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REVIEW

The Role of Iron in Type 1 Diabetes Etiology: A Systematic Review of New Evidence on a Long-Standing Mystery

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■ Abstract

BACKGROUND: The incidence of type 1 diabetes (T1D) is rising, which might be due to the influence of environmental factors. Biological and epidemiological evidence has shown that excess iron is associated with beta-cell damage and impaired insulin secretion. **AIM**: In this review, our aim was to assess the association between iron and the risk of T1D. **METHODS**: A systematic literature search was performed in PubMed and EMBASE in July 2016. Studies investigating the effect of iron status/intake on the risk of developing T1D later were included, and study quality was evaluated. The results have been summarized in narrative form. **RESULTS**: From a total of 931 studies screened, we included 4 observational studies evaluating iron intake from drinking water or food during early life and the risk of T1D. The quality of the studies was moderate to high assessed via the nine-star Newcastle Ottawa Scale. One out of the four studies included in this review found estimates of dietary iron intake to be associated with risk of T1D development, whereas three studies found no such relationship for estimates of iron in drinking water. **CONCLUSIONS**: The limited number of studies included found dietary iron, but not iron in drinking water, to be associated with risk of T1D. Further studies are needed to clarify the association between iron and risk of T1D, especially studies including measurements of body iron status.

Keywords: type 1 diabetes · iron overload · iron intake · risk factor · insulin · systematic review

1. Introduction

Type 1 diabetes (T1D) is a chronic disease that arises from an autoimmune, primarily T-cell-mediated, destruction of insulin-producing beta-cells in the human pancreas [1]. T1D is one of the most common chronic diseases in childhood and adolescence [2], and for reasons that remain unknown, the incidence is increasing, which can be observed especially in Western countries [3]. In contrast, the prevalence of high-risk HLA haplotypes is not increasing. This coincidence suggests the existence of strong environmental forces contributing to the development and increasing incidence of T1D [4, 5].

The autoimmune destruction of beta-cells in T1D happens when islet-infiltrating immune cells release pro-inflammatory cytokines, which is believed to contribute to beta-cell dysfunction and death, in part by triggering intracellular formation of reactive oxidative species (ROS) [6, 7]. Beta-cells are very sensitive to oxidative stress, and the generation of ROS in beta-cells may cause oxidative damage and cell death [6, 8].

Iron is a key trace element in metabolism, and is essential for beta-cell insulin secretion [6, 8], but excessive amounts may be toxic to the beta-cell as iron generates ROS by participating in the Fenton reaction [9]. No actual threshold for iron toxicity has been proposed, but in adult populations, beta-cell dysfunction and diabetes have been observed in relation to serum ferritin level >150 µg/l [10, 11]. The normal range in healthy adults is 20-300 µg/l [12].

We aimed to conduct a systematic review of published studies on the association between elevated concentrations of iron parameters or elevated iron intake, and increased risk of T1D in both pediatric and adult populations.

Abbreviations:

BmP-6	bone morphogenetic protein 6
BMPR	bone morphogenetic protein signaling receptors type 1 and 2
DMT1	divalent metal transporter 1
GAD	glutamate decarboxylase
GDM	gestational diabetes mellitus
Gpx4	glutathione peroxidase 4
HCP-1	heme carrier protein 1
HFE	high iron (Fe)
HH	hereditary hemochromatosis
HJV	hemojuvelin
HLA (DR)	human leukocyte antigens (antigen D-related)
HO1	heme oxygenase 1
IAA	islet auto antigen
IRR	adjusted incidence rate ratio
LIP	labile iron pool
NHH	non-hereditary hemochromatosis
NOS	Newcastle-Ottawa Scale
NTBI	non-transferrin-bound iron
OR	adjusted odds ratio
ROS	reactive oxidative species
SD	standard deviation
SIR	standardized incidence rate
SMAD	mothers against decapentaplegic
T1D	type 1 diabetes
T2D	type 2 diabetes
TF	transferrin
TfR1/TfR2	transferrin receptor 1 and 2
Zip14	ZRT/IRT-like protein 14

2. Materials and methods

The PRISMA guidelines were used in this systematic review [13].

2.1 Data sources and searches

The protocol, including the full search string, has been registered with the international prospective register of systematic reviews (PROSPERO) (http://www.crd.york.ac.uk/PROSPERO/) under ID Number 42016042680. We conducted a systematic search of published literature in the PubMed and EMBASE databases on July 4, 2016. Our search combined MeSH terms and keywords related to exposure (iron status markers) and outcome (T1D). The search string was as follows:

"Iron OR irons OR ferric compound OR ferric compounds OR ferrous compound OR ferrous compounds OR ferritin OR ferritins OR transferrin OR transferrins OR transferrin receptor OR transferrin receptors OR iron isotope OR iron isotopes OR iron compounds OR hemosiderin OR hemosiderin OR hemosiderosis OR haemosiderosis OR transferrin saturation

AND

Type 1 diabetes OR diabetes mellitus, type 1 OR autoimmune diabetes OR insulin-dependent diabetes mellitus"

2.2 Study selection and eligibility criteria

The potentially eligible records from the systematic search were imported into Covidence (www.covidence.org), and duplicates were removed. Titles and abstracts were screened by two persons (KLS and SUT) for eligibility. If assessments were inconsistent, the opinion of a third author (JS) was obtained. The following inclusion criteria were pre-specified:

- Human studies (clinical trials, cohort, case-control, or cross-sectional studies)
- Measurements of body iron status (e.g. ferritin, transferrin, transferrin saturation, and iron intake)
- T1D or risk for T1D as an outcome of interest
- T1D duration not longer than 6 months before exposure assessment
- English language articles

We excluded animal studies, non-English language articles, non-original papers (letters, editorials, conference abstracts, reviews, or commentaries) and duplicate publications. Other related outcomes (i.e. type 2 diabetes (T2D), gestational diabetes (GDM)) were excluded as well.

2.3 Data extraction and quality assessment

From each study, we extracted the following information: first author's name, year of publication, study design, number of participants, exposition to

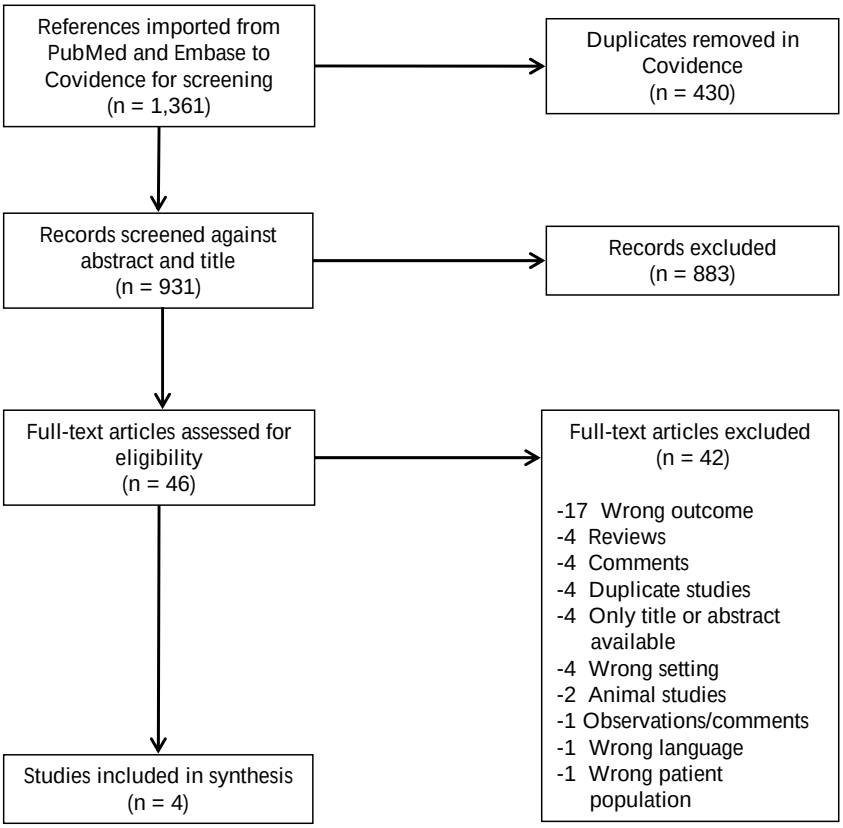

Figure 1. Flow diagram on study search

iron, country, and the associations between iron and risk of T1D indicated as adjusted incidence rate ratio (IRR) or adjusted odds ratios (OR).

We evaluated and scored the quality of each study using the nine-star Newcastle-Ottawa Scale (NOS), a tool used for quality assessment of non-randomized studies [14]. Studies were evaluated based on selection, comparability, and outcome, and judged by a maximum of nine stars. Scores above five stars indicate moderate to high study quality. The NOS for cohort and case-control studies was retrieved from: http://www.ohri.ca/programs/clinical_epidemiology/nosgen.pdf.

3. Results

We decided not to conduct a meta-analysis in this review because of the small number of eligible studies, which would have complicated the estimation of between-studies variance, an important factor in statistical analysis. Instead, we restricted our review to descriptive counts of occasions where iron exposure was found to be significantly associated with the risk of T1D. We followed a narrative approach to the review, including the use of key tables and figures and the description and summary of the results in a qualitative manner.

3.1 Literature search results

A flow diagram of the literature search and study selection for body iron status markers and T1D is presented in **Figure 1**. Of the 931 separate abstracts reviewed independently by two authors, 883 were excluded. Of the 46 full-text articles reviewed thereafter, 42 were excluded. A total of four publications, including one retrospective cohort study and three case-control studies, met the inclusion criteria, and were therefore included.

3.2 Characteristics of the studies included

Table 1 shows the characteristics of the studies included. The number of participants in the studies ranged from 95 to 517. Three studies looked at drinking water components (e.g. iron) as risk factors for T1D, and one study looked at dietary iron intake as a risk factor for T1D. The median bias risk score of the studies included was 7.5 (range: 6-9). The results of the quality assessments are shown in **Table 2**.

3.3 Iron content in drinking water and risk of T1D

A total of three studies focused on the content and amount of trace metals in drinking water at the time of T1D diagnosis [15-17]. This included one retrospective cohort study and two case-control studies of moderate to high assessment quality (6-9). The participants were children with islet autoimmunity [16] or T1D [15-17] diagnosed before the age of 16 and matched controls. The number of participants ranged from 95 to 517 in the cases and from 67 to 323 in the controls [16, 17]. The retrospective cohort study by Zhao *et al.* had no

Table 1. Summary of included studies

Study (yr)	Country	Study design	Subjects T1D/controls	Age at diagnosis, yr (range)	Iron indices	Risk of T1D, OR, or SIR (95% CI)	Adjustment Variates
Samuelsson et al. (2011)	Sweden	Case-control	130/323	≤16	Iron in tap water	OR 1.56 (0.99-2.44)	Municipality of residents
Ashraf et al. (2010)	USA	Case-control	128/67	<6	Dietary iron intake the first 4 month of life.	OR 2.01 (1.18-3.41)	Birth weight, age at time of survey, and birth order
Winkler et al. (2008)	Germany	Case-control	95/139	4.89 (2.73-7.91)	Iron in tap water	OR 1.0 (0.4-2.3)	HLA DR 3/4, 4/4 and maternal T1D
Zhao et al. (2001)	UK	Retrospective cohort	517/0	<15	Iron in tap water	IRR 98.2 (84-115). P for linear trend between SIR of T1D and iron concentration (p = 0.44)	Not indicated

Legend: CI - confidence interval, HLA - human leukocyte antigen, IRR - adjusted incidence rate ratio, OR - adjusted odds ratio, SIR - adjusted standardized incidence ratio, T1D - type 1 diabetes.

control population [15]. The studies analyzed the drinking water from the region or municipality in which the children had been resident at the time of diagnosis. In one study, all families received and filled a 200 ml plastic bottle with tap water, which was then analyzed for trace elements including iron [17].

In the two other studies, the data on drinking water compositions were received from the geographically linked water supply [15, 16]. Samuelsson *et al*. suggested that a high concentration of iron in the water increases the risk of T1D (OR = 1.56, 95% CI = 0.99-2.44), although not significantly [17]. Winkler *et al*. found that there was no significant difference in the risk of developing islet autoimmunity (IAA) and/or progression of IAA to T1D in children exposed to high vs. low iron concentration in drinking water (OR for development of IAA = 0.8, 95% CI = 0.5-1.2, and OR for progression to T1D = 1.0, 95% CI = 0.4-2.3) [16]. Zhao *et al*. concluded that iron had no significant relation with the standardized incidence rate (SIR) of T1D [15].

3.4 Dietary iron intake and risk of T1D

One case-control study investigated the effect of iron intake on the development of T1D [18]. The assessment quality of this study was 7. The study found that the OR for developing T1D increased by 2.01 (95% CI = 1.18-3.41) at any increase in standard deviation (SD) of iron intake by one, during the first four months of life, among all participants (128 cases and 67 controls). The OR increased by 2.26 (95% CI = 1.27-4.03) in a restricted sample of T1D cases with control siblings (59 cases and 59 controls).

4. Discussion

This review included a total of four observational studies investigating and reporting results regarding the association between exposure to high iron concentration during early childhood and risk of T1D. The studies found that iron concentrations in drinking water did not have an effect on the risk of developing T1D [15-17], whereas one study found that high dietary iron intake was significantly associated with increased risk of T1D [18].

None of the studies included measured plasma iron status in their study populations, but instead estimated the intake via food or drinking tap water. Iron uptake is tightly regulated, and the absorption of iron intake depends on several factors [19, 20], including the amount of iron, bioavailability of iron from the food source, competing mechanisms at the di-metal luminal transporters, and genetic factors regulating the luminal and basolateral iron transport. However, high absorption of iron may cause an elevated body iron status because excretion mechanisms for iron are limited [19]. As presented in the studies, iron concentration in drinking water was 0.01-0.08 mg/l [15-17], while iron concentration in infant formula was 4.5-12 mg/l [18], demonstrating a substantial difference in iron exposure between the studies. Furthermore, estimation of dietary iron intake may be problematic and possibly confounded. Consequently, measures of iron status would have improved the comparability of the studies. Due to these limitations and the small number of studies on this topic, no conclusions regarding the effect of iron on T1D pathology can be made based on our findings.

Table 2. Study-specific Newcastle-Ottawa quality assessment

Case-control study	**Selection**				**Comparability**	**Exposure**			**No. of stars**
	S1	S2	S3	S4	C1	E1	E2	E3	
Ashraf *et al.*	a*	a*	a*	a*	a+b**	d	a*	c	7
Samuelsson *et al.*	a*	a*	a*	a*	b*	a*	a*	a*	8
Winkler *et al.*	a*	a*	a*	a*	a+b**	a*	a*	a*	9
									Median = 8
Cohort study	**Selection**				**Comparability**	**Outcome**			**No. of stars**
	S1	S2	S3	S4	C1	O1	O2	O3	
Zhao *et al.*	b*	a*	a*	a*	b*	b*			6
									Median = 6

Legend: A minimum number of one star (i.e. asterisk) is awarded within category "Selection", and a maximum of two stars within "Comparability". Answers "a" and/or "b" within the sub-categories S1-S4, C1, E1-E3, and O1-O3 meet the requirement for a star. Sub-categories evaluate definitions and selection of cases and controls, representation and selection of cohorts, ascertainment of exposure, and outcome.

The results by Ashraf *et al*. suggest that elevated iron levels during early life may play a role in the development of T1D. Similarly, a prospective cohort study by Størdal *et al*. found a significantly higher risk of developing celiac disease, a disease with autoimmune features, in the offspring of mothers treated with an iron supplement during pregnancy [21]. Research confirms the fact that newborns of iron-treated mothers have a higher serum ferritin level than newborns of non-treated mothers [22, 23]. As there is a genetic overlap between celiac disease and T1D [24-26] it seems relevant to investigate whether excess iron, as a result of iron supplementation during pregnancy, could be a risk factor for T1D in the offspring.

Several studies have dealt with iron intake or physiological high iron levels and the risk of other types of diabetes. Two meta-analyses investigating risk factors for T2D have found a strong association between iron and risk of T2D [27, 28]. Also, several studies have found that elevated maternal iron indices and/or dietary iron intake are significantly associated with the risk of GDM [29-33]. Järvelä *et al*. found that women who develop GDM have an increased risk of both T1D and T2D later in life [34]. This study also found that the majority of patients with GDM who later developed T1D had autoantibodies, reflecting GDM resulting from immune-mediated beta-cell destruction [34]. This demonstrates a potential overlap in the pathogenesis of GDM, T2D, and T1D, and provides another argument for investigating the possible role of iron in T1D pathogenesis.

It is widely documented that extreme iron overload due to conditions such as hereditary hemochromatosis (HH) and non-hereditary hemochromatosis (NHH) is associated with the risk of diabetes [20, 35, 36], and diabetes secondary to these diseases is mimicking both T1D and T2D [37]. Beta-cell destruction in HH and NHH is generally assumed to be a result of iron-mediated ROS production and oxidative stress [6, 8, 20]. However, to the best of our knowledge, no proper classification of diabetes type in HH and NHH, e.g. by examining the prevalence of islet-specific autoantibodies, has been performed.

Experimental animal and human studies support the hypotheses that iron is related to the risk of T1D, and that iron impacts beta-cell function (secretion), insulin sensitivity, and the immune system [20]. Different mechanisms have been proposed:

1. Iron contributes to the acceleration of immune initiated beta-cell damage. This theory was presented in a study by Hansen *et al*., concluding that cytokine-dependent upregulation of divalent metal transporter 1 (DMT1) (**Figure 2**) and the resulting raise in iron import causes increased ROS-mediated damage in the beta-cell [38]. Furthermore, the study found that knock-out of the DMT1 gene protects against islet inflammation and T1D in a murine model [38].
2. Iron accumulation and subsequent tissue damage accelerate or trigger an autoimmune process. This hypothesis is based on a study in hepcidin knock-out mice which found iron accumulation in exocrine pancreas to be accompanied by severe infiltration of macrophages and neutrophils, but no significant difference in lymphocyte content [39]. However, the inflammatory state in the pancreas may potentially initiate an autoimmune response in the parapancreatic lymph nodes by transport of islet-specific autoantigens via professional antigen-presenting cells, thereby triggering T1D.

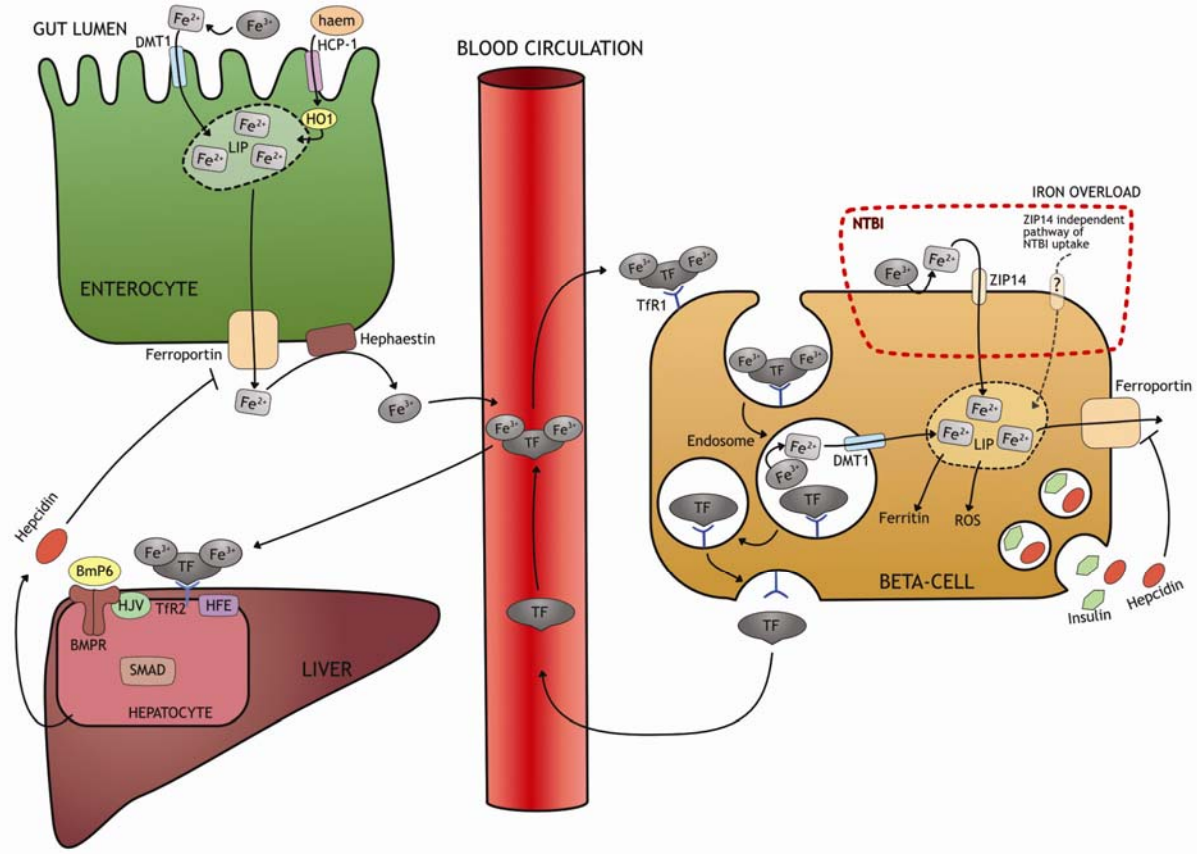

Figure 2. Several transporters and iron-sensing mechanisms modulate intra- and extracellular iron load. Iron (inorganic and hem-bound) is absorbed from the intestine, and enters the LIP in cytoplasma. Iron is excreted through ferroportin, oxidized by hephestin, and released into the circulation. Iron is transported in the bloodstream bound to transferrin (TF), ensuring controlled delivery of iron to the recipient cell (here beta-cell). On the cell surface, the TF-iron complex binds to transferrin receptor 1 (TfR1) and is internalized into endosomes. Via acidification, iron is released from TF and transported by divalent metal transporter 1 (DMT1) into the labile iron pool (LIP). The unbound TF is released into the bloodstream. In case of iron overload, TF-binding capacity may be exceeded and non-transferrin-bound iron (NTBI) will be present. NTBI is taken up by and transported into the beta-cell in a nonregulated process via ZRT/IRT-like protein 14 (ZIP14) or a ZIP14-independent pathway. Excess iron is stored as ferritin or excreted via ferroportin, but may also cause ROS formation and beta-cell damage (not shown). Hepcidin, secreted by hepatocytes and beta-cells, inhibits iron release by breaking down ferroportin. On the hepatocyte, TF-iron complex binds TfR2 and HFE protein, and by mechanisms not completely understood involving bone morphogenetic protein 6 (BmP6), hemojuvelin (HJV), and mothers against decapentaplegic (SMAD)-mediated intracellular signaling, production of hepcidin is upregulated. In the beta-cell, hepcidin is secreted with insulin and inhibit iron release via ferroportin. *Abbreviations*: BmP6 - bone morphogenetic protein 6, BMPR - bone morphogenetic protein signaling receptors type 1 + 2, DMT1 - divalent metal transporter 1, HCP-1 - heme carrier protein 1, HO1 - hemeoxygenase 1, LIP - labile iron pool, HJV - hemojuvelin, NTBI - non-transferrin-bound iron, ROS - reactive oxidative species, TF - transferrin, TfR1/TfR2 - transferrin receptor 1 and 2, Zip14 - ZRT/IRT-like protein 14.

3. Iron overload is widely documented to cause oxidative stress and beta-cell death [6, 20, 38]. However, the mechanism is not fully understood. Ferroptosis is a recently defined non-apoptotic cell death mechanism, which is dependent on iron and ROS formation [40]. Although it has been described in cancer cells almost exclusively, antioxidant enzymes like heme oxygenase 1 (HO1) [41] and glutathione peroxidase 4 (Gpx4) [40, 42] connect ferroptosis to the beta-cell. Therefore, it would be interesting to investigate whether ferroptosis may be an initiator of cell death due to iron overload and oxidative stress in the beta-cell as another pathogenic pathway.

4. Iron has a direct effect on autoimmunity. This hypothesis is based on studies showing damage of specific proteins caused by iron-catalyzed ROS, which may lead to beta-cell autoantigens stimulating T cell autoreactivity [43, 44]. This is another mechanism of deregulating self-tolerance in T1D. In this context, autoantibodies from patients with T1D recognize glutamate decarboxylase (GAD) modified by iron-catalyzed oxidation [45]. If this holds true *in vivo* the autoimmune response may be triggered or accelerated by such epitope modifications.
5. Iron influences the number of beta-cells. In a study by Vukicevic *et al.*, the number of Langerhans islets was reduced in mice with knock-out of bone morphogenetic protein-6 (BmP-6) [46], a protein involved in iron homeostasis that is maintained by hepcidin regulation [47] (**Figure 2**).
6. Iron also influences other risk factors of T1D in such way that it affects the progression of T1D development. Iron overload is related to insulin resistance [20, 35], and obesity and insulin resistance have been hypothesized previously to be accelerators of T1D development [48, 49]. It is presumed that the damaged or reduced beta-cells in these conditions are unable to meet the insulin need.
7. Finally, beta-cells are found to be an extra-hepatic source of hepcidin, with hepcidin stored in and released from the insulin granules [50, 51] (**Figure 2**). Thus, to some extent, blood glucose may act as a regulator of iron concentration in the blood as increased insulin release will result in hepcidin release and consequently decreased iron release into the circulation. Furthermore, as ferroportin also seems to be located in the membrane of beta-cells [40, 51], the insulin/hepcidin release may result in an autocrine inhibition of iron efflux, thereby increasing the intracellular iron accumulation and ROS formation in beta-cells (**Figure 2**). We speculate, if this is an additional mechanism behind the association of diabetes with increased food intake (e.g. high glucose) and overweight [52-54], it would support the hypothesis that insulin resistance (and subsequent high blood glucose) accelerates T1D development [48, 49].

It is important to consider that murine models, which were used in the above studies, have been found to be more resistant to the damaging consequences of iron overload [55, 56]. Therefore, more human studies are needed to clarify the effects of excess iron in humans.

In summary, excess iron due to environmental factors or genetic variation is associated with many downstream biochemical and cellular effects on beta-cell function and intracellular ROS formation. Furthermore, it has been proposed that the (high iron) HFE gene has an immunological function independent of iron regulation [57]. The consequences of this function on T1D has not yet been described.

There are several unresolved questions about the relationship of elevated iron indices (or exposure to iron) and risk of T1D:

1. Are elevated maternal iron indices (because of iron supplementation during pregnancy, for example) a risk factor for islet autoimmunity or T1D in the offspring?
2. Can elevated iron status in the child trigger islet autoimmunity?
3. Can elevated iron status in the child influence the progression from islet autoimmunity to T1D during childhood?
4. Are there any specific sensitive periods during life where iron might be especially influential?

Owing to the limited quantity of studies included, and the fact that there are no papers dealing with pre-onset measurements of body iron markers and T1D risk in children, further studies are needed, especially pregnancy/offspring cohort studies examining the role of iron intake during pregnancy on mother and offspring, iron levels/status in neonates, and the risk of accelerating and/or developing T1D during childhood. The optimal method for quantifying body iron status is still debated [58]. The “gold-standard” for body iron estimation is liver biopsy, but this invasive procedure is risky and therefore not practicable [59]. Serum ferritin correlates closely with body iron stores in healthy individuals [60, 61], and has a good sensitivity in diagnosing iron deficiency, but it generally fails to rise until iron stores are exceptionally high [62]. In contrast, a lesser degree of iron overload is required to increase transferrin saturation levels [63], which potentially makes transferrin saturation a more suitable choice for estimating physiological iron overload. Of note, the body iron load (intra- and extracellular) is a result of a complex combination of cellular transporters, receptors, importers, and regulative mechanisms (**Figure 2**) [35, 39, 47, 64], and the interactions

between all these components have not yet been fully explored. Therefore, future studies should also include genotyping for polymorphisms in these iron transporters and regulators to identify individuals genetically disposed to harmful effects from moderate degrees of iron overload.

5. Strengths and limitations

One of the strengths of this review is that it is a combined systematic and narrative review. The way it has been conducted is transparent and reproducible because of the systematic literature search and the criterion-based selection of relevant evidence, while the data analysis is conducted in a narrative manner, allowing a more comprehensive approach to study populations and outcomes. Because of the narrow scope of evidence in this field, we found this to be the most appropriate way of presenting this review. Furthermore, we also performed a quality assessment for each study.

The present systematic review is limited as the studies in this field are rare, and most of them are on a relatively small scale. Furthermore, the included studies rely on estimates of iron intake from diet and drinking water only, which may be problematic because of confounding factors. Based on the limited number of studies, a meta-analysis did not seem feasible and the retrospective designs of the studies preclude causal conclusions. However, this emphasizes the need for more research in this field, preferably by performing large prospective cohort studies, e.g. with children at high genetic risk of developing T1D.

6. Conclusions

Iron is an essential trace element, but at moderate to high levels it may be associated with T1D risk. One of four studies included in this review found estimates of dietary iron intake to be associated with the risk of T1D development, whereas three studies found no such relationship for estimates of iron in drinking water.

More studies are needed to clarify the association between iron overload and T1D in humans. Such studies should include sensitive iron status markers and if possible iron-pathway genotyping for in-depth understanding of complex iron-genotype interactions, which could identify specific at-risk groups.

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