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Circulating ferritin concentrations and risk of type 2 diabetes in Japanese individuals

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ABSTRACT

Aims/Introduction: Higher iron storage has been linked to an increased risk of type 2 diabetes, but little is known about the mediator of this association. Here, we prospectively investigated the association between circulating ferritin, a marker of iron storage, and the incidence of type 2 diabetes among Japanese individuals.

Materials and Methods: The participants were 4,754 employees who attended a comprehensive health check-up in 2008–2009 and donated blood for the study. During 5 years of follow up, diabetes was identified based on plasma glucose, glycated hemoglobin and self-report. Two controls matched to each case on sex, age and date of check-up were randomly chosen using density sampling, giving 327 cases and 641 controls with ferritin measurement. Cox proportional hazards regression was used to estimate the hazard ratio while adjusting for a series of potential confounders or mediators.

Results: Elevated serum ferritin levels were associated with a significantly increased risk of type 2 diabetes, with the hazard ratio adjusted for known risk factors in the highest vs lowest quartile of 1.42 (95% confidence interval: 1.03–1.96). This association was unchanged after adjustment for C-reactive protein and adiponectin, but attenuated after adjustment for liver enzyme and insulin resistance (hazard ratio 1.04). The ferritin–diabetes association was confined to non-obese participants.

Conclusions: These results suggest that elevated iron storage is associated with increased risk of type 2 diabetes in normal weight individuals, and that this association is partly mediated through liver dysfunction and resulting insulin resistance.

INTRODUCTION

Although iron is an essential mineral necessary for numerous physiological processes, concerns have been raised that it is a health hazard because of its high reactivity, by which it induces oxidative stress[1]. However, epidemiological evidence for an association of iron status with cancer[2] and cardiovascular disease[3] in the general population is inconsistent. With regard to glucose metabolism, experimental studies have shown that iron-induced oxidative stress decreases insulin sensitivity and causes a deterioration in pancreatic β-cell function[4]. Clinical studies have also showed that iron reduction by phlebotomy decreased insulin resistance[5], blood glucose[6] and glycated hemoglobin (HbA1c)[6]. In humans, higher levels of circulating iron have been associated with insulin resistance[7], hyperglycemia[8] and metabolic syndrome[9], and several prospective studies in the general population have reported an increase in the risk of type 2 diabetes among those with higher levels of iron markers, mainly ferritin[10–17]. These data lend support to a significant role of iron metabolism in the development of type 2 diabetes, even within its normal range.

Despite the accumulating evidence linking diabetes to iron, several issues remain to be solved. The interpretation of the observed association between ferritin, a marker of iron storage[18], and type 2 diabetes is complicated by the fact that ferritin concentrations increase in the presence of risk factors for type 2 diabetes, including obesity[19], alcohol drinking[20] and inflammation[21]. Although most previous studies found a significant association even after adjustment of these factors, Jehn

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et al.[22] reported that the ferritin–diabetes association disappeared after additional adjustment for components of metabolic syndrome. Furthermore, no studies on this issue adjusted for visceral fat accumulation, which would be more relevant to glucose metabolism than body mass index (BMI) or waist circumference[23]. It thus remains elusive which higher concentrations of circulating iron are associated with type 2 diabetes risk independently of factors that could determine iron levels. Furthermore, epidemiological data on this issue are derived mainly from Western populations, and only a few have been carried out in Asia[11,16]. Interestingly, a large European study reported a more pronounced ferritin–diabetes association in leaner individuals[14], prompting more research in Asians, who are on average much leaner than Westerners[24].

Here, to address these issues, we prospectively examined the association between serum ferritin concentration and the incidence of type 2 diabetes in a cohort of Japanese employees.

METHODS

Study design

The Hitachi Health Study is an ongoing prospective study among employees, their spouses and retired employees who participated in a comprehensive health examination at Hitachi Health Care Center (Hitachi, Japan). Details of this study have been described elsewhere[23]. Briefly, from 17,606 examinees between April 2008 and March 2009, we asked men who underwent abdominal computed tomography (CT) examination and all women (irrespective of CT examination) to participate in the study and donate a blood specimen, of whom 7,993 examinees agreed. Of these, we excluded participants who reported a history of cancer, stroke or myocardial infarction (n = 365); those who had diabetes, or no measurement of both fasting plasma glucose and HbA1c at baseline (n = 882); and those who did not attend any subsequent health check-up (n = 347). Of the remaining 6,399 participants, we further excluded retired employees and spouses (n = 1,645). Finally, 4,754 were entered as the biomarker study cohort, from which cases and controls were sampled (Figure S1). Blood samples were stored at −80°C until biochemical analysis, including ferritin measurement. This study was approved by the ethics review committee of Hitachi Health Care Center, and the National Center for Global Health and Medicine. Written informed consent was obtained from all participants.

Health check-up and laboratory measurement

Health-related lifestyle, including smoking, alcohol drinking, and leisure time and commuting physical activity, as well as own and family history of disease, were ascertained through a questionnaire. Height and weight were measured using an automated scale (BF-220; Tanita, Tokyo, Japan). BMI was calculated as the weight (kg) divided by the square of the height (m). The visceral fat area at the umbilical level was measured using a CT scanner (Radix Turbo; Hitachi Medico, Tokyo, Japan) while the examinee was in a supine position, and calculated using a software application (fatPointer; Hitachi Medico). Imaging conditions were 120 kV and 50 mA, using a 5-mm thick slice. Plasma glucose was measured using the glucose oxidase enzyme-electrode method (A&T, Tokyo, Japan). Fasting serum immunoreactive insulin (μU/mL) was determined by an immunoenzymatic method using the AxSYM insulin assay (Abbott, Tokyo, Japan). The homeostasis model assessment of insulin resistance (HOMA-IR), an index of insulin resistance, was calculated as fasting plasma glucose (mg/dL) multiplied by fasting insulin (μU/mL) divided by 405. The homeostasis model assessment of β-cell function (HOMA-β), an index of insulin secretion, was calculated as 360 multiplied by fasting insulin (μU/mL) divided by (fasting glucose [mg/dL] − 63). HbA1c was measured using a high-performance liquid chromatography method (HLC723-G9; TOSOH, Tokyo, Japan). The value for HbA1c (%) was estimated as a National Glycohemoglobin Standardization Program equivalent value (%) calculated by the formula HbA1c (%) = HbA1c (Japan Diabetes Society) (%) + 0.4%[25]. Aspartate aminotransferase, alanine transaminase (ALT) and γ-glutamyl transpeptidase (GGT) were measured by a standard method using an autoanalyzer (Hitachi 7600; Hitachi Co. Ltd., Tokyo, Japan). Serum C-reactive protein (CRP) was measured using a latex-enhanced turbidimetric immunoassay (CRP-LATEX(II)X2 'SEIKEN'; Denka Seiken Co. Ltd., Tokyo, Japan) on an automatic analyzer (LABOSPECT 008; Hitachi Hightechnologies Co. Ltd., Tokyo, Japan). Adiponectin levels were measured using an immunoturbidimetric method (Adiponectin Latex Kit for humans; Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan). Triglyceride and high-density lipoprotein (HDL) cholesterol levels were measured using an enzymatic colorimetric method (Cholestest TG; Sekisui Medical, Tokyo, Japan) and a non-settling enzymatic method (Cholestest NHDL; Sekisui Medical). Ferritin was measured using a chemiluminescence immunoassay (Chemilumi ACS-Ferritin II; Siemens Healthcare Diagnostics, Tokyo, Japan) on an ADVIA Centaur (Bayer, Tarrytown, New York, USA), with a lower limit of detection of 0.5 μg/L, and coefficients of variation of 3.4% at 36.7 μg/L, 3.4% at 83.3 μg/L and 4.2% at 255 μg/L.

Ascertainment of diabetes and control selection

Participants were followed on the basis of annual health check-up until March 2013. Diabetes was defined as HbA1c ≥6.5% (48 mmol/mol), fasting plasma glucose ≥126 mg/dL (7.0 mmol/L) or random plasma glucose ≥200 mg/dL (11.1 mmol/L), or currently under medical treatment for diabetes, according to the American Diabetes Association criteria for the diagnosis of diabetes[26]. Individuals without diabetes at baseline who satisfied at least one of these criteria in subsequent check-ups were considered as incident cases of type 2 diabetes.

Control participants were selected using the incident density method. For each case of diabetes, two controls individually matched on sex, age (≤2 years) and the date of health check-up (≤2 weeks) were randomly chosen from the cohort members who were under observation and free of diabetes, as

confirmed by fasting glucose, HbA1c and self-report, at the time of case detection. We allowed a control of a case to be again chosen as a control of other cases, and a case to be served as a control before diabetes diagnosis.

As shown in Figure S1, we identified 357 cases of diabetes during a maximum of 5 years of follow up; of these, five cases had no matched control. For each of the remaining 352 cases, we listed candidates of matched controls and assigned sequential numbers in a random manner. After excluding four cases with no or insufficient volume of serum, we selected serum samples of cases and their matched controls with sequential numbers of 1 and 2. If either of the control samples was lacking or with insufficient volume, we moved on to the sample with the next sequential number, and then repeated this step until two controls were selected for each case, resulting in 348 cases and 694 controls with a minimum volume of serum sample (1:1 matching for 2 sets). Of these, 18 cases were selected as a control of other cases before a diabetes diagnosis, and 54 controls were selected more than once for other cases. Using the serum remaining after fatty acid measurement, we measured ferritin concentrations for 327 cases and 641 matched controls.

Statistical analysis

Differences in baseline characteristics between cases and controls were assessed using the χ^2-test for categorical variables, and the Student's *t*-test or Mann–Whitney test for continuous variables. Baseline characteristics of individuals in the control group either in proportion, mean or median were presented according to sex-specific quartiles of serum ferritin concentration. Trend associations were assessed by assigning ordinal numbers to each quartile.

We estimated hazard ratios (HRs) and 95% confidence intervals (CIs) for incident type 2 diabetes using a Cox proportional hazards regression model, in which matching variables were treated as covariates. Such unconditional data analysis for a study with matched design has recently been recommended, because it is not only more valid and easier in practice, but also yields better statistical precision than matched (conditional) analysis[27]. After log-transformation of variables with skewed distribution (CRP, adiponectin, ALT, GGT, triglyceride, HDL cholesterol and HOMA-IR), we fitted several different models with increasing levels of adjustment for key potential confounders or mediators. Model 1 was adjusted for age (years), sex and month of examination (April–June, July–September, October–December or January–March). Model 2 was additionally adjusted for leisure time physical activity (min/week, tertiles), occupational physical activity (sedentary or active), smoking (never smoker, former smoker or current smoker consuming <20 or ≥20 cigarettes/day), alcohol drinking (non-drinker or drinker consuming <23, 23 to <46 or ≥46 g ethanol/day), shift work (yes or no), sleep duration (<6, 6 to <7 or ≥7 h/day), family history of diabetes (yes or no) and hypertension (yes or no). Model 3 was additionally adjusted for BMI (kg/m^2). Model 4 was additionally adjusted for CRP (mg/L) and adiponectin (μg/mL). Model 5 was additionally adjusted for ALT (U/L) and GGT (U/L). Model 6 was additionally adjusted for triglyceride and HDL cholesterol. Model 7 was additionally adjusted for HOMA-IR. An indicator variable for the missing data was created for the covariates shift work (n = 9) and occupational activity (n = 12). Trend association was assessed by assigning to each quartile of ferritin ordinal numbers that were treated as a continuous variable. We repeated the above analysis while entering ferritin as a continuous variable. We carried out a series of analyses with additional adjustment for the following variables: waist circumference (cm; model 2 + waist circumference), subcutaneous fat (cm^2; model 2 + subcutaneous fat), visceral fat (cm^2; model 2 + visceral fat), HbA1c (%; model 5 + HbA1c) and fasting glucose (mg/dL; model 5 + fasting glucose).

We carried out several sensitivity analyses in a restricted sample. We assessed the association after excluding individuals with high ferritin concentration (ferritin >1,000 μg/L), a history of chronic liver disease, high liver enzyme concentrations (ALT >40 U/L, aspartate aminotransferase >40 U/L or GGT >60 U/L for men and >40 U/L for women), or high alcohol consumption (≥46 g ethanol/day). We repeated the analysis after excluding patients with diabetes who were diagnosed during the first 2 years of follow up to avoid reverse causation.

Because men had much higher ferritin concentrations than women (just 10% of study population; 32 cases, 64 controls), we reported the results for men and women separately. To examine whether the association differs by the level of obesity and insulin resistance, we carried out analyses stratified by BMI (<25 or ≥25 kg/m^2), visceral fat (<116 or ≥116 cm^2 for men, <68 or ≥68 cm^2 for women), subcutaneous fat (<126 or ≥126 cm^2 for men, <150 or ≥150 cm^2 for women) and HOMA-IR (<1.22 or ≥1.22); all cut-offs except for BMI were medians among controls. An interaction term was generated by multiplying the aforementioned dichotomized variables with log-transformed ferritin and added to the multivariate model.

We checked the collinearity among covariates. We included each of the predictor variables, in turn, as a response variable for a regression model, and estimated the tolerance and variance inflation factor. No collinearity was found among predictors (all variance inflation factors <2).

RESULTS

Compared with controls, participants who newly developed diabetes had higher baseline means of BMI, waist circumference, visceral fat areas, markers of glucose metabolism (fasting glucose, HbA1c, fasting insulin and HOMA-IR), CRP, liver enzymes and triglyceride, but a lower mean of adiponectin and HDL cholesterol (Table 1). Among controls, individuals with higher ferritin concentrations were more likely to be alcohol drinkers and to report a history of hypertension. They also had higher means for BMI, waist circumference, visceral fat area, subcutaneous fat, fasting glucose, fasting insulin, HOMA-IR,

HOMA-β, liver enzymes and triglyceride, but a lower mean of adiponectin and HDL cholesterol (Table 2).

As shown in Table 3, ferritin concentrations were significantly and positively associated with the risk of type 2 diabetes in a basic model adjusted for age, sex and month of examination (model 1). This association was virtually unchanged after further adjustment for known risk factors for type 2 diabetes, including leisure time physical activity, occupational physical activity, smoking, alcohol drinking, shift work, sleep duration, family history of diabetes and hypertension (model 2); HR in the highest vs lowest quartile was 1.68 (95% CI: 1.22, 2.31) (*P* for trend <0.001). The association was attenuated after further adjustment for BMI (model 3: HR 1.42; 95% CI: 1.03–1.96). Results were similar when waist circumference, subcutaneous fat or visceral fat was adjusted instead of BMI; HRs (95% CI) for the highest quartile were 1.43 (1.04–1.98), 1.45 (1.03–2.05) and 1.39 (0.99–1.98) in models with adjustment for waist circumference, subcutaneous fat or visceral fat, respectively. The association was also materially unchanged after further adjustment for CRP and adiponectin (model 4). Additional adjustment for ALT and GGT moderately attenuated the association (model 5), with HR for the highest quartile of 1.25 (95% CI: 0.89–1.76; *P* for trend = 0.09). Additional adjustment for triglyceride and HDL cholesterol did not change the association materially (model 6). Additional adjustment for HOMA-IR further attenuated the association (model 6), with HR for the highest quartile of 1.04 (95% CI: 0.73–1.48). The adjustment for fasting glucose instead of HOMA-IR gave similar results, whereas the adjustment for HbA1c instead of HOMA-IR strengthened the association, with HR for the highest quartile of 1.39 (95% CI: 0.99–1.96).

In sensitivity analysis, results were materially unchanged after excluding individuals who developed diabetes within the first 2 years of follow up (data not shown). Similar results were obtained after excluding individuals with a very high ferritin concentration, any sign of liver disease or high alcohol consumption; or individuals with history of chronic liver disease (data not shown).

In sex-specific analyses, HRs in the highest vs lowest quartile was 1.34 (95% CI: 0.95–1.89) for men and 2.53 (95% CI: 0.61–

Table 1 | Baseline characteristics of cases and controls

	Case	Control	*P*-value*
n	327	641	
Age (years)	51.4 ± 7.3	51.3 ± 7.1	0.96
Men (%)	90.7	89.8	0.65
Current smoker (%)	37.9	34.1	0.23
Heavy alcohol drinking, ≥46 g of ethanol/day (%)	13.7	10.7	0.17
Sleeping time, <6 h/day (%)	48.1	47.7	0.89
Shift work (%)†	12.7	10.6	0.34
Occupational physical activity, sedentary (%)‡	61.4	65.4	0.22
Leisure time physical activity, ≥150 min/week (%)	16.9	14.4	0.30
Family history of type 2 diabetes (%)	24.2	18.1	0.02
Hypertension (%)	28.9	17.0	<0.001
Body mass index (kg/m^2)	25.2 ± 3.3	23.4 ± 2.6	<0.001
Waist circumference (cm)	87.4 ± 8.8	83.1 ± 7.8	<0.001
Visceral fat areas (cm^2)§	138.6 ± 49.8	116.9 ± 49.2	<0.001
Subcutaneous fat (cm^2)§	153.0 ± 59.4	131.7 ± 52.4	<0.001
Fasting glucose (mg/dL)	111.3 ± 7.9	99.6 ± 7.9	<0.001
HbA1c, % (mmol/mol)	6.0 (42) ± 0.28 (3.1)	5.7 (39) ± 0.27 (3.0)	<0.001
Fasting insulin (μU/mL)¶	6.5 (4.3–10.2)	4.9 (3.3–7.1)	<0.001
HOMA-IR¶	1.8 (1.2–2.9)	1.2 (0.8–1.8)	<0.001
HOMA-β¶	50.7 (31.8–76.3)	46.9 (33.9–68.2)	0.30
Adiponectin (μg/mL)††	5.6 (4.5–7.8)	7.1 (5.3–9.7)	<0.001
CRP (mg/L)	0.06 (0.03–0.13)	0.04 (0.02–0.09)	<0.001
ALT (U/L)	28.0 (20.0–40.0)	21.0 (16.0–29.0)	<0.001
AST (U/L)	23.0 (19.0–29.0)	21.0 (18.0–25.0)	<0.001
GGT (U/L)	45.0 (28.0–81.0)	34.0 (21.0–53.0)	<0.001
HDL cholesterol (mg/dL)	50.0 (43.0–58.0)	54.0 (46.0–66.0)	<0.001
Triglyceride (mg/dL)	132.0 (92.0–185.0)	110.0 (76.0–155.0)	<0.001

Data are presented as mean ± standard deviation or median (interquartile range) unless otherwise specified. *The χ^2-test for categorical variables, and Student's *t*-test or Mann–Whitney test for continuous variables. The number of missing data: †*n* = 9; ‡*n* = 12; §*n* = 86; ¶*n* = 70; ††*n* = 1. ALT, alanine aminotransferace; AST, aspartate aminotransferace; CRP, C-reactive protein; GGT, γ-glutamyl transpeptidase; HDL, high density lipoprotein; HOMA-β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance.

Table 2 | Baseline characteristics according to quartiles of serum ferritin concentrations in the control group

	Ferritin (μg/L)				*P* for trend*
	Q1 <88 for men <17 for women	Q2 88–141 for men 17–31 for women	Q3 142–212 for men 32–61 for women	Q4 >212 for men >61 for women	
No. men/women	143/16	148/16	138/16	148/16	
Median of serum ferritin concentrations (μg/L)	51.7	112.5	173.0	271.0	
Age (years)	52.0 ± 7.0	50.1 ± 7.1	50.9 ± 7.3	51.9 ± 7.2	0.93
Men (%)	89.9	90.2	89.6	90.2	0.97
Current smoker (%)	35.8	35.4	37.7	26.8	0.13
Heavy alcohol drinking, ≥46 g of ethanol/day (%)	5.7	8.5	15.6	13.4	0.01
Sleeping time, <6 h/day (%)	44.6	39.0	56.5	49.4	0.07
Shift work (%)†	10.7	11.0	12.4	8.7	0.68
Occupational physical activity, sedentary (%)‡	67.3	60.9	62.7	69.2	0.65
Leisure time physical activity, ≥150 min/week (%)	14.4	15.8	14.9	11.6	0.42
Family history of type 2 diabetes (%)	20.1	16.5	13.0	21.9	0.85
Hypertension (%)	13.8	14.0	18.2	25.0	0.01
Body mass index (kg/m^2)	22.7 ± 2.3	23.0 ± 2.5	23.8 ± 2.7	24.2 ± 2.8	<0.001
Waist circumference (cm)	80.9 ± 7.0	81.7 ± 7.7	84.3 ± 7.6	85.3 ± 8.1	<0.001
Visceral fat areas (cm^2)§	104.5 ± 48.4	108.3 ± 49.4	118.7 ± 46.2	133.2 ± 48.9	<0.001
Subcutaneous fat (cm^2)§	114.7 ± 46.7	124.3 ± 49.2	135.8 ± 52.3	150.7 ± 54.6	<0.001
Fasting glucose (mg/dL)	98.5 ± 7.8	99.3 ± 8.0	99.6 ± 7.6	101.2 ± 7.9	0.003
HbA1c, % (mmol/mol)	5.7 (39) ± 0.3 (3.3)	5.7 (39) ± 0.3 (3.3)	5.7 (39) ± 0.3 (3.3)	5.7 (39) ± 0.3 (3.3)	0.34
Fasting insulin (μU/mL)¶	3.7 (2.8–5.9)	4.7 (3.3–6.9)	5.2 (3.5–7.1)	6.2 (4.4–8.7)	<0.001
HOMA-IR¶	0.9 (0.7–1.5)	1.2 (0.8–1.7)	1.3 (0.8–1.9)	1.5 (1.0–2.2)	<0.001
HOMA-β¶	39.1 (29.6–53.2)	44.6 (31.2–63.7)	47.7 (37.1–68.4)	54.5 (43.7–80.6)	<0.001
Adiponectin (μg/mL)††	7.7 (5.9–10.7)	7.3 (5.2–10.5)	6.7 (5.0–9.2)	6.6 (4.9–9.0)	0.002
CRP (mg/L)	0.04 (0.02–0.08)	0.04 (0.02–0.07)	0.04 (0.02–0.14)	0.04 (0.03–0.10)	0.95
ALT (U/L)	17.0 (14.0–22.0)	20.0 (15.0–28.0)	22.0 (16.0–30.0)	26.5 (20.5–42.0)	<0.001
AST (U/L)	19.0 (17.0–22.0)	20.0 (18.0–24.0)	21.0 (17.0–25.0)	23.5 (20.0–30.5)	<0.001
GGT (U/L)	27.0 (16.0–46.0)	32.5 (20.0–50.0)	35.0 (22.0–51.0)	41.0 (27.0–71.0)	<0.001
HDL cholesterol (mg/dL)	57.0 (48.0–67.0)	56.0 (48.0–67.0)	51.5 (46.0–65.0)	53.0 (45.0–62.0)	0.01
Triglyceride (mg/dL)	98.0 (66.0–142.0)	97.5 (73.0–136.0)	113.5 (83.0–150.0)	128.0 (93.5–179.0)	<0.001

Total $n = 641$. Data are presented as mean ± standard deviation or median (interquartile range) unless otherwise specified. *Linear regression analysis for continuous variables, and Mantel–Haenszel test for trends for categorical variable. The number of missing data: †$n = 5$; ‡$n = 6$; §$n = 60$; ¶$n = 49$; ††$n = 1$. ALT, alanine aminotransferace; AST, aspartate aminotransferace; CRP, C-reactive protein; GGT, γ-glutamyl transpeptidase; HDL, high density lipoprotein; HOMA-β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance.

10.52) for women after adjustment for known risk factors and BMI (model 3), and there was no significant interaction between ferritin and sex (*P* for interaction = 0.73).

In stratified analyses (Table 4), the ferritin–diabetes association was statistically significant among normal weight individuals (*P* for trend <0.001), but not among overweight and obese individuals (*P* for trend = 0.57), with a significant interaction (*P* for interaction = 0.04). Similarly, ferritin concentrations were significantly associated with type 2 diabetes only among individuals with low visceral fat (*P* for trend = 0.001) or low subcutaneous fat (*P* for trend <0.001), and not among those with high visceral fat (*P* for trend = 0.34) or high subcutaneous fat (*P* for trend = 0.71). A similar differential association was also observed on analysis by HOMA-IR level (low: *P* for trend = 0.048; high: *P* for trend = 0.51).

DISCUSSION

In the present nested case–control study, we found that a higher serum ferritin concentration was significantly associated with an increased risk of type 2 diabetes, independently of known risk factors. The association was virtually unchanged after further adjustment for CRP and adiponectin, but attenuated after adjustment for ALT, GGT and HOMA-IR. Furthermore, an increased risk of type 2 diabetes associated with higher ferritin was observed in non-obese participants, but not in obese participants.

Table 3 | Association between serum ferritin concentrations and risk of type 2 diabetes

	HR (95% CI) by quartile				*P* for trend*	HR (95% CI) per 100 μg/L increment
	Q1 <88 for men <17 for women	Q2 88–141 for men 17–31 for women	Q3 142–212 for men 32–61 for women	Q4 >212 for men >61 for women		
No. cases/controls	58/159	61/164	77/154	131/164		
Model 1†	1.00 (Reference)	1.00 (0.70–1.43)	1.33 (0.95–1.88)	1.83 (1.34–2.50)	<0.001	1.19 (1.11–1.28)
Model 2‡	1.00 (Reference)	0.97 (0.67–1.40)	1.28 (0.90–1.81)	1.68 (1.22–2.31)	<0.001	1.15 (1.07–1.24)
Model 3§	1.00 (Reference)	0.98 (0.68–1.41)	1.16 (0.81–1.64)	1.42 (1.03–1.96)	0.01	1.09 (1.01–1.18)
Model 4¶,††	1.00 (Reference)	0.97 (0.67–1.40)	1.11 (0.78–1.58)	1.40 (1.01–1.93)	0.02	1.08 (1.00–1.18)
Model 5‡‡	1.00 (Reference)	0.93 (0.64–1.34)	1.08 (0.76–1.54)	1.25 (0.89–1.76)	0.09	1.04 (0.95–1.14)
Model 6§§	1.00 (Reference)	0.92 (0.64–1.32)	1.06 (0.75–1.51)	1.20 (0.86–1.67)	0.16	1.03 (0.94–1.13)
Model 7¶¶,†††	1.00 (Reference)	0.90 (0.61–1.32)	0.97 (0.67–1.40)	1.04 (0.73–1.48)	0.65	1.01 (0.92–1.11)

*Based on Cox proportional hazards regression with the assignment of ordinal numbers to each category of serum ferritin as a continuous variable. †Model 1: adjusted for age (years), sex, and month of examination (April–June, July–September, October–December or January–March). ‡Model 2: additionally adjusted for leisure time physical activity (min/week, tertiles), occupational physical activity (sedentary or active), smoking (never smoker, former smoker or current smoker consuming <20 or ≥20 cigarettes/day), alcohol drinking (non-drinker or drinker consuming <23, 23 to <46 or ≥46 g ethanol/day), shift work (yes or no), sleep duration (<6, 6 to <7 or ≥7 h/day), family history of diabetes (yes or no) and hypertension (yes or no). §Model 3: additionally adjusted for body mass index (kg/m^2). ¶Model 4: additionally adjusted for C-reactive protein (mg/L) and adiponectin (μg/mL). ††Total *n* = 967, excluding one participant with data missing on adiponectin. ‡‡Model 5: additionally adjusted for alanine aminotransferace (U/L) and γ-glutamyl transpeptidase (U/L). §§Model 6: additionally adjusted for high-density lipoprotein cholesterol (mg/dL) and triglyceride (mg/dL). ¶¶Model 7: additionally adjusted for homeostasis model assessment of insulin resistance. †††Total *n* = 898, excluding 70 participants with missing data on homeostasis model assessment of insulin resistance. CI, confidence interval; HR, hazard ratio.

The strength of association between ferritin and diabetes risk in the present study (HR adjusted for diabetes risk factors in the highest vs lowest quartile of ferritin 1.42) was similar to that of the latest meta-analysis of Western prospective studies, which reported a pooled relative risk in the highest vs lowest quartile of ferritin of 1.49 (95% CI: 1.19–1.86)[17]. In a model with adjustment for visceral fat, which would be more relevant to glucose metabolism than BMI or waist circumference[23], we observed only slightly greater attenuation of association (HR for the highest vs lowest quartile of ferritin 1.38) than that of the BMI-adjusted model (HR 1.42). The present study not only confirms a significant association, which is independent of traditional risk factors, between circulating ferritin concentrations and risk of type 2 diabetes among the Japanese population, but also suggests that the association is independent of visceral fat accumulation.

Ferritin is a marker of inflammation[21], which has been linked to type 2 diabetes risk[28]. This has hampered interpretation of the observed ferritin-diabetes association. Here, however, we confirmed that additional adjustment of CRP did not materially change the association, a finding consistent with those of a meta-analysis of studies with adjustment for inflammatory markers[17]. Circulating adiponectin concentrations have been reported to be inversely associated with both circulating ferritin concentrations[29] and type 2 diabetes risk[30], and thus could confound the association. Again, we observed no difference in association after adjustment for adiponectin. In the Nutrition and Health of Aging Population in China study[16], the relative risk for the highest vs lowest quintile of plasma ferritin was 1.65 in models both with and without simultaneous adjustment for adiponectin and CRP. In the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study[13], relative risks for the highest vs lowest quintile of serum ferritin were 1.95 and 2.00 in models with and without adjustment for adiponectin, respectively. In the EPIC-Norfolk study[31], although the odds ratio for the clinically raised ferritin category (≥300 μg/L for men and ≥150 μg/L for women) compared with the lowest quartile was attenuated after adjustment for adiponectin, it nevertheless remained significantly higher (odds ratios 3.2 and 4.0 in models with and without adjustment for adiponectin). Taken together, these findings show that the association between ferritin and type 2 diabetes risk might not be ascribable to mechanisms related to inflammation or adiponectin.

Among other findings, we found that the association between ferritin and type 2 diabetes was moderately attenuated after adjustment for ALT and GGT, with the HR for the highest vs lowest quartile of ferritin decreasing from 1.40 to 1.25. Similar results have been reported elsewhere[11,13,14,31]. For example, in the EPIC-Potsdam study[13], the relative risk for the highest vs lowest quintile of ferritin was attenuated from 2.00 to 1.74 after adjustment for ALT and GGT. These findings suggest that the association between ferritin and type 2 diabetes might be partly mediated through liver dysfunction caused by iron accumulation. In the present study, further adjustment for lipoproteins did not materially change the association. We also found that the association was further attenuated and lost statistical significance after additional adjustment for HOMA-IR (HR 1.04), a

Table 4 | Association between serum ferritin concentrations and risk of type 2 diabetes[§] stratified by body mass index, visceral fat, subcutaneous fat and homeostasis model assessment of insulin resistance

	n of cases/ controls	HR (95% CI) by quartile				*P* for trend	HR (95% CI) per 100 μg/L increment
		Q1 <88 for men <17 for women	Q2 88–141 for men 17–31 for women	Q3 142–212 for men 32–61 for women	Q4 >212 for men >61 for women		
Body mass index							
<25 kg/m^2	157/486	1.00 (Reference)	1.19 (0.69–2.06)	1.97 (1.17–3.34)	2.68 (1.65–4.37)	<0.001	1.29 (1.16–1.43)
≥25 kg/m^2	170/155	1.00 (Reference)	0.83 (0.50–1.39)	0.73 (0.45–1.19)	0.87 (0.56–1.35)	0.57	0.98 (0.87–1.10)
P for interaction = 0.04							
Visceral fat[†]							
Low	93/289	1.00 (Reference)	0.93 (0.47–1.84)	1.56 (0.82–2.97)	2.59 (1.41–4.76)	0.001	1.30 (1.14–1.49)
High	208/292	1.00 (Reference)	0.94 (0.58–1.52)	0.99 (0.62–1.56)	1.16 (0.77–1.76)	0.34	1.06 (0.95–1.17)
P for interaction = 0.40							
Subcutaneous fat[‡]							
Low	119/292	1.00 (Reference)	1.21 (0.65–2.25)	1.65 (0.91–2.99)	2.73 (1.55–4.80)	<0.001	1.25 (1.11–1.39)
High	182/289	1.00 (Reference)	0.74 (0.44–1.24)	0.85 (0.53–1.38)	0.97 (0.63–1.50)	0.71	1.04 (0.93–1.17)
P for interaction = 0.05							
HOMA-IR							
Low (<1.22)	78/293	1.00 (Reference)	1.26 (0.65–2.43)	1.07 (0.52–2.19)	1.97 (1.01–3.85)	0.048	1.20 (1.02–1.42)
High (≥1.22)	228/299	1.00 (Reference)	0.83 (0.51–1.35)	1.00 (0.65–1.55)	1.04 (0.70–1.56)	0.51	1.02 (0.93–1.13)
P for interaction = 0.004							

The cut-off for visceral fat and subcutaneous fat were defined by median among controls ([†]visceral fat: 116 cm^2 for men or 68 cm^2 for women; [‡]subcutaneous fat: 126 cm^2 for men or 150 cm^2 for women). [§]Adjusted for age (years), sex, month of examination (April–June, July–September, October–December or January–March), leisure time physical activity (min/week, tertiles), occupational physical activity (sedentary or active), smoking (never smoker, former smoker, or current smoker consuming <20 or ≥20 cigarettes/day), alcohol drinking (non-drinker or drinker consuming <23, 23 to <46 or ≥46 g ethanol/day), shift work (yes or no), sleep duration (<6, 6 to <7 or ≥7 h/day), family history of diabetes (yes or no) and hypertension (yes or no). CI, confidence interval; HOMA-IR, homeostasis model assessment of insulin resistance; HR, hazard ratio.

finding compatible with a suggested mechanism whereby iron induces insulin resistance[32].

Previously, we found a significant association of serum ferritin with insulin resistance, leptin and visfatin in men, but not in women.[33,34] In the present study, however, there was no statistically significant sex difference in association between ferritin and type 2 diabetes. Two meta-analyses (one based on prospective studies in Western countries[35], and another based on various types of studies in Western and Asian countries[17]) reported no evidence of sex difference in association between ferritin and type 2 diabetes. In a recent European study[14], the ferritin–type 2 diabetes association was statistically significant in both men and women, although the association was stronger in women than men. Taken together, despite a marked sex difference in the distribution of ferritin concentrations, the ferritin–diabetes association appears to be consistent in both men and women.

In the present study, an increased risk of type 2 diabetes associated with high ferritin was evident among non-obese participants, but not in obese participants. Similar findings have been reported in some[14,36], but not all[16], studies. In the EPIC-InterAct Study[14], a more pronounced association of ferritin with type 2 diabetes risk was observed among leaner individuals; HRs of type 2 diabetes per 100 μg/L of ferritin were 1.35, 1.15 and 1.12 for participants with BMI <25, 25–30 and ≥30 kg/m^2, respectively. In the Nurses' Health Study[36], the relative risk of type 2 diabetes in the highest vs lowest quintile of plasma ferritin was somewhat higher among participants with BMI <25 kg/m^2 (relative risk 3.00) compared with overweight (relative risk 2.40) or obese participants (relative risk 2.67). The stronger association among non-obese people might be because any detrimental effect of ferritin tends to be masked in the presence of obesity, a strong risk factor of type 2 diabetes. Alternatively, iron-induced oxidative stress might have a greater impact on glucose metabolism among leaner individuals, who have lower β-cell function than obese individuals[37].

Although the mechanism linking ferritin and type 2 diabetes is unclear, the following have been suggested. Iron is a catalyst for reactive oxygen spices, such as hydroxyl radical, leading to oxidative stress. Iron-induced oxidative stress mediates the apoptosis of pancreatic islets with a resulting decrease in insulin secretory capacity[38]. Iron deposition induces insulin resistance by inhibiting glucose uptake in fat and muscle tissues[39,40]. In addition, iron overload induces mitochondrial dysfunction and accelerates the oxidative stress response, resulting in gluconeogenesis and hepatic insulin resistance[41].

The strengths of the present study include its prospective design in a well-defined cohort, annual assessment of diabetes using both HbA1c and blood glucose, and adjustment for known and suspected risk factors (including CT-measured visceral fat) of type 2 diabetes. However, the present study also had some limitations. First, we defined type 2 diabetes according to HbA1c and plasma glucose as well as self-report, but not oral glucose tolerance test. HbA1c does not require fasting and reflects long-term glycemic status, making this test suitable for use in epidemiological studies. The International Expert Committee has recommended using HbA1c to diagnose diabetes[42]. Second, we measured serum ferritin, but no other markers of iron status, such as transferrin saturation. Third, serum ferritin was measured at baseline only, probably resulting in underestimation of the association due to random misclassification. Fourth, despite adjustment for a number of potential confounders, we cannot rule out the possibility of bias due to unmeasured confounders or residual confounding. For example, high intake of red meat increases the risk of type 2 diabetes[43], and is also associated with increased circulating ferritin concentrations[44]; thus, red meat intake might confound the ferritin–type 2 diabetes association. Finally, caution should be exercised in generalizing the study findings to populations with a different background.

In conclusion, higher serum ferritin concentrations were associated with an increased risk of type 2 diabetes, independently of known risk factors, among normal-weight Japanese employees. This association was attenuated after adjustment for liver enzymes and a marker of insulin resistance, suggesting liver dysfunction and resulting insulin resistance as an underlying mechanism. Further investigation is required to confirm whether a reduction in body iron storage can decrease the risk of type 2 diabetes.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 | Flow diagram of the study population.