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RESEARCH ARTICLE

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 $\log_2$ 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 ameliorates 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 [1]. 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 quinquennial 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 $\geq$126 mg/dL ($\geq$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 $\leq$100 mg/dL ($\leq$5.6 mmol/L).

Body mass index was calculated as weight in kilograms over height in metres squared. Glomerular filtration rate

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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 2 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 1. Baseline characteristics of the study population

	Reference	Incident diabetes	*p* value	Prevalent diabetes	*p* value
n	566	33	—	76	—
Demographic variables					
Age, years	65.4 ± 10.3	65.0 ± 9.9	0.809	70.6 ± 9.1	<0.001
Female sex, *n* (%)	295 (52.1)	19 (57.6)	0.667	36 (47.4)	0.512
Lifestyle variables					
Smoking, *n* (%)	86 (15.5)	7 (21.9)	0.473	12 (16.2)	1.000
No. of cigarettes smoked	2.1 ± 5.6	2.6 ± 6.3	0.580	2.1 ± 5.9	0.997
Alcohol intake, g/d	11.3 (1.1, 31.6)	6.7 (0.4, 16.8)	0.188	15.7 (1.3, 42.8)	0.266
Baecke sports score	2.39 ± 0.78	2.27 ± 0.78	0.390	2.05 ± 0.76	<0.001
Metabolic variables					
Body mass index, kg/m^2	25.1 ± 3.8	27.2 ± 3.6	0.002	27.6 ± 4.6	<0.001
Waist-to-hip ratio, cm/cm	0.91 ± 0.07	0.92 ± 0.07	0.324	0.96 ± 0.08	<0.001
Fasting glucose, mg/dL	94 (89, 101)	106 (100, 110)	<0.001	135 (125, 160)	<0.001
Glycosylated haemoglobin, %	5.7 (5.5, 5.9)	5.9 (5.9, 6.1)	<0.001	6.4 (5.9, 7.5)	<0.001
Glycosylated haemoglobin, mmol/mol	39.0 (37.1, 41.0)	41.0 (41.0, 43.1)	<0.001	46.0 (41.3, 58.8)	<0.001
Gamma glutamyl transferase, ukat/L	23 (16, 38)	36 (21, 56)	0.007	35 (23, 67)	<0.001
hs-CRP, mg/L	1.74 (0.89, 3.64)	2.50 (1.22, 5.28)	0.043	2.38 (1.16, 7.29)	0.002
Fibrinogen, mg/dL	288.0 ± 58.3	300.4 ± 53.6	0.239	298.2 ± 71.1	0.167
eGFR-MDRD, mL/min/1.73 m^2	82.3 ± 14.6	80.6 ± 15.7	0.528	79.4 ± 13.9	0.113
Haemoglobin, g/L	144.0 ± 12.0	147.3 ± 10.7	0.112	145.4 ± 13.5	0.321
Anaemia, *n* (%)	20 (3.5)	0 (0.0)	0.619	4 (5.3)	0.513
HFE C282Y mutation [a], *n* (%)	29 (5.1)	1 (3.0)	1.000	1 (1.3)	0.240

eGFR-MDRD, estimated glomerular filtration rate using the Modification of Diet in Renal Disease formula.
Values are given as mean ± standard deviation, median (first quartile and third quartile) or count (percentage).
P values test against a reference group of subjects without incident and free of prevalent diabetes
[a]30 subjects were heterozygous, and 1 subject was homozygous for the mutation

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Table 2. Differences in serum iron parameters in subjects with incident or with prevalent diabetes compared with reference subjects

Adjustment →	Age and sex		+ Serum Ferritin	
Serum iron parameter by group	% difference	*p* value	% difference	*p* value
Incident diabetes, $n = 33$				
Ferritin, μg/L	28.1 (−5.6, 73.8)	0.111	—	—
Hepcidin, nmol/L	−10.6 (−43.9, 42.4)	0.636	−30.2 (−51.0,−0.6)	0.046
Hepcidin-ferritin ratio, μmol/μg	−29.8 (−50.7,−0.2)	0.049	—	—
Iron, μg/dL	−0.9 (−12.3, 12.0)	0.882	−3.2 (−14.1, 9.0)	0.590
Transferrin, mg/dL	1.2 (−4.3, 7.0)	0.672	2.9 (−2.3, 8.4)	0.279
Transferrin saturation, %	−2.2 (−14.4, 11.7)	0.741	−6.2 (−17.0, 6.2)	0.312
Soluble transferrin receptor, mg/L	4.0 (−2.9, 11.5)	0.257	5.5 (−1.4, 12.7)	0.119
Prevalent diabetes, $n = 76$				
Ferritin	39.4 (12.4, 73.0)	0.003	—	—
Hepcidin	4.9 (−24.2, 45.3)	0.772	−23.7 (−40.8,−1.6)	0.037
Hepcidin-ferritin ratio	−24.7 (−41.5,−3.2)	0.027	—	—
Iron	−1.3 (−9.7, 7.8)	0.764	−5.2 (−12.9, 3.3)	0.224
Transferrin	4.4 (0.3, 8.7)	0.037	7.2 (3.3, 11.2)	<0.001
Transferrin saturation	−5.5 (−14.3, 4.2)	0.258	−11.5 (−19.0,−3.3)	0.007
Soluble transferrin receptor	1.0 (−3.8, 6.0)	0.694	3.0 (−1.8, 7.9)	0.223

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

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

Hazard ratios are for a 1-unit increase in $\log_2$ 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.

Time-to-event analysis revealed a significant inverse association between baseline serum hepcidin levels and risk for incident T2DM between 2000 and 2010 under adjustment for age, sex and serum ferritin. As is detailed in Table 3, this association was not significantly modified by sex and was robust to comprehensive multivariable adjustment. Crude incidence rates (events per 1000 person-years, 95% confidence interval) of T2DM in those with hepcidin–ferritin ratio below *versus* above the median were 7.6 (4.5, 11.1) and 4.8 (2.6, 7.7).

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

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type 1 diabetes mellitus [17]. In overweight or obese subjects, an inverse relationship between the hepcidin–ferritin ratio and insulin resistance, quantified by homeostasis 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 observations remain elusive but may include genetic polymorphisms of iron regulating genes as found in other genetic iron loading diseases [18,19] along with the inhibitory effects of hormones including insulin and glucagon, growth factors and (adipo)cytokines on hepcidin expression [13,14,34–37]. Of interest, a recent study in mice revealed that gluconeogenic signals directly affect hepcidin synthesis and that during states of starvation hepcidin expression is induced to preserve sufficient iron concentrations 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 generally healthy elderly community dwellers of Western European 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 subjects with incident T2DM. However, even under the limitation of low statistical power, a significant association was detected, which was robust to comprehensive multivariable 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 diabetes and of other variables and representativeness for the general population. Moreover, in this community sample, hepcidin levels were likely only marginally affected by pathological hepcidin determinants such as inflammation, anaemia, kidney disease, the HFE C282Y polymorphism or obesity (Table 1), making respective confounding unlikely.

In conclusion, inadequately low hepcidin was a significant and independent predictor of incident T2DM.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Simcox JA, McClain DA. Iron and diabetes risk. *Cell Metab* 2013; **17**(3): 329–41.
2. Rajpathak SN, Crandall JP, Wylie-Rosett J, Kabat GC, Rohan TE, Hu FB. The role of iron in type 2 diabetes in humans. *Biochim Biophys Acta* 2009; **1790**(7): 671–81.
3. Fernández-Real JM, López-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. *Diabetes* 2002; **51**(8): 2348–54.
4. Ellervik C, Mandrup-Poulsen T, Andersen HU, *et al*. Elevated transferrin saturation and risk of diabetes. *Diabetes Care* 2011; **34**(10): 2256–8.
5. Fernández-Real JM, Peñarroja G, Castro A, García-Bragado F, Hernández-Aguado I, Ricart W. Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and beta-cell function. *Diabetes* 2002; **51**(4): 1000–4.
6. Fernández-Real JM, López-Bermejo A, Ricart W. Iron stores, blood donation, and insulin sensitivity and secretion. *Clin Chem* 2005; **51**(7): 1201–5.
7. Equitani F, Fernandez-Real JM, Menichella G, Koch M, Calvani M, Nobili V, Mingrone G, Manco M. Bloodletting ameliorates insulin sensitivity and secretion in parallel to reducing liver iron in carriers of HFE gene mutations. *Diabetes Care* 2008; **31**(1): 3–8.
8. Aigner E, Felder TK, Oberkofler H, *et al*. Glucose acts as a regulator of serum iron by increasing serum hepcidin concentrations. *J Nutr Biochem* 2013; **24**(1): 112–7.
9. Aigner E, Theurl I, Theurl M, *et al*. Pathways underlying iron accumulation in human nonalcoholic fatty liver disease. *Am J Clin Nutr* 2008; **87**(5): 1374–83.
10. Luque-Ramírez M, Alvarez-Blasco F, Botella-Carretero JI, Sanchón R, San Millán JL, Escobar-Morreale HF. Increased body iron stores of obese women with polycystic ovary syndrome are a consequence of insulin resistance and hyperinsulinism and are not a result of reduced menstrual losses. *Diabetes Care* 2007; **30**(9): 2309–13.
11. Vecchi C, Montosi G, Garuti C, *et al*. Gluconeogenic signals regulate iron homeostasis via hepcidin in mice. *Gastroenterology* 2014; **146**(4): 1060–9.e3.
12. Ganz T. Systemic iron homeostasis. *Physiol Rev* 2013; **93**(4): 1721–41.
13. Wang H, Li H, Jiang X, Shi W, Shen Z, Li M. Hepcidin is directly regulated by insulin and plays an important role in iron overload in streptozotocin-induced diabetic rats. *Diabetes* 2014; **63**(5): 1506–18.
14. Le Guenno G, Chanséaume E, Ruivard M, Morio B, Mazur A. Study of iron metabolism disturbances in an animal model of insulin resistance. *Diabetes Res Clin Pract* 2007; **77**(3): 363–70.
15. Bekri S, Gual P, Anty R, *et al*. Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. *Gastroenterology* 2006; **131**(3): 788–96.

DOI: 10.1002/dmrr

16. Sonnweber T, Ress C, Nairz M, *et al*. High-fat diet causes iron deficiency via hepcidin-independent reduction of duodenal iron absorption. *J Nutr Biochem* 2012; **23**(12): 1600–8.
17. Sam AH, Busbridge M, Amin A, *et al*. Hepcidin levels in diabetes mellitus and polycystic ovary syndrome. *Diabet Med* 2013; **30**(12): 1495–9.
18. Weiss G. Genetic mechanisms and modifying factors in hereditary hemochromatosis. *Nat Rev Gastroenterol Hepatol* 2010; **7**(1): 50–8.
19. Pietrangelo A. Hemochromatosis: an endocrine liver disease. *Hepatology* 2007; **46**(4): 1291–301.
20. Bonora E, Kiechl S, Oberhollenzer F, Egger G, Bonadonna RC, Muggeo M, Willeit J. Impaired glucose tolerance, type II diabetes mellitus and carotid atherosclerosis: prospective results from the Bruneck Study. *Diabetologia* 2000; **43**(2): 156–64.
21. Kiechl S, Wittmann J, Giaccari A, *et al*. Blockade of receptor activator of nuclear factor-κB (RANKL) signaling improves hepatic insulin resistance and prevents development of diabetes mellitus. *Nat Med* 2013; **19**(3): 358–63.
22. Bonora E, Kiechl S, Willeit J, *et al*. Bruneck study. Population-based incidence rates and risk factors for type 2 diabetes in white individuals: the Bruneck study. *Diabetes* 2004; **53**(7): 1782–9.
23. Bonora E, Kiechl S, Willeit J, *et al*. Insulin resistance as estimated by homeostasis model assessment predicts incident symptomatic cardiovascular disease in Caucasian subjects from the general population: the Bruneck study. *Diabetes Care* 2007; **30**(2): 318–24.
24. Bansal SS, Abbate V, Bomford A, *et al*. Quantitation of hepcidin in serum using ultra-high-pressure liquid chromatography and a linear ion trap mass spectrometer. *Rapid Commun Mass Spectrom* 2010; **24**(9): 1251–9.
25. Kroot JJC, Kemna EHJM, Bansal SS, *et al*. Results of the first international round robin for the quantification of urinary and plasma hepcidin assays: need for standardization. *Haematologica* 2009; **94**(12): 1748–52.
26. Selvin E, Steffes MW, Gregg E, Brancati FL, Coresh J. Performance of A1C for the classification and prediction of diabetes. *Diabetes Care* 2011; **34**(1): 84–9.
27. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *Am J Clin Nutr* 1982; **36** (5): 936–42.
28. Ainsworth BE, Haskell WL, Whitt MC, *et al*. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc* 2000; **32** (9 Suppl): S498–504.
29. Willett WC, Sampson L, Stampfer MJ, *et al*. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985; **122**(1): 51–65.
30. Ramos E, Ruchala P, Goodnough JB, *et al*. Minihepcidins prevent iron overload in a hepcidin-deficient mouse model of severe hemochromatosis. *Blood* 2012; **120**(18): 3829–36.
31. Dongiovanni P, Ruscica M, Rametta R, *et al*. Dietary iron overload induces visceral adipose tissue insulin resistance. *Am J Pathol* 2013; **182**(6): 2254–63.
32. Gabrielsen JS, Gao Y, Simcox JA, *et al*. Adipocyte iron regulates adiponectin and insulin sensitivity. *J Clin Invest* 2012; **122**(10): 3529–40.
33. Luque-Ramírez M, Álvarez-Blasco F, Alpañés M, Escobar-Morreale HF. Role of decreased circulating hepcidin concentrations in the iron excess of women with the polycystic ovary syndrome. *J Clin Endocrinol Metab* 2011; **96**(3): 846–52.
34. Chung B, Matak P, McKie AT, Sharp P. Leptin increases the expression of the iron regulatory hormone hepcidin in HuH7 human hepatoma cells. *J Nutr* 2007; **137**(11): 2366–70.
35. Latour C, Kautz L, Besson-Fournier C, *et al*. Testosterone perturbs systemic iron balance through activation of epidermal growth factor receptor signaling in the liver and repression of hepcidin. *Hepatology* 2014; **59**(2): 683–94.
36. Ramos E, Kautz L, Rodriguez R, *et al*. Evidence for distinct pathways of hepcidin regulation by acute and chronic iron loading in mice. *Hepatology* 2011; **53** (4): 1333–41.
37. Sonnweber T, Nachbaur D, Schroll A, *et al*. Hypoxia induced downregulation of hepcidin is mediated by platelet derived growth factor BB. *Gut* 2014 gutjnl – 2013–305317.
38. Theurl I, Schroll A, Sonnweber T, *et al*. Pharmacologic inhibition of hepcidin expression reverses anemia of chronic inflammation in rats. *Blood* 2011; **118** (18): 4977–84.
39. Sun CC, Vaja V, Babitt JL, Lin HY. Targeting the hepcidin-ferroportin axis to develop new treatment strategies for anemia of chronic disease and anemia of inflammation. *Am J Hematol* 2012; **87**(4): 392–400.

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