported by recent result of our group, where HBCs were isolated, and conditions of simulated iron overload (Ferric ammonium citrate treatment) and depletion (Deferoxamine treatment) were investigated. In high iron overload treatment HBCs show upregulated TFR1 expression and therefore probably function as a storage and buffer for the placenta (Bajtela, 2021). These findings suggest that the placenta itself plays a significant role in regulating iron homeostasis. Together with the observation that in maternal IDA TFR1 was upregulated, but the IDA itself did not transfer to the child, it indicates that the placenta has a substantial compensatory capacity.

Table 4. Current knowledge field of TFR1 expression in the placenta in the context of ID and IDA

Autor (year)	Organism	Definitions	Groups	Results of relevance to current topic	N	Extra info
Sangkhae et al.(2020)	C57BL/6J mice (human & PHT/BeWo cell line)	Standard chow diet 185 ppm iron, low-iron diet 4 ppm iron, injected with 20 mg iron dextran (at the time of mating) Human: ID Ft <10 µg/l	Mice: 1) pregnant iron-replete (standard chow), 2) pregnant iron-deficient (low-iron diet), 3) pregnant iron loaded (standard chow + 1 time iron injection) 4-6) control: same procedures but not pregnant Human: women were grouped by maternal serum ferritin levels, as cutoff for ID.	Mice: mRNA & protein expression of TFR1 did mostly not differ between iron groups significantly but had tendencies. Human: Protein expression of TFR1 was mildly but significantly increased in ID compared to control, mRNA differences were not statistically significant. BeWo/PHT: upregulation of TFR1 in iron-deficient cells	39 (human)	Mice: Study included expression patterns over the course of pregnancy: Groups 1-3 -> 3 harvest times: E12.5, E15.5, and E18.5. Protein level of TFR1 and FPN1 increased with gestational age. Mice as main model, different confirmation models: Human and cell culture (PHT and BeWo) Mice: Term & non-term placenta (humans: n.a.)
Cao et al.(2021)	129S6/SvEvTac mice	Iron adequate (IA) diet 50 ppm iron, iron deficient (ID) diet 2–5 ppm iron	1) IA diet (N=8), 2) ID diet (N=8)	*TFR1* mRNA was upregulated approximately 2-fold in both male and female ID placentas compared with IA placentas. *TFR1* expression highly increased from E12.5 to E19.5 regardless of group	16	Study included expression patterns over the course of pregnancy (E12.5, E14.5, E16.5, and E19.5). Results split in male and female placentas. Term & non-term placenta
Gambling et al. (2001)	Lister hooded rat (& BeWo cells)	Control group diet: 50 mg/kg iron; decreasing iron diets in the 3 other groups: 37.5 mg/kg, 12.5 mg/kg and 7.5 mg/kg iron	4 groups (N each = 8): 1 control group, 3 experimental groups with decreasing iron in their diet	Transferrin receptor expression was increased at both mRNA and protein levels with increasing iron deficiency	32	Confirmation of findings in BeWo cells. Term & non-term placenta
Present study	Human	IDA: Hb <11 g/dl & Ft <30 µg/l	1) Non-IDA (N=33), 2) IDA (N=14)	TFR1 mRNA and protein upregulated in IDA, backed up by lower FTL1 in these placentas	97, 47 placentas analysed	Additional data from FFQ. Term placenta

Liu et al. (2007)	Human	n.a.	1) normal, 2) ID, 3) IDA	*TFR1* mRNA expression is increased, especially when maternal ID is progressed	n.a.	Article only available in Chinese. Term placenta
Best et al.(2016)	Human	Anemia: Hb <11 g/dl and <10.2 g/dl in African American	No groups, factor analysis & correlations between maternal Fe status and TFR1; Anemia at birth N=27	Across statistical analyses, deficient maternal Fe status was consistently associated with high expression of TFR1 protein and mRNA	154	No groups, no specification for IDA, only for anemia. Term & non-term placenta
Li et al. (2008)	Human	Mild IDA: Hb <11to ≤9 g/dl & hematocrit <33.0% & Ft <16 mg/l, moderate IDA: Hb <9 g/dl & hematocrit <33.0% & Ft < 16 mg/l.	1) non-IDA (N=18), 2) mild IDA (N=17), 3) moderate IDA (N=5)	Expression of *TFR1* mRNA increased significantly in mild IDA and decreased near to normal level in moderate IDA. Same trend for TFR1 protein expression	40	Inverted U-shape: Up-regulation in mild IDA compared to control group, but almost same mRNA level in moderate IDA compared to control group. Term placenta
Young et al. (2010)	Human	Anemia: Hb <11 g/dl, iron deficient Ft <12 µg/l, deplete iron stores Ft <20 µg/l	No groups, correlations between maternal Fe status, anemia and TFR1	Placental TFR1 expression was inversely associated with maternal iron status (Ft)	92, 80 placentas analysed	No groups. Term & non-term placenta
Tussing-Humphreys et al. (2021)	Human	Anemia: Hb <11 g/dl and <10.2 g/dl in African American	1) obese (N=20), 2) non-obese (N=22) No iron-specific groups, correlations within their total group	Maternal ferritin and total body iron were significantly inversely correlated with placental TFR1 protein expression	42	No difference of TFR1 and FPN1 expression among women with or without obesity. Term /non-term placenta: n.a.
Garcia-Valdes et al. (2015)	Human	Iron sufficient: Hb ≥11 g/dl & Ft ≥12 µg/l, IDA: Hb <11 g/dl, & Ft <12µg/l ID: Hb ≥ 11 g/dl & Ft <12µg/l	1) normal weight (N=90), 2) overweight (N=37), 3) obese (N=31)	mRNA and protein levels of placental TFR1 were both inversely related to maternal iron status regardless of weight, although, low iron stores were more common in obese women	158, 86 placentas analysed	ID and IDA were combined and compared to iron sufficient. TFR1: No differences in weight sub-groups. Term /non-term placenta: n.a.

Abbreviations: Hemoglobin (Hb), Transferrin Receptor 1 (TFR1), ferritin (Ft), ferroportin 1 (FPN1), iron deficiency (ID), non-iron deficiency anemia (non-IDA), iron deficiency anemia (IDA), iron adequate (IA), primary human trophoblast (PHT). derived human choriocarcinoma cell (BeWo), embryonic day (E), food frequency questionnaire (FFQ), not available (n.a.).

5 Conclusion and Outlook

Recruiting the participants along with collecting questionnaire data, tissues, and blood posed a significant effort, particularly due to restrictions during the COVID-19 pandemic. In this work, data on iron consumption were linked with blood values of mothers and children, as well as placental markers. There was almost no correlation found between nutrition and blood iron levels, except that in the IDA group, vitamin C consumption was higher. Subsequent studies must include higher numbers of ID and IDA cases; the quite low numbers represent a limitation in this study. Additionally, attention should be given to the precise documentation of supplements and iron medications when investigating oral iron intake. As a matter of interest, future studies could also focus on vegans, as this group has been relatively understudied in the context of questions related to iron homeostasis between mother, child, and placenta. As described, overweight and, especially, obesity play a role in connection with iron metabolism during pregnancy. While not the primary focus of this study, it was observed that in the IDA group, a significantly higher proportion had higher pre-pregnancy BMI values than the non-IDA group and several correlations between iron status of mother as well as cord to BMI could be seen, although the effects were small. As mentioned before, the underlying mechanism of this outcome is likely to involve a proinflammatory status and subsequently altered hepcidin levels, but further research is necessary to elucidate this hypothesis. With regard to the question of whether IDA is transmitted from mother to child, there were several significant correlations in the analysis, but the observed effects were very small. These tendencies might become more pronounced in larger study groups. However, even in comparative studies, there are no conclusive results on this topic, as discussed earlier. Presumably, the unborn child may inherit parameters related to blood iron status when maternal IDA is severe; however, cases of severe anemia were not observed in the current study cohort, and no data can be added to the discourse of severe anemia. The present study demonstrates that placental TFR1 is upregulated in women with IDA. TFR1 is not only upregulated on mRNA level and translated to protein, the transporters are functional, as supported by the data from FTL1. This result aligns with findings from other described studies (Table 4). Assuming that maternal IDA is not transmitted to the child or only in severe cases, and the placenta counteracts with compensatory measures such as altered expression of TFR1 and FTL1, the significant question emerges regarding how these mechanisms are regulated and which of the involved components (maternal liver, placenta, newborn) play a key role. This should be a primary focus for further studies on this topic.

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7 Appendix

7.1 Protocols

SDS-Page gel composition

Reagent	Resolving gel (12,5%)	Stacking gel (5%)
Acrylamide (30%) (ml)	5	0,5
Resolving gel buffer pH 8,8 (ml)	3	-
Stacking gel buffer pH 6,8 (ml)	-	0,75
H2O (ml)	4	1,75
10 APS (µl)	120	30
TEMED (µl)	10	2,5

Protocol: RNA Extraction

Centrifugation at 10 000 x g at room temperature for 1 min, if not described in detail

1. Remove RNAlater using absorbent paper
2. Lyse tissue (~75 mg) on ice in 700 µl ice cold Cell Disruption Buffer using a motorized rotor-stator homogenizer (TissureRupture II, QIAGEN)
3. Split the lysate and transfer 400 µl of the lysate to a new tube with 400 µl prepared 2X Lysis/Binding Solution (13,8 µl 2-mercaptoethanol / 1ml 2X Lysis/Binding Solution). The remainder of the lysate is incubated on ice for protein analysis (see Protein isolation)
4. Transfer first lysate to a new tube with an QIAshredder (QIAGEN, Cat.No. 79656) and centrifuge for 2 min to raise yield and remove debris
5. Remove QIAshredder and add 400 µl 100% EtOH and invert tube
6. Transfer 700 µl of sample mixture to a Collection Tube with a Filter Cartridge (both supplied in the Kit), centrifuge then discard flow through
7. Transfer rest of sample mixture to the Collection Tube, centrifuge then discard flow through
8. Apply 700 µl Wash Solution 1, centrifuge then discard flow through
9. Apply 500 µl prepared Wash Solution 2/3 (add 64 ml 100% EtOH to Wash solution 2/3 before first use), centrifuge then discard flow through, repeat step 9 one time
10. Centrifuge for 30 s to remove traces of Wash Solution 2/3
11. Transfer the Filter Cartridge in a new Collection Tube
12. Place 30 µl of preheated (95°C) Elution Solution in the centre of the filter then centrifuge for 30 s, repeat step 12 one time and store RNA at -80°C

Protocol: cDNA Synthesis

1. Determine RNA concentration with Nanodrop-1000 (Thermo Fisher Scientific)
2. Add nuclease-free water to RNA to adjust the RNA concentration for reverse transcription to a total of 10 µl

Component	Volume (µl)
RNA	X (up to 1000 ng)
Nuclease-free water	X (to a total of 10 µl)

3. Incubate RNA samples at 70°C for 5 min
4. Add 10 µl of Master Mix

Component	Volume (µl)
Nuclease-Free Water	4
GoScript™ Reaction Buffer, Random Primer	4
GoScript™ Enzyme Mix	2

5. Place samples into thermocycler (Peqlab)

Step	Time (min)	Temperature (°C)
Anneal primer stage	5	25
Extension stage	60	72
Inactivation stage	15	70

Protocol: Quantitative RT PCR

1. Prepare working dilution, dilute cDNA:nuclease-free water 1:10
2. Reverse-pipette 2 µl of prepared cDNA in 96-well plate in a twofold approach
3. Add 13 µl PCR master mix

Component	Volume (µl)
Primer	0,75
iTaq™ Universal Probes Supermix (Bio-Rad)	7,5
Nuclease- free water	4,75

4. Seal the plate and vortex briefly to mix contents, then vortex briefly to collect the mixture at the bottom of the wells
5. Load and read plate with StepOnePlus™ Real-Time PCR System (applied biosystems):

Step	Time	Temperature (°C)
holding stage	2 min	50
denaturation stage	10 min	95
cycling stage a)	15 sec	95
cycling stage b)	1 min	60

Cycling stage is repeated 40x

Protocol: Protein Isolation

1. Placenta tissue is lysed in Cell Disruption Buffer beforehand (see Protocol: RNA extraction)
2. Incubate for 10 min on ice
3. Centrifugate at 15 000 x g at 4°C for 10 min
4. Transfer supernatant into new tube
5. Add 1 µl of protein mixture to 1 ml of Bradford 1x Dye Reagent (Bio-Rad)
6. Add 1 µl of Cell Disruption Buffer to 1 ml of Bradford 1x Dye Reagent for the blank
7. Incubate for 5 min in the dark at RT
8. Measure protein concentration with spectrometer (Bio-Rad Smartspec 3000 Spectrophotometer, Bio-Rad) at 595 nm

9. Store protein at -20°C

Protocol: Western Blot & und Immunoblotting

Washing, blocking and incubation of antibodies took place on a 2D shaker

1. Prepare 20 µg protein
2. Supplement 4X Protein Loading Buffer (LI-COR) with 10% of β-mercaptoethanol (Merck)
3. Mix up prepared 4X Protein Loading Buffer with protein 1:4
4. Incubate 10 min at 72°C
5. Transfer samples on gel
6. Use PageRuler™ Prestained Protein Ladder (Thermo Fisher Scientific) as marker
7. Run gel at 70 V until separation of marker bands is visible
8. Switch to 120 V and run gel for 1 h
9. Blot on a nitrocellulose membrane at 350 mA for 1h
10. Stain total protein with Revert 700 Total Protein Stain (LI-COR) and wash with Revert 700 Wash Solution (LI-COR), then rinse with dH2O
11. Total protein assessment: Scan membrane with Odyssey CLx imager (LI-COR)
12. Block for 1 h in Intercept (TBS) Blocking Buffer
13. Wash 3 x 5 min with TBST
14. Incubate with primary antibody (diluted as described above) in Intercept T20 (TBS) Antibody Diluent over night at 4°C
15. Wash 3 x 5 min with TBST
16. Incubate with secondary antibody (diluted as described above) in Intercept T20 (TBS) Antibody Diluent for 1 h at 4°C protected from light
17. Wash 3 x 5 min with TBST
18. Antibody assessment: Scan membrane with Odyssey CLx imager (LI-COR)

7.2 Antibodies and Chemicals

Antibody	**Company**	**Catalogue Number**	**Dilution**
Ferritin Light Chain, rabbit pAb	Abcam	69090	WB 1:1 000
Transferrin Receptor 1, rabbit mAb	Cell Signaling Technology	13113	WB 1:1 000
IRDyes 800CW Goat anti-Mouse IgG1-Specific	LI-COR	926-32350	WB 1:20 000
IRDyes 800CW Goat anti-Rabbit IgG Secondary Antibody	LI-COR	926-32211	WB 1:20 000

Substance	**Company**	**Catalogue Number**
RNAlater	Thermo Fisher Scientific	AM7021
Cell Disruption Buffer	Thermo Fisher Scientific	AM1921 (PARIS™ Kit)
2X Lysis/Binding Solution	Thermo Fisher Scientific	AM1921 (PARIS™ Kit)
Wash Solution 1	Thermo Fisher Scientific	AM1921 (PARIS™ Kit)

Wash Solution 2/3 Concentrate	Thermo Fisher Scientific	AM1921 (PARIS™ Kit)
Elution Solution	Thermo Fisher Scientific	AM1921 (PARIS™ Kit)
Ethanol 100%	VWR	20821.330
β-mercaptoethanol	Merck	8057401000
GoScript™ Reverse Transcriptase	Promega	A5000 GoScript™ Reverse Transcription System
GoScript™ 5X Reaction Buffer	Promega	A5000 GoScript™ Reverse Transcription System
PCR Nucleotide Mix	Promega	A5000 GoScript™ Reverse Transcription System
Random Primers	Promega	A5000 GoScript™ Reverse Transcription System
RNAse-free water	Promega	A5000 GoScript™ Reverse Transcription System
Nuclease-free water	Promega	P1197
Resolving gel: 1.5 M Trizma® Base; 0.4% SDS; pH 8.8	Sigma/VWR	93362;307128.0500P
Stacking gel: 0.5 M Trizma® Base; 0.4% SDS; pH 6.8	Sigma/VWR	93362;307128.0500P
Acrylamide 30% Acrylamide/bis-Acrylamide	Sigma	A3699
10% Ammoniumpersulfat	Sigma	A3678
PageRuler™ Prestained Protein Ladder	Thermo Scientific	26617
TBST 1X TBS; 0.1% Tween® 20x	VWR	J77500.K8
TEMED N,N,N',N'-Tetramethylethan-1,2-diamin	Sigma	T9281
Quick Start™ Bradford 1x Dye Reagent	Bio-Rad	5000205
4X Protein Loading Buffer	LI-COR	928-40004
Revert™ 700 Total Protein Stain Kit	LI-COR	926-11010
Intercept® (TBS) Blocking Buffer	LI-COR	927-60001
Intercept® T20 (TBS) Antibody Diluent	LI-COR	927-65001
iTaq™ Universal Probes Supermix	Bio-Rad	1725130

7.3 Questionnaire

Fragebogen Eisenstudie Plazenta

Allgemein

Datum: .....

PatientInnenetikett

Name: ..... Geburtsdatum: .....

Wohnort: ..... Geburtsland: .....

SS Woche: ..... Größe (cm): .....

Gewicht vor SS (kg): ..... Gewicht aktuell (kg): .....

Größe und Gewicht des Vaters des Kindes: Größe (cm): .....
Gewicht aktuell (kg): .....

Höchste abgeschlossene Ausbildung:
- ☐ kein Schulabschluss
- ☐ Volksschule
- ☐ Hauptschule/AHS-Unterstufe/Neue Mittelschule
- ☐ Polytechnische Schule/Berufsschule/ Berufsbildende höhere Schule ohne Matura
- ☐ Berufsbildende höhere Schule/AHS mit Matura
- ☐ Akademischer Abschluss

Berufsstatus:
- ☐ In Ausbildung
- ☐ Berufstätig
- ☐ Nicht berufstätig/Hausfrau

Rauchverhalten:
- ☐ Nicht-Raucherin
- ☐ Ex-Raucherin/vor SS aufgehört
  ..... Zigaretten pro Tag
  ..... Jahre
- ☐ Raucherin
  ..... Zigaretten pro Tag
  ..... Jahre

Alkoholkonsum während SS:
- ☐ Nein
- ☐ Ja Wie häufig: .....

Drogenkonsum während SS:
- ☐ Nein
- ☐ Ja Wie häufig: .....

Medizinische Vorgeschichte

Anzahl der SS:	Anzahl der Lebendgeburten: ..... Davon Sectio: .....
Anzahl der Fehlgeburten:	Anzahl der Abtreibungen: ..... Curettage ☐ nein ☐ ja
Zeugung bei dieser SS	☐ Spontan ☐ Künstliche Befruchtung
Bluttransfusion im letzten Monat	☐ Nein ☐ Ja Letzte Transfusion: .....
Blutspende im letzten Monat	☐ Nein ☐ Ja Letzte Spende: .....
Magen-Darm-Erkrankungen	☐ Nein ☐ Ja Welche: .....
Allergien	☐ Nein ☐ Ja Welche: .....
Nahrungsmittelunverträglichkeiten	☐ Nein ☐ Ja Welche: .....

Medikamente und Nahrungsergänzungsmittel (während SS)

Ferrogradumet / Fol	☐ Dosis:	Aktiferrin	☐ Dosis: .....
Tardyferon / Fol	☐ Dosis:	Ferinject	☐ Dosis: .....
Fermed	☐ Dosis:	Venofer	☐ Dosis: .....
Femibion	☐ Dosis:	Pregnavit	☐ Dosis: .....
Elevit	☐ Dosis:		

Andere: ..... Dosis: .....
..... Dosis: .....

Ernährungsprofil

Die folgenden Fragen beziehen sich auf <u>das letzte Monat Ihrer Schwangerschaft.</u> Bitte geben Sie Durchschnittswerte für diesen Zeitraum an.

Ausfüllhilfe: In einer Zeile können mehrere Lebensmittel stehen. Bitte zählen Sie all jene Lebensmittel in einer Zeile, die konsumiert wurden, zusammen. Geben Sie dann die Anzahl der Portionen pro Woche an, außer es trifft „nie" oder „<1 /Woche" zu. Im angeführten Beispiel wird täglich ein Glas Orangensaft und ein Glas Johannisbeersaft konsumiert. Das entspricht 2 Portionen täglich, bzw. 14 Portionen pro Woche.

Beispiel:

Orangensaft, Grapefruitsaft & Beerensäfte	☐ nie	☐ <1 /Woche	☐ 14 x pro Woche

<u>Generelle Ernährungsform:</u>

☐ Mischkost
☐ vegetarisch
☐ vegan
☐ Andere .....

<u>Fleisch, Fisch & Eier</u>

1 Portion **Fleisch** entspricht der **Handfläche ohne Fingern**, 1 Portion **Wurst** entspricht **5-6 Scheiben** (Durchmesserbeispiel: Salami), 1 Portion **Wurstwaren** entspricht **einem Paar**, 1 Portion **Fisch** entspricht der **Handfläche mit Fingern**, 1 Portion **Ei** entspricht **einem Ei**.

Rotes Fleisch (Rindfleisch, Schweinefleisch, Kalbfleisch, Lammfleisch, Wild & Faschiertes)	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Geflügelfleisch	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Leber, Leberwurst & Blutwurst	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Wurst & Speck	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Wurstwaren (Frankfurter, Debreziner etc.)	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Fisch & Shrimps	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Eier, inklusive verarbeitete Eier	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche

Brot & Getreideprodukte

1 Portion **Brot** entspricht der **Handfläche mit Fingern**, 1 Portion **Frühstücksflocken** entspricht **6 Esslöffel**, 1 Portion **Nudeln/Reis gekocht** bzw. **Getreide gekocht** entspricht 2 **Schöpflöffel**, 1 Portion **Kuchen/Torte** entspricht **1 Stück drei Finger breit**.

Weißbrot & Weißmehlgebäck (Semmel, Kornspitz, Croissant, etc.)	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Schwarzbrot, Mischbrot & Grahamweckerl	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Vollkornbrot & Pumpernickel	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Haferflocken & Müslimischungen	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Eierteigwaren (Nudeln)	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Weißer Reis	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Vollkornnudeln & Vollkornreis	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Hirse, Buchweizen, Amaranth & Quinoa	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Kuchen & Torte	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche

Hülsenfrüchte

1 Portion **Hülsenfrüchte gekocht** oder **aus der Dose** entspricht **2 geballten Fäusten**, 1 Portion **Tofu** entspricht einer **Handfläche mit Fingern**.

Bohnen, Linsen, Erbsen & Kichererbsen	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Tofu	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche

Gemüse, Pilze & Obst

1 Portion entspricht einer **geballten Faust Rohprodukt** bzw. bei **Blattgemüse** dem **Schälchen mit beiden Händen**.

Spinat, Mangold & Vogerlsalat	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Zucchini, rote Rübe, Grünkohl & Kürbis	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Paprika, Broccoli, Kohlrabi & Karfiol	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Eierschwammerl/Pfifferlinge	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Andere Pilze (Champignons, Steinpilz, etc.)	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Beeren & Kiwi	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Orangen, Grapefruit, Papaya & Mango	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche

Nüsse, Samen, Trockenfrüchte & Schokolade

1 Portion **Nüsse/Samen/Trockenfrüchte** entspricht einer **Handinnenfläche**, bzw. 1 Portion **Schokolade** entspricht **2 Rippen**.

Sesam, Pinienkerne & Leinsamen	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Kürbiskerne, Sonnenblumenkerne & Cashewkerne	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Andere Nüsse (Walnusskerne, Mandeln, Pistazien, Haselnuss, Erdnuss & Paranusskerne)	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Trockenfrüchte	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Zartbitterschokolade (über 70% Kakaoanteil)	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Vollmilchschokolade	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche

Milch & Milchprodukte

1 Portion **Milch** entspricht einem **Glas 250ml**, 1 Portion **Joghurt** einem Becher **250g**, 1 Portion **Käse** entspricht **2 Scheiben** (z.B. Gouda) ca. 50g.

Milch, Joghurt & Käse	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche

Getränke

1 Portion **Tee/Kaffee** entspricht einer Tasse **250ml** bzw. bei **Säften** ungespritzt einem Glas **250ml**.

Orangensaft, Grapefruitsaft & Beerensäfte	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Schwarzer & grüner Tee	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche
Kaffee	☐ nie	☐ <1 /Woche	☐ ..... x pro Woche

7.4 Supplementary Tables

Supplementary Table 1.

Lebensmittel	BLS Code	Zusatzinfo	Kcal/100g	KH(g)/100g	F(g)/100g	EW(g)/100g	Fe(mg)/100g	VitC(mg)/100g
Rind	U170100	Bratenfleisch roh	128,5	0,0	5,3	20,2	2,3	0,0
Schwein	U570100	Bratenfleisch roh	218,5	0,0	16,5	18,5	1,8	0,0
Kalb	U370100	Bratenfleisch roh	93,9	0,0	1,0	21,2	2,0	0,0
Faschiertes	U050100	Rind/Schwein Hackfleisch roh	225,7	0,1	16,4	19,4	1,7	0,0
Geflügelfleisch	V416111	roh	101,8	0,0	0,7	23,5	1,1	0,0
Wurst	W235300	Wurst gemischt	285,1	1,1	24,8	15,0	1,8	16,7
Speck	W411011	Schwein, roh geräuchert	320,0	0,0	28,9	16,0	0,6	0,0
Leber Kalb	V532100	roh	86,4	4,1	1,2	14,9	7,9	37,0
Leber Schwein	V533100	roh	129,0	0,9	4,5	21,2	17,3	23,0
Kalbsleberwurst	W314000		345,1	1,7	32,0	13,6	5,6	25,7
Blutwürste	W343000		367,8	0,6	33,7	15,5	10,8	0,1
Frankfurter	W211000	Würstchen/Bockwurst/Wiener Würstchen	271,5	0,3	24,5	13,1	0,8	23,0
Debreziner	W185000		334,3	0,2	30,1	15,6	1,0	0,0
Bratwurst	W222611	Rostbratwurst	329,1	0,3	29,5	16,5	0,8	0,1
Käsekrainer	W205910	aus ÖNWT	256,1	0,7	19,0	20,0	1,4	0,0
Leberkäse	W256100		292,1	0,3	27,4	11,8	1,7	23,8
Fisch	T400100	roh	84,9	0,0	1,7	17,4	0,9	3,0
Eier	E110111	roh	136,7	1,5	9,3	11,9	1,8	0,0
Weißbrot	B31000		242,0	48,0	1,2	8,2	0,7	0,0
Schwarz-/Mischbrot	B251000		236,4	46,3	1,5	8,6	1,4	0,0
Vollkornbrot	B101000		197,9	38,7	1,2	7,3	2,0	0,0
Haferflocken	C133001		354,4	59,5	6,7	13,2	4,4	0,0
Müsli	C512011		346,1	59,0	6,8	11,0	3,3	1,6
Eierteigwaren	E430001	gegart	134,3	25,9	1,0	4,9	0,8	0,0
Reis	C352022	weiß, gegart	105,6	23,5	0,2	2,2	0,3	0,0

Fortsetzung								
Lebensmittel	**BLS Code**	**Zusatzinfo**	**Kcal/100g**	**KH(g)/100g**	**F(g)/100g**	**EW(g)/100g**	**Fe(mg)/100g**	**VitC(mg)/100g**
Vollkornteigwaren	E500022	gegart	143,4	26,9	1,1	6,0	1,3	0,0
Vollkornreis	C351022	gegart	125,7	26,5	0,7	2,7	1,2	0,0
Hirse	C330022	gegart	129,1	24,7	1,3	3,8	2,6	0,0
Buchweizen	C320022	gegart	123,7	25,4	0,6	3,5	1,4	0,0
Amaranth		keine BLS Daten, gegart	102,0	18,7	1,6	3,8	2,1	n.a.
Quinoa	C118032	gegart	103,3	16,7	1,8	3,5	0,9	1,1
Kuchen	D400011		380,3	55,1	14,9	6,1	0,4	0,2
Torten	D300011		278,0	30,6	14,5	4,7	1,5	0,7
Bohnen	H74290n	Kidneybohnen Konserve abgetropft	106,4	15,5	0,6	9,4	2,6	0,4
Linsen	H73090n	Konserve abgetropft	131,2	21,0	0,6	10,0	3,0	0,1
Erbsen	H710902	Konserve abgetropft	133,7	17,8	0,6	9,7	2,0	0,2
Kichererbsen	G770902	Konserve abgetropft	134,8	17,4	2,6	7,3	2,2	6,8
Tofu	J381011		77,2	0,5	4,8	8,1	1,9	0,1
Spinat	G211111	roh	21,7	0,7	0,4	3,3	3,6	32,3
Mangold	G230111	roh	16,0	0,7	0,3	2,1	2,7	39,0
Vogerlsalat	G104111	Feldsalat roh	14,8	0,8	0,4	1,8	2,0	35,0
Zucchini	G582111	roh	20,8	2,3	0,3	2,0	1,0	17,6
Rote Rübe	G613111	roh	41,8	8,4	0,1	1,5	0,9	10,0
Grünkohl	G322100	roh	45,2	2,5	0,9	4,3	1,9	105,0
Kürbis	G581100	roh	28,9	4,6	0,1	1,1	0,8	12,0
Paprika	G543111	roh rot	36,8	6,4	0,5	1,3	0,6	140,0
Broccoli	G312100	roh	33,9	2,7	0,2	3,8	0,8	0,8
Kohlrabi	G331111	roh	24,9	3,7	0,2	1,9	0,5	63,3
Karfiol	G311111	Blumenkohl roh	22,7	2,3	0,3	2,5	0,5	64,0
Eierschwammerl	K713111	Pfifferlinge roh	14,6	0,2	0,5	2,4	6,5	6,0
Pilze	K700100	roh	24,7	0,6	0,2	4,1	1,2	4,9
Beeren	F300000	Beerenobst roh	35,8	5,5	0,4	0,8	0,6	57,0
Kiwi	F514111	roh	54,3	9,1	0,6	1,0	0,8	43,7

Fortsetzung								
Lebensmittel	**BLS Code**	**Zusatzinfo**	**Kcal/100g**	**KH(g)/100g**	**F(g)/100g**	**EW(g)/100g**	**Fe(mg)/100g**	**VitC(mg)/100g**
Orange	F603111	roh	43,3	8,3	0,2	1,0	0,2	45,5
Grapefruit	F604111,	roh	43,7	7,4	0,2	0,6	0,2	40,9
Papaya	F517111	roh	32,0	7,1	0,1	0,5	0,4	80,3
Mango	F616111	roh	58,8	12,4	0,4	0,6	0,4	37,3
Sesam	H420111	roh	571,9	10,2	50,4	20,9	10,0	0,0
Pinienkerne	H320100	roh	595,9	7,3	50,7	24,0	9,2	1,9
Leinsamen	H410111	roh	444,6	7,7	36,5	22,3	6,8	0,0
Kürbiskerne	H31060n	geröstet	564,7	2,7	46,4	35,5	4,9	0,1
Sonnenblumenkerne	H430111	roh	479,9	34,7	26,3	26,1	5,7	0,0
Cashewkerne	H170100	roh	602,9	22,2	47,1	21,0	6,4	0,0
Walnusskerne	H120100	roh	733,2	6,1	70,6	16,1	2,8	0,0
Mandeln	H210101	süß, roh	589,6	5,7	53,0	24,0	3,1	0,0
Pistazien	H250601	geröstet	627,2	15,9	55,4	17,9	3,1	7,3
Haselnuss	H130101	roh	650,1	6,0	63,3	16,3	3,4	0,0
Erdnuss	H110601	geröstet	615,4	9,3	53,0	26,9	2,3	0,0
Paranuss	H180101	roh	687,9	4,1	68,1	17,0	2,8	0,0
Trockenfrüchte	F01D400		268,0	59,0	0,6	2,7	2,7	16,6
Vollmilchschokolade	S560011		496,7	46,3	31,1	8,1	17,0	0,0
Zartbitterschokolade	S530000	über 70% Kakaoanteil	536,6	54,1	31,5	9,2	1,5	0,0
Vollmilch	M111311	Kuhmilch, 3,5% Fett	54,0	4,7	3,6	3,4	0,1	1,7
Joghurt	M141311	Kuhmilch, 3,6% Fett	69,1	4,4	3,8	3,9	0,0	1,0
Käse	M400000	Schnittkäse 45% F.i.Tr.	354,2	0	28,3	24,3	0,3	0,0
Orangensaft	F603600		43,3	8,7	0,1	0,7	0,3	41,5
Grapefruitsaft	F604600		54,0	10,1	0,1	0,5	0,6	36,0
Beerensäfte	F021600	Roter Multivitamin Saft (ÖNWT)	42,9	9,9	0,3	0,3	0,7	30,6
Tee	N600100	schwarz, grün	0,5	0,0	0,0	0,1	0,0	0,0
Kaffee	N410100		2,2	0,3	0,0	0,2	0,2	0,0

In dieser Tabelle befinden sich alle Lebensmittel, die in der nachfolgenden Tabelle in eine einzelne oder zusammengesetzte Variable gemündet sind.

Supplementary Table 2.

Variable	Lebensmittel	BLS CODEs	Kcal/100g	KH(g)/100g	F(g)/100g	EW(g)/100g	Fe(mg)/100g	VitC(mg)/100g	Port.größe(g)
RotFleisch	Rotes Fleisch (Rindfleisch, Schweinefleisch, Kalbfleisch, Lammfleisch, Wild & Faschiertes)	U170100, U570100, U370100, U050100	166,7	0,0	9,8	19,8	2,0	0,0	100
Geflügel	Geflügelfleisch	V416111	101,8	0,0	0,7	23,5	1,1	0,0	100
Leber	Leber, Leberwurst & Blutwurst	V532100, V533100, W314000, W343000	232,1	1,8	17,9	16,3	10,4	21,5	100
Wurst	Wurst & Speck	W235300, W411011	302,6	0,6	26,9	15,5	1,2	8,4	50
Wurstwaren	Wurstwaren (Frankfurter, Debreziner etc.)	W211000, W185000, W222611, W205910, W256100	296,6	0,4	26,1	15,4	1,1	9,4	100
Fisch	Nur Fisch, Shrimp verworfen	T400100	84,9	0,0	1,7	17,4	0,9	3,0	150
Eier	Eier, inklusive verarbeitete Eier	E110111	136,7	1,5	9,3	11,9	1,8	0,0	60
Wbrot	Weißbrot & Weißmehlgebäck (Semmel, Kornspitz, Croissant, etc.)	B31000	242,0	48,0	1,2	8,2	0,7	0,0	50
Sbrot	Schwarzbrot, Mischbrot & Grahamweckerl	B251000	236,4	46,3	1,5	8,6	1,4	0,0	50
Vbrot	Vollkornbrot & Pumpernickel	B101000	197,9	38,7	1,2	7,3	2,0	0,0	60
Haferfl	Haferflocken & Müslimischungen	C133001, C512011	350,3	59,3	6,8	12,1	3,9	0,8	40
Eierteigw	Eierteigwaren (Nudeln)	E430001	134,3	25,9	1,0	4,9	0,8	0,0	150
Wreis	Weißer Reis	C352022	105,6	23,5	0,2	2,2	0,3	0,0	170
Vreis	Vollkornnudeln & Vollkornreis	E500022, C351022	134,6	26,7	0,9	4,4	1,3	0,0	170
Hirse	Hirse, Buchweizen, Amaranth & Quinoa	C330022, C320022, Fehlt, C118032	114,5	21,4	1,3	3,7	1,8	0,4	150
Kuchen	Kuchen & Torte	D400011, D300011	329,2	42,9	14,7	5,4	1,0	0,5	50
Bohnen	Bohnen, Linsen, Erbsen & Kichererbsen	H74290n, H73090n, H710902, G770902	126,5	17,9	1,1	9,1	2,5	1,9	150
Tofu	Tofu	J381011	77,2	0,5	4,8	8,1	1,9	0,1	100
Spinat	Spinat, Mangold & Vogerlsalat	G211111, G230111, G104111	17,5	0,7	0,4	2,4	2,8	35,4	40
Zucchini	Zucchini, rote Rübe, Grünkohl & Kürbis	G582111, G613111, G322100, G581100	34,2	4,5	0,4	2,2	1,2	36,2	150

Fortsetzung									
Variable	**Lebensmittel**	**BLS CODEs**	**Kcal/100g**	**KH(g)/100g**	**F(g)/100g**	**EW(g)/100g**	**Fe(mg)/100g**	**VitC(mg)/100g**	**Port.größe(g)**
Paprika	Paprika, Broccoli, Kohlrabi & Karfiol	G543111, G312100, G331111, G311111	29,6	3,8	0,3	2,4	0,6	67,0	150
Eierschw	Eierschwammerl/Pfifferlinge	K713111	14,6	0,2	0,5	2,4	6,5	6,0	100
Pilze	Andere Pilze (Champignons, Steinpilz, etc.)	K700100	24,7	0,6	0,2	4,1	1,2	4,9	100
Beeren	Beeren & Kiwi	F300000, F514111	45,1	7,3	0,5	0,9	0,7	50,4	150
Orangen	Orangen, Grapefruit, Papaya & Mango	F603111, F604111, F517111, F616111	44,5	8,8	0,2	0,7	0,3	51,0	150
Sesam	Sesam, Pinienkerne & Leinsamen	H420111, H320100, H410111	537,5	8,4	45,9	22,4	8,7	0,6	20
Kürbisk	Kürbiskerne, Sonnenblumenkerne & Cashewkerne	H31060n, H430111, H170100	549,2	19,9	39,9	27,5	5,7	0,0	20
Nüsse	Andere Nüsse (Walnusskerne, Mandeln, Pistazien, Haselnuss, Erdnuss & Paranusskerne)	H120100, H210101, H250601, H130101, H110601, H180101	650,6	7,9	60,6	19,7	2,9	1,2	20
Trockenfr	Trockenfrüchte	F01D400	268,0	59,0	0,6	2,7	2,7	16,6	20
Zartbitters	Zartbitterschokolade (über 70% Kakaoanteil)	S560011	496,7	46,3	31,1	8,1	17,0	0,0	30
Vollmilchs	Vollmilchschokolade	S530000	536,6	54,1	31,5	9,2	1,5	0,0	30
Milch a)	Vollmilch 3,5% F.	M111311	54,0	4,7	3,6	3,4	0,1	1,7	250
Milch b)	Joghurt 3,6% F.	M141311	69,1	4,4	3,8	3,9	0,0	1,0	250
Milch c)	Schnittkäse 45% F.i.Tr.	M400000	354,2	0	28,3	24,3	0,3	0,0	50
Osaft	Orangensaft, Grapefruitsaft & Beerensäfte	F603600, F604600, F021600	46,7	9,6	0,2	0,5	0,5	36,0	250
SwTee	Schwarzer & grüner Tee	N600100	0,5	0,0	0,0	0,1	0,0	0,0	200
Kaffee	Kaffee	N410100	2,2	0,3	0,0	0,2	0,2	0,0	200

Diese Tabelle stellt alle Variablen dar, die für die Berechnung von Eisen- und Vitamin C -verzehr herangezogen wurden. Zusammengesetzte Variablen wurden aus den Einzellebensmittel der oberen Tabelle berechnet; z.B. Variable „Osaft“ besteht aus dem arithmetischen Mittelwert von Orangensaft, Grapefruitsaft und Beerensäfte. Abk.: Porttionsgröße (Port.größe).

Supplementary Table 3. Iron status parameters of mothers and newborns.

	Total			*Non-IDA*			*IDA*		
	N (%)	**Mean (SD)**	**MED (Min-Max)**	**N (%)**	**Mean (SD)**	**MED (Min-Max)**	**N (%)**	**Mean (SD)**	**MED (Min-Max)**
Maternal blood and serum									
Hb (g/dl)	97	11.7 (1.1)	11.7 (8.5-14.6)	77	12.1 (0.9) [c]	11.9 (10.5-14.6)	20	10.4 (0.6) [c]	10.6 (8.5-10.9)
sFe (µg/dl)	97	90 (39)	86 (23-200)	77	95 (37) [a]	91 (31-200)	20	72 (39) [a]	67 (23-179)
Ft (µg/l)	97	34.1 (42.5)	22.4 (<15-322.0)	77	46.7 (25.0) [c]	25.0 (<15-322.0)	20	17.0 (3.4) [c]	<15 (<15-27.7)
Tf (mg/dl)	97	377 (66)	368 (148-545)	77	374 (66)	362 (148-545)	20	390 (68)	406 (245-492)
Tfsat (%)	97	18.1 (10.4)	16.7 (3.6-73.3)	77	19.2 (10.3) [b]	17.4 (5.1-73.3)	20	13.0 (9.6) [b]	11.5 (3.6-42.6)
sTfR (mg/l)	96	1.4 (0.5)	1.3 (0.6-3.3)	76	1.3 (0.4) [a]	1.2 (0.6-2.9)	20	1.6 (0.6) [a]	1.4 (0.7-3.3)
CRP (mg/dl)	97	0.4 (0.4)	0.3 (<0.03 -2.9)	77	0.4 (0.4)	0.3 (<0.03 -2.9)	20	0.3 (0.2)	0.3 (0.1-0.9)
Cord blood and serum									
Hb (g/dl)	87	15.3 (1.5)	15.2 (11.2-19.2)	68	15.4 (1.5)	15.4 (12.5-19.2)	19	14.8 (1.4)	14.9 (11.2-16.9)
sFe (µg/dl)	92	157 (35)	155 (80-247)	73	158 (35)	155 (82-247)	19	154 (398)	149 (80-220)
Ft (µg/l)	92	209 (109)	196 (<15-659)	73	220 (113)	203 (16-659)	19	165 (85)	178 (<15-355)
Tf (mg/dl)	92	165 (29)	161 (112-261)	73	163 (25)	161 (112-256)	19	173 (39)	168 (122-261)
Tfsat (%)	92	69.2 (16.6)	74.3 (21.7-92.7)	73	69.9 (15.7)	74.4 (24.9-92.7)	19	66.3 (19.7)	73.7 (21.7-90.7)
sTfR (mg/l)	86	1.8 (0.5)	1.7 (1.1-4.4)	67	1.8 (0.4)	1.7 (1.1-3.4)	19	2.1 (0.8)	1.8 (1.2-4.4)
CRP (mg/dl)	92	0.0 (0.0)	0.0 (<0.03 -0.1)	73	0.0 (0.0)	0.0 (<0.03 -0.1)	19	0.0 (0.0)	0.0 (<0.03 -0.1)

Abbreviations: Standard deviation (SD), median (MED), minimum (Min), maximum (Max), hemoglobin (Hb), serum Iron (sFe), serum Ferritin (Ft), transferrin saturation (Tfsat), soluble transferrin receptor (sTfR), C-reactive protein (CRP), non-iron deficiency anemia (non-IDA), iron deficiency anemia (IDA). [a]P<0.05; [b] P<0.01; [c] P<0.0001 from Mann-Whitney-U test.