Inadequate hepcidin serum concentrations predict incident type 2 diabetes mellitus

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Abstract

Background Type 2 diabetes mellitus (T2DM) is closely associated with elevated body iron stores. The hormone hepcidin is the key regulator of iron homeostasis. Inadequately low hepcidin levels were recently reported in subjects with manifest T2DM. We investigated whether alterations of hepcidin levels precede the manifestation of T2DM and predict T2DM development independently of established risk conditions.

Methods This prospective population-based study included 675 subjects aged 50–89 years, 51.9% of whom were female. Hepcidin levels were measured by gold standard tandem mass spectrometry. Diabetes was diagnosed according to American Diabetes Association criteria, and incident diabetes was recorded between baseline in 2000 and 2010.

Results The baseline hepcidin-to-ferritin ratio in subjects that subsequently developed diabetes during follow-up was reduced on average by 29.8% as compared with subjects with normal glucose tolerance (95% confidence interval, −50.7% to −0.2%; p = 0.049). After adjustment for age, sex, and serum ferritin, higher hepcidin levels were associated with reduced risk of incident diabetes (hazard ratio per 1-unit higher log₂ hepcidin, 0.80; 95% confidence interval, 0.64–0.98; p = 0.035; 33 events). Additional adjustment for established diabetes risk factors and determinants of hepcidin concentration did not appreciably change these results (HR, 0.81; 95% CI, 0.66–0.99). Likewise, inadequately low hepcidin levels were also detected in subjects with prevalent T2DM (n = 76).

Conclusions Hepcidin levels that are inadequately low in relation to body iron stores are an independent predictor for incident T2DM and may contribute to diabetes-related tissue iron overload. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords type 2 diabetes mellitus; hepcidin; iron overload; risk factors; iron

Abbreviation T2DM, type 2 diabetes mellitus

Introduction

Disturbances of iron homeostasis and of insulin signalling are intertwined [1–3]. Iron overload is commonly featured by insulin resistance and predisposes to type 2 diabetes mellitus (T2DM) [1,2,4], and interventional reduction of iron stores...
ameliorsates insulin sensitivity [5–9]. Likewise, metformin was reported to reduce body iron stores in women with polycystic ovary syndrome, in association with a marked increase in insulin sensitivity [10].

The mechanisms linking iron and glucose metabolism are, however, incompletely understood [11]. The small peptide hormone hepcidin, which is the master regulator of iron homeostasis, may be of central importance in this context because it determines tissue iron homeostasis by regulating cellular iron export [11]. The expression of hepcidin is positively regulated by circulating iron levels, and thus, hepcidin concentrations are closely linked to serum ferritin levels, a measure of body iron stores [12]. Hepcidin activity decreases intestinal iron absorption and iron egress from hepatocytes, macrophages, and enterocytes [12], whereas hepcidin deficiency causes iron overload [12]. Animal studies have shown that hepcidin is directly induced by insulin [13] and down-regulated in high-fat/high-energy diet-induced insulin resistance [14]. Moreover, hepcidin levels have been found to be increased in obesity because of autonomous production in adipose tissue [15], independently of insulin resistance, whereas a high-fat diet reduced hepcidin levels because of impaired iron absorption [16].

Hepcidin levels that were inadequately low in relation to body iron status have recently been reported in subjects with T2DM [17] and suggest relative hepcidin deficiency as a potential cause of diabetes-related iron overload similar to that observed in genetic hemochromatosis [18,19]. Because of its cross-sectional design, this study [17] could not define the temporal relationship between hepcidin deficiency and diabetes onset. We thus investigated here whether inadequate hepcidin levels precede and predict incident T2DM in a prospective population-based study, the Bruneck Study.

Materials and methods

Study population and definition of clinical variables

The Bruneck Study is a prospective, population-based survey on the epidemiology and pathogenesis of cardiovascular disease and its risk factors [20–23]. In 1990, the study population was recruited as an age-stratified and sex-stratified random sample of all inhabitants of Bruneck (125 men and 125 women from each of the fifth through eighth decades of age, all of Western European descent). The participation rate was high at 93.4%. In 2000, the baseline of the current study, 702 of the 766 surviving subjects (91.6%) participated in the second quinquenniual re-examination. There were two follow-up examinations, one in 2005 and one at end of follow-up in 2010. The study protocol was approved by the ethics committees of Bolzano and Verona and conforms to the Declaration of Helsinki. All study subjects provided written informed consent. Risk factors were assessed by means of validated standard procedures as described previously [20]. Serum samples were drawn in the morning after an overnight fast and 12 h of abstinence from smoking. In subjects with acute infection, blood sampling was delayed for at least 6 weeks. Samples were divided into aliquots and immediately stored at −80 °C. Serum samples for hepcidin measurement were available for 694 individuals. Serum hepcidin was measured by tandem mass spectrometry [24] in one of the reference laboratories that participated in the first international round robin for hepcidin quantification [25]. The lower limit of quantitation was 0.35 nmol/L. Hepcidin measurement was successful for 675 of 694 subjects (97.3%) for which samples were available. Ferritin concentrations were determined by electrochemiluminescence on a Roche Elecsys system. Serum iron was measured on an Olympus AU640 analyser, transferrin and soluble transferrin receptor were measured using a Behring BNA II nephelometer system, and transferrin saturation was derived from iron and transferrin concentrations. Anaemia was defined as a haemoglobin concentration <120 mg/L in women and <130 mg/L in men.

During follow-up from 2000 to 2010, detailed information about new-onset T2DM was carefully collected for all subjects free of T2DM at baseline (follow-up rate, 100%). Incident diabetes was defined as diabetes diagnosed after baseline and before end of the 10-year follow-up time period, while prevalent diabetes was defined as diabetes diagnosed before or during study baseline. Both incident and prevalent diabetes were defined by the need of pharmacotherapy with insulin or with oral hypoglycaemic agents or by fasting plasma glucose ≥126 mg/dL (≥7.0 mmol/L) in at least two separate examinations, in line with American Diabetes Association criteria [22]. In individuals who reported a diagnosis of diabetes at baseline or at the 5- or 10-year follow-up, the presence of the disease was obligatorily confirmed and its time of diagnosis ascertained by reviewing the medical records of their general practitioners and of the Hospital of Bruneck. No self-reported case of diabetes was accepted without validation using medical records. If subjects’ laboratory measurements at any follow-up were suggestive of impaired glucose metabolism their general practitioner was informed for further investigation. Our method of diabetes ascertainment was thus visit-based [26]. There were no diagnoses of type 1 diabetes. Normal fasting glucose was defined as fasting plasma glucose ≤100 mg/dL (≤5.6 mmol/L).

Body mass index was calculated as weight in kilograms over height in metres squared. Glomerular filtration rate
was estimated based on serum creatinine according to the Modification of Diet in Renal Disease formula. Physical activity was ascertained by the Baecke questionnaire [27], and intensities of activities were rated according to the compendium of physical activities [28]. Alcohol consumption was assessed through a standardized and validated questionnaire [29].

Statistical analysis

Baseline characteristics are presented as mean ± standard deviation, median (first quartile and third quartile) or count (percentage) (Table 1). *P* values were calculated by *t*-test, Wilcoxon–Mann–Whitney test, chi-squared test or Fisher’s exact test as appropriate.

Percent differences in iron parameters of subjects with prevalent T2DM or subjects with incident T2DM compared with reference subjects were estimated by linear regression (Table 2). All iron parameters were log-transformed, which reduced absolute skewness in all cases, and regression coefficients were back-transformed.

Associations between hepcidin and incident T2DM under varying multivariable adjustment were analysed using Cox regression (Table 3). The proportional hazards assumption was tested by computing the correlation between Schoenfeld residuals and follow-up time and was not refuted. Hepcidin, ferritin, fasting glucose, and C-reactive protein were log-transformed for these analyses.

An alpha level of 0.05 is used throughout, and all *p* values are two-sided. Analyses were conducted with R 3.1.1.

Results

At baseline in 2000, 766 participants of the Bruneck study were alive, 702 participated in the baseline examination, serum samples were available for 694 and hepcidin could be measured in samples of 675 subjects. Their baseline characteristics are shown according to diabetes status in Table 1. Subjects that were newly diagnosed with T2DM between 2000 and 2010 compared with reference subjects had higher body mass index and higher serum levels of fasting glucose, glycosylated haemoglobin, gamma glutamyl transferase and C-reactive protein. Similar alterations were also present in subjects with prevalent diabetes at baseline in 2000.

Table 1 displays the percent differences in serum iron parameters between subjects with incident or with prevalent T2DM and subjects with normal fasting glucose. Of note, subjects with incident T2DM had decreased hepcidin–ferritin ratios and, likewise, decreased serum hepcidin concentrations conditional on serum ferritin. Similar associations emerged in subjects with prevalent T2DM, who additionally showed elevated serum ferritin and transferrin levels.
Table 2. Differences in serum iron parameters in subjects with incident or with prevalent diabetes compared with reference subjects

<table>
<thead>
<tr>
<th>Adjustment →</th>
<th>Age and sex</th>
<th>+ Serum Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron parameter by group</td>
<td>% difference</td>
<td>p value</td>
</tr>
<tr>
<td>Incident diabetes, n = 33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin, μg/L</td>
<td>28.1 (−5.6, 73.8)</td>
<td>0.111</td>
</tr>
<tr>
<td>Hepcidin, nmol/L</td>
<td>−10.6 (−43.9, 42.4)</td>
<td>0.636</td>
</tr>
<tr>
<td>Haptoglobin-ferritin ratio, μmol/μg</td>
<td>−29.8 (−50.7, −0.2)</td>
<td>0.049</td>
</tr>
<tr>
<td>Iron, μg/dL</td>
<td>−0.9 (−12.3, 12.0)</td>
<td>0.882</td>
</tr>
<tr>
<td>Transferrin, mg/dL</td>
<td>1.2 (−4.3, 7.0)</td>
<td>0.672</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>−2.2 (−14.4, 11.7)</td>
<td>0.741</td>
</tr>
<tr>
<td>Soluble transferrin receptor, mg/L</td>
<td>4.0 (−2.9, 11.5)</td>
<td>0.257</td>
</tr>
<tr>
<td>Prevalent diabetes, n = 76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin</td>
<td>39.4 (12.4, 73.0)</td>
<td>0.003</td>
</tr>
<tr>
<td>Heparin</td>
<td>4.9 (−24.2, 45.3)</td>
<td>0.772</td>
</tr>
<tr>
<td>Haptoglobin-ferritin ratio</td>
<td>−24.7 (−41.5, −3.2)</td>
<td>0.027</td>
</tr>
<tr>
<td>Iron</td>
<td>−1.3 (−9.7, 7.8)</td>
<td>0.764</td>
</tr>
<tr>
<td>Transferrin</td>
<td>4.4 (0.3, 8.7)</td>
<td>0.037</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>−5.5 (−14.3, 4.2)</td>
<td>0.258</td>
</tr>
<tr>
<td>Soluble transferrin receptor</td>
<td>1.0 (−3.8, 6.0)</td>
<td>0.694</td>
</tr>
</tbody>
</table>

Values represent the average percent change in the row variable for each group compared with a reference group of 414 subjects free of incident diabetes, prevalent diabetes and impaired fasting glucose.

Table 3. Association of serum hepcidin concentration with risk of incident type 2 diabetes mellitus

<table>
<thead>
<tr>
<th></th>
<th>n (events)</th>
<th>Hazard ratio (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>675 (33)</td>
<td>0.80 (0.64, 0.98)</td>
<td>0.035</td>
</tr>
<tr>
<td>Men</td>
<td>325 (14)</td>
<td>0.83 (0.62, 1.12)</td>
<td>0.232</td>
</tr>
<tr>
<td>Women</td>
<td>350 (19)</td>
<td>0.77 (0.60, 0.99)</td>
<td>0.042</td>
</tr>
<tr>
<td>Interaction by sex</td>
<td>—</td>
<td>—</td>
<td>0.665</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>675 (33)</td>
<td>0.78 (0.63, 0.96)</td>
<td>0.022</td>
</tr>
<tr>
<td>Men</td>
<td>325 (14)</td>
<td>0.84 (0.62, 1.14)</td>
<td>0.274</td>
</tr>
<tr>
<td>Women</td>
<td>350 (19)</td>
<td>0.74 (0.58, 0.95)</td>
<td>0.018</td>
</tr>
<tr>
<td>Interaction by sex</td>
<td>—</td>
<td>—</td>
<td>0.465</td>
</tr>
<tr>
<td><strong>Model 3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>675 (33)</td>
<td>0.81 (0.66, 0.99)</td>
<td>0.041</td>
</tr>
<tr>
<td>Men</td>
<td>325 (14)</td>
<td>0.88 (0.65, 1.18)</td>
<td>0.398</td>
</tr>
<tr>
<td>Women</td>
<td>350 (19)</td>
<td>0.76 (0.60, 0.98)</td>
<td>0.031</td>
</tr>
<tr>
<td>Interaction by sex</td>
<td>—</td>
<td>—</td>
<td>0.423</td>
</tr>
</tbody>
</table>

Hazard ratios are for a 1-unit increase in log₂ hepcidin serum concentration, or equivalently for a doubling of hepcidin serum concentration.

Model 1: Adjustment for age, sex, and serum ferritin.
Model 2: Further adjustment for physical activity, alcohol consumption, number of cigarettes smoked, body mass index and waist-to-hip ratio.
Model 3: Further adjustment for C-reactive protein, triglycerides, HDL cholesterol, systolic blood pressure and fasting glucose.

Discussion

This study is the first to demonstrate that baseline serum hepcidin levels that are inadequately low in relation to body iron stores significantly predict the risk for T2DM, independently of a broad range of diabetes risk factors and determinants of hepcidin levels (Table 3). Inadequate hepcidin levels may foster diabetes by causing iron overload and subsequent tissue iron accumulation [1,2,12,30]. Iron-generated reactive oxygen species can then cause beta cell failure, reduced insulin expression, reduced insulin receptor binding and insulin resistance [1]. Recent experimental studies have shown that iron overload can also affect the expression of adipokines, increasing serum levels of resistin [31] and decreasing those of adiponectin [32], changes that both favour insulin resistance.

Conversely, a number of experimental and epidemiological studies have found insulin signalling to affect iron homeostasis and hepcidin expression in particular. Experimental studies in mice found decreased hepcidin levels in high-fat/high-energy diet-induced insulin resistance [14] and found hepcidin to be directly induced by insulin activity [13]. Epidemiological studies proposed insulin resistance or hyperinsulinemia rather than hyperglycaemia to contribute to inadequate hepcidin levels based on observations of inadequate hepcidin levels in prevalent T2DM [17] and in polycystic ovary syndrome [17,33] but not in...
type 1 diabetes mellitus [17]. In overweight or obese sub-
jects, an inverse relationship between the hepcidin–
ferritin ratio and insulin resistance, quantified by homeo-
stasis model assessment-estimated insulin resistance, was
detected [17], and iron depletion by phlebotomy was able to
improve homeostasis model assessment-estimated insulin
resistance [9].

Hepcidin levels that are too low in relation to body iron
stores are thus an intriguing new player in the reciprocal
process in which iron loading and insulin resistance beget
each other [1,10]. The mechanisms underlying these ob-
servations remain elusive but may include genetic poly-
morphisms of iron regulating genes as found in other
generic iron loading diseases [18,19] along with the inhib-
itory effects of hormones including insulin and glucagon,
growth factors and (adipo)cytokines on hepcidin expres-
sion [13,14,34–37]. Of interest, a recent study in mice re-
vealed that gluconeogenic signals directly affect hepcidin
synthesis and that during states of starvation hepcidin ex-
pression is induced to preserve sufficient iron concentra-
tions in tissue for metabolic activities [11]. Clinical
relevance emerges from the fact that hepcidin modulating
drugs are currently being developed [30,38,39] and that
these new drugs may, at least hypothetically, ameliorate
endocrine diabetic function by reducing tissue iron
retention.

The Bruneck Study features a random sample of gener-
ally healthy elderly community dwellers of Western Euro-
pean descent. It is thus uncertain to what extent our
finding applies to diseased subjects, younger subjects, or
subjects with a different genetic background.

One weakness of this study is the low number of sub-
jects with incident T2DM. However, even under the limi-
tation of low statistical power, a significant association
was detected, which was robust to comprehensive multi-
variable adjustment, and a corresponding association
was found for prevalent T2DM (n = 76). Strengths of
our study are gold standard measurement of hepcidin by
tandem mass spectrometry, high-quality assessment of di-
betes and of other variables and representativeness for
the general population. Moreover, in this community sam-
ple, hepcidin levels were likely only marginally affected by
pathological hepcidin determinants such as inflammation,
anæmia, kidney disease, the HFE C282Y polymorphism
or obesity (Table 1), making respective confounding
unlikely.

In conclusion, inadequately low hepcidin was a signifi-
cant and independent predictor of incident T2DM.

Acknowledgements
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Science Fund) [TRP 188]. The Bruneck Study is supported by
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Conflicts of interest
The authors declare that they have no conflicts of interest.

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