Hepatic iron stores are increased as assessed by magnetic resonance imaging in a Chinese population with altered glucose homeostasis[1–4]

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ABSTRACT

Background: Emerging scientific evidence has disclosed a correlation between iron metabolism and type 2 diabetes (T2D).

Objective: The objective of this study was to test the hypothesis that body iron stores are higher in a Chinese population with altered glucose homeostasis.

Design: Serum iron, ferritin, and soluble transferrin receptor concentrations were measured in 298 subjects, including 70 subjects with normal glucose tolerance (NGT group), 60 subjects with prediabetes (prediabetes group), and 168 subjects with T2D (T2D group). Hepatic iron stores in 88 subjects were assessed by using a magnetic resonance imaging (MRI) T2* gradient-recalled-echo technique. A general linear model ANOVA was performed for comparisons between groups after adjustment for age and BMI. Stepwise multiple linear regression analysis was used to identify factors associated with the MRI-estimated hepatic iron concentration (M-HIC).

Results: Mean (±SD) M-HIC and R2* values in the prediabetes and T2D groups were significantly higher than in the NGT group (M-HIC: 40.6 ± 8.6 and 39.3 ± 10.7 μmol/g compared with 27.8 ± 9.1 μmol/g; R2* values: 47.9 ± 11.9 and 47.3 ± 11.5 s^{-1} compared with 34.9 ± 7.0 s^{-1}; all $P < 0.01$). No significant difference was shown in M-HIC and R2* values between prediabetes and T2D groups. The M-HIC independently contributed to 43.3% of the glycated hemoglobin variance after adjustment for main clinical indexes ($P < 0.001$). The proportions of subjects with mild hepatic iron overload in the NGT, prediabetes, and T2D groups were 12.5%, 70.6%, and 63.6%, respectively.

Conclusions: To our knowledge, our findings provide novel evidence to support the hypothesis of a mild iron overload in patients with prediabetes and T2D. A cohort study concerned with the effect of the attenuation of excess iron on glucose metabolism in a prediabetic population is warranted. *Am J Clin Nutr* 2011;94:1012–9.

INTRODUCTION

Diabetes mellitus has become a major public health problem as reported by the latest epidemiologic research in China (1). Emerging scientific evidence has disclosed unsuspected influences between iron metabolism and T2D[5] (2). Several studies reported increased serum ferritin concentration in Chinese T2D patients, even though the intake and bioavailability of iron in the Chinese diet is relatively low compared with that in Western countries (3, 4). Serum ferritin is a generally accepted biomarker of body iron stores (5). However, it is also an acute-phase reactive protein that reflects systemic inflammation that coexists with diabetes rather than high iron storage (6). In some studies, although the ferritin concentration was adjusted by the inflammatory marker C-reactive protein or was taken into the empirical formula to calculate total-body iron (6, 7), it is not prudent to conclude that there is an increase in overall body iron in T2D patients simply because of changes in serum ferritin concentrations. Therefore, sTfR, which is a novel marker of body iron that is less affected by systemic inflammation (8), was investigated in addition to ferritin in T2D patients. But the results were controversial (7–11).

In recent years, the HIC has generally been accepted as a good surrogate means of the assessment of total-body iron stores (12). The liver is a crucial organ at the crossroads of iron and glucose metabolism. As the main iron-storage organ, it also plays a pivotal role in glucose metabolism by regulating glycogen synthesis, glucose output, and insulin catabolism (13). Therefore, a biochemical evaluation of the HIC is of more significance in the study of the pathogenesis of T2D. An autopsy study showed no significant difference in the mean amount of hepatic iron between the T2D and matching NGT groups (14). This result rendered the iron status in T2D patients to be more disputable. With consideration that dietary restrictions after the diagnosis of T2D and diabetes nephropathy are common in the advanced stage of T2D, we believe that the HIC in autopsy specimens cannot reflect the

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[5] Abbreviations used: FPG, fasting plasma glucose; GRE, gradient-recalled-echo; Hb A_{1c}, glycated hemoglobin; HIC, hepatic iron concentration; HOMA-IR, homeostatic model assessment of insulin resistance; IV-C, collagen IV; M-HIC, magnetic resonance imaging–estimated hepatic iron concentration; MRI, magnetic resonance imaging; NGT, normal glucose tolerance; PPG, 2-h postload plasma glucose in an oral-glucose-tolerance test; ROI, region of interest; sTfR, soluble transferrin receptor; TC, total cholesterol; TIBC, total-iron-binding capacity; T2D, type 2 diabetes.

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real iron status in a living body, especially in the early stage of T2D. Thus, whether body iron stores are increased in T2D patients is disputable and warrants further investigation.

With the notable development of MRI, a novel MRI T2* GRE technique has been established to evaluate the HIC (15). Because of its noninvasiveness, an MRI can overcome the weakness of a biopsy and is a more practical method for clinical investigations. More importantly, a good correlation was reported between MRI and the biochemical assessment of iron storage even in patients with a mild hepatic iron overload (15–17). Therefore, we performed this study in subjects with NGT, prediabetes, and T2D through the evaluation of body iron status by using hepatic MRI variables and serum biomarkers to test the hypothesis that body iron stores are increased in a Chinese population with altered glucose homeostasis.

SUBJECTS AND METHODS

Study sample

A total of 298 subjects, aged 30–80 y, were eligible and recruited from the data pool established in a diabetes screening study (2006–2007) in Shanghai Communities from 1 May 2008 to 1 May 2009. Subjects were allocated into 3 groups as follows: *1*) the NGT group, which was defined as patients with an FPG concentration <6.1 mmol/L and a 2-h PPG concentration <7.8 mmol/L after a 75-g oral-glucose-tolerance test, *2*) the prediabetes group, which included patients with either an impaired FPG concentration between 6.1 and 7.0 mmol/L or an impaired 2-h PPG concentration between 7.8 and 11.1 mmol/L, and *3*) a T2D group, which included patients who had a definite clinical diagnosis of T2D or were newly diagnosed with T2D according to the 1999 WHO criteria (1). People with the following conditions were excluded: *1*) hemachromatosis, anemia (hemoglobin concentration <110 g/L for women; hemoglobin concentration <120 g/L for men), or other diseases that feature severely altered iron metabolism; *2*) viral hepatitis, hepatic cirrhosis, gastrointestinal surgery, and other severe digestive system diseases; *3*) a history of the use of iron preparations in ≤6 mo of the study; *4*) malignant tumors; and *5*) a macroalbuminuria stage of diabetic nephropathy or impaired renal function (albumin-to-creatinine ratio >300 mg/g or estimated glomerular filtration rate <90 mL · min^{-1} · 1.73 m^{-2}). A detailed inquiry of medical history, unenhanced hepatic MRI screening before MRI T2* GRE sequences, and serum type III procollagen concentration (normal range <120 μg/L), IV-C concentration (normal range <140 μg/L), and hyaluronic acid concentration (normal range <120 mg/L) were used to ensure no candidates with hepatic cirrhosis were included.

With consideration that there was a high prevalence (>55%) of nonalcoholic fatty liver disease in T2D patients (18), which may affect the magnetic hepatic iron evaluation, the expected number of subjects enrolled in the MRI determination in the T2D group was 3 times the number of subjects in the other 2 groups. Of the 298 subjects, 88 subjects completed an MRI examination (*see* supplemental Figure 1 under "Supplemental data" in the online issue). There were 16, 17, and 55 subjects who completed MRI screening in the NGT, prediabetes, and T2D groups, respectively. People with metal implants, pacemakers, blood vessel stents, or claustrophobia were excluded from the MRI examination.

Peripheral blood was obtained from all patients after an overnight fast. Participants with no history of diabetes were given a standard 75-g glucose solution, whereas participants with a self-reported history of diabetes were given a steamed bun that contained ~80 g complex carbohydrates for safety reasons (1). Glucose concentrations were measured 120 min after glucose or carbohydrate loading. Serum biomarkers were measured at ADICON Clinical Laboratories, which was accredited by the Colleges of American Pathologists. Serum iron and TIBC were measured with a biochemical autoanalyzer by using a ferene reaction reagent for the iron test. Plasma ferritin was measured with a commercially available particle-enhanced immunoturbidimetric kit (Gensource Co Ltd). Fasting insulin was detected by iodine [^{125}I] insulin radioimmunoassay. Serum sTfR and hepcidin were measured by using a double monoclonal sandwich-enzyme immunoassay (BoShun Co Ltd). Intra-and interassay CVs were <5%. Insulin resistance was calculated as

$$\text{HOMA} - \text{IR} = [\text{fasting insulin (mU/L)} \times \text{FPG (mmol/L)}] \div 22.5 \quad (1)$$

The insulin sensitivity index was defined as the reciprocal of HOMA-IR. Patients who received insulin therapy were excluded from this calculation. Insulin resistance was defined as HOMA-IR >4.65 (19). A threshold of 36 μmol/g dry liver weight was referred to as the limit between normal and minor iron overload in liver iron concentration. Mild hepatic iron overload was defined as 36–85 μmol/g, moderate iron overload was defined as 86–125 μmol/g, and severe iron overload was defined as >125 μmol/g according to previous studies (20, 21).

MRI examination

Subjects underwent an MRI with a 1.5-T clinical system (Excite Signa HDx MRI Scanner; GE) with 8-channel body phased-array coils with a gradient strength of 40 mT/m. For the evaluation of liver-iron overload in people with altered glucose homeostasis, both M-HIC and R2* values were used according to previous reports (15).

For M-HIC imaging, 5 axial GRE breath-hold sequences were performed to ensure the accuracy of the measurement of the M-HIC. The variables were as follows: repetition time 120 ms; slice thickness: 10 mm; interslice gap: 2 mm; matrix size: 128 × 256; field of view: 380 mm; number of signals acquired: 2; bandwidth: 12.5 KHz; 4–6 slices acquired through midliver; and acquisition time, 16–20 s. With different echo time (4–21 ms) and 2 flip angles (20–90°) according to the desired weighting, 5 axial gradient echo sequences including 3 T2-weighted imaging, one proton density–weighted imaging and one T1-weighted imaging were used to measure the signal intensity of the liver and paraspinal muscles.

Definitions of T2* variables and the model used for generating maps are described as before (22). For T2*-weighted imaging, an axial gradient echo breath-hold multiecho T2* sequence was performed. The variables were as follows: flip angle 20°; slice thickness, 10 mm; interslice gap, 2 mm; matrix size, 128 × 256; field of view, 380 mm; number of signals acquired, 2; bandwidth, 12.5 KHz; 4–6 slices acquired through the midliver; acquisition time, 15–20 s; 6 echo times (2.6–17.0 ms, with 2.4-ms

increments); and repetition time (169–247 ms, with 20-ms increments) within a single-breath hold.

To access the presence of fatty liver and other liver lesions, all subjects underwent a routine liver protocol that included the following breath-hold sequences: coronal T2 weighted with single-shot fast spin-echo sequence, transverse fast spin-echo T2 weighted with fat saturation sequence, and transverse T1-weighted gradient echo in-phase and out-phase sequence (MRI chemical shift imaging technique).

Image processing and evaluation

Data were processed at a workstation (Advantage Windows 4.2; GE Medical Systems) with functional tools (GE Medical Systems). R2* was calculated as the slope of the mono-exponential fit of the natural log of signal intensity compared with echo time. The reciprocal of R2* is T2* (T2* = 1 ÷ R2*). R2* functional maps were generated with a commercial workstation (T2* map software, GE Workstation 4.2; GE). Two observers, each of whom had 8 y of experience in radiology, including 3 y of experience in gastrointestinal radiology, were blinded to clinical information and independently analyzed the same 2 consecutive slices at the midliver (midportal vein level). Axial slices closer to the liver dome were excluded because of risk of a susceptibility artifact from the air in the adjacent lung.

The observers manually placed 4 ROIs in the liver parenchyma to measure the signal intensity and R2* values in the right anterior, right posterior, left medial, and left lateral hepatic segments of each slice and avoided the adjacent normal vasculature and fat. ROIs measured were ~2–3 cm^2 according to the description of Virtanen et al (17). Means and SDs of signal intensity and R2* values for all 8 ROIs in each patient were recorded, and values were averaged for all 8 ROIs in each patient. With the use of the same method, the averaged signal intensity in bilateral paraspinal muscles was measured. Data sets were processed by a statistical professional with an online-available tool (http://www.radio.univ-rennes1.fr/Sources/FR/Hemo Calc15.html) to calculate the M-HIC on the basis of the signal-intensity ratio between the liver and paraspinal muscles. This protocol allowed a fully approved acquisition and analysis to act as an international standard (15). R2* functional maps were also represented in a color-coding scheme in a rainbow format that ranged from blue to red, with blue as the lower range and red as the higher range of display for the R2* value (color range: 10–100) to obtain comparable color maps as varying shades of blue, green, yellow, and red in order of increasing R2* values.

Statistical analyses

Continuous variables were shown as means ± SDs, and categoric variables were summarized as percentages. Comparisons of iron-related indexes in the 3 groups were performed by using a general linear model ANOVA after adjustment for age and BMI. Tukey's tests were performed for post hoc analysis. Correlations between M-HIC and biochemical variables were analyzed by using Spearman's partial correlation test. Multiple linear regression analysis with stepwise variable selection was used to identify factors associated with sTfR or the M-HIC after the main clinical and biochemical indexes were controlled for. All analyses were performed with SPSS statistical software (version 18.0; SPSS). $P < 0.05$ was considered to indicate a significant difference.

RESULTS

Descriptive characteristics of subjects

Characteristics of subjects and comparisons between sexes in the 3 groups are shown in **Table 1**. There was no significant difference in the sex ratio between the 3 groups ($P = 0.602$). Subjects in the T2D group were significantly older than subjects in the NGT group and had a higher BMI, FPG, Hb A_{1c}, TC, and triglycerides than did the other 2 groups. Subjects in the prediabetes group were older and had higher FPG and Hb A_{1c} than did subject in the NGT group. The duration and medication of T2D are shown in **Table 2**. Albumin-to-creatinine ratios in the T2D group were higher than in the other 2 groups ($P < 0.001$; Table 1).

With consideration of the attendant effect of fatty liver on the HIC, subjects with fatty liver in the 3 groups were analyzed separately. The number of subjects with fatty liver in NGT, prediabetes, and T2D groups were 1 (6.25%), 2 (11.76%), and 18 (32.73%), respectively. All of these subjects had mild fatty liver evaluated by using the MRI chemical shift imaging technique. No characteristic morphologic alterations of cirrhotic liver were detected by using unenhanced MRI. Although there were significant differences in IV-C between groups, means of serum type III procollagen, IV-C, and hyaluronic acid concentrations were all within the normal range in the 3 groups (Table 1).

Comparisons of iron-related biochemical indexes

After adjustment for age and BMI, serum iron concentrations in the prediabetes group were significantly higher than in the NGT and T2D groups ($P < 0.001$), whereas no difference was observed between the latter 2 groups (*see* supplemental Figure 2 under "Supplemental data" in the online issue). Serum ferritin concentrations in the T2D group were significantly higher than in the prediabetes and NGT groups ($P < 0.001$). Serum sTfR concentrations in the T2D group were significantly lower than in the NGT group after adjustment for age and BMI ($P < 0.01$), and there was no significant difference in sTfR between T2D and prediabetes groups. No difference in serum hepcidin was observed between the 3 groups (Table 1).

Serum iron, transferrin saturation, and ferritin concentrations in women were significantly lower than in men (Table 1). No differences were observed in MRI R2* values and M-HICs between sexes ($P = 0.356$ and $P = 0.66$, respectively).

MRI assessment of hepatic iron stores

From MRI R2* functional maps, we observed more green, yellow, and red areas in prediabetes and T2D subjects than in NGT subjects (**Figure 1**). M-HIC and R2* values in the prediabetes and T2D groups were significantly higher than those in the NGT group (M-HIC: 40.6 ± 8.6 and 39.3 ± 10.7 compared with 27.8 ± 9.1 μmol/g, respectively; R2* value: 47.9 ± 11.9 and 47.3 ± 11.5 compared with 34.9 ± 7.0 s^{-1}, respectively; all $P < 0.01$). However, no significant difference in M-HIC and R2* values was observed between prediabetes and T2D groups (Table 1).

TABLE 1
Characteristics of and iron-related biomarkers in subjects[1]

	NGT group (n = 70)		Prediabetes group (n = 60)		T2D group (n = 168)		P		
	Men (n = 21)	Women (n = 49)	Men (n = 23)	Women (n = 37)	Men (n = 56)	Women (n = 112)	Between groups	Between sexes	Interaction between group and sex
Age (y)	52.8 ± 10.7	53.0 ± 6.7	66.0 ± 12.9**	63.1 ± 10.2**	65.7 ± 9.3**	61.2 ± 8.1**	0.234	0.003	<0.001
BMI (kg/m^2)	24.0 ± 1.1	24.4 ± 1.3	24.5 ± 3.1	24.6 ± 3.0	25.0 ± 3.1*	25.6 ± 3.9*	0.841	0.133	0.02
Hemoglobin (g/L)	141.7 ± 11.5	137.3 ± 10.4	139.9 ± 13.1	134.9 ± 12.2	143.2 ± 14	138.5 ± 14.4	0.147	0.020	0.977
Hb A_{1c} (%)	5.3 ± 0.2	5.3 ± 0.2	6.0 ± 0.4**	6.0 ± 0.3**	7.1 ± 1.2**,‡	7.3 ± 1.0**,‡	<0.001	0.723	0.592
FPG (mmol/L)	4.8 ± 0.5	4.7 ± 0.5	6.1 ± 0.6**	5.9 ± 0.5**	7.0 ± 1.7**,‡	7.9 ± 2.3**,‡	<0.001	0.319	0.064
PPG (mmol/L)	6.0 ± 1.2	5.8 ± 1.1	9.2 ± 1.1**	9.3 ± 1.0**	15.8 ± 1.9**,‡	15.8 ± 2.0**,‡	<0.001	0.950	0.813
FIN (mU/L)	17.1 ± 7.3	16.9 ± 5.6	19.0 ± 7.1	19.5 ± 8.7	45.3 ± 10.9**,‡	46.8 ± 13.1**,‡	<0.001	0.683	0.785
HOMA-IR	3.6 ± 1.7	3.5 ± 1.2	5.3 ± 1.7*	5.2 ± 1.8*	14.4 ± 10.6*,‡	17.3 ± 11.3*,‡	<0.001	0.672	0.961
TC (mmol/L)	4.5 ± 0.7	5.0 ± 1.1	4.8 ± 0.7	5.4 ± 0.8	5.1 ± 1.0**,†	5.9 ± 1.4**,†	<0.001	<0.001	0.535
Triglycerides (mmol/L)	1.3 ± 0.5	1.3 ± 0.5	1.3 ± 0.6	1.4 ± 0.9	1.7 ± 1.4*,†	2.0 ± 1.8*,†	0.010	0.435	0.618
HDL cholesterol (mmol/L)	1.3 ± 0.2	1.4 ± 0.4	1.2 ± 0.2	1.4 ± 0.3	1.2 ± 0.3	1.4 ± 0.4	0.402	0.001	0.586
LDL cholesterol (mmol/L)	2.6 ± 0.6	2.8 ± 0.8	2.9 ± 0.7	3.1 ± 0.8	2.9 ± 0.8*	3.3 ± 1.0*	0.050	0.013	0.612
ACR (mg/g)	6.4 ± 2.5	6.9 ± 1.8	16.5 ± 16.6*	16.5 ± 15.5*	61.7 ± 23.0**,‡	56.2 ± 26.2**,‡	<0.001	0.526	0.505
PCIII (μg/L)	74.8 ± 32.8	80.2 ± 23.0	71.2 ± 31.5	78.8 ± 26.2	76.8 ± 23.2	75.9 ± 22.2	0.947	0.285	0.427
IV-C (μg/L)	56.8 ± 28	63.8 ± 26.6	71.9 ± 22.6*	62.9 ± 28.2*	69.0 ± 30.7**	68.6 ± 29.9**	0.023	0.620	0.261
HA (mg/L)	52.9 ± 16.9	57.9 ± 15.0	58.1 ± 18.9	60.5 ± 15.3	57.6 ± 16.9	58.7 ± 18.6	0.612	0.212	0.791
SI (μmol/L)	16.8 ± 7.9	19.6 ± 7.9	24.1 ± 6.6**	29.9 ± 9.6**	16.7 ± 6.1‡	19.1 ± 6.6‡	<0.001	<0.001	0.285
TIBC (μmol/L)	56.0 ± 7.7	55.9 ± 11.0	59.3 ± 7.0*	58.2 ± 7.0*	57.9 ± 8.7**	61.7 ± 11.2**	0.004	0.698	0.255
TS (%)	36 ± 14	31 ± 12	51 ± 15**	41 ± 8**	33 ± 10*,‡	27 ± 9*,‡	<0.001	<0.001	0.496
Ferritin (μg/L)	133.8 ± 56.1	89.4 ± 68.0	149.1 ± 80.4*	116.4 ± 68.8*	242.2 ± 172.3**,‡	148.6 ± 109.4**,‡	<0.001	<0.001	0.155
Hepcidin (μg/L)	80.2 ± 56.1	94.6 ± 121.7	92.6 ± 109.1	76.1 ± 56.1	80.5 ± 81.9	94.9 ± 104.8	0.851	0.633	0.548
sTfR (nmol/L)	31.4 ± 17.4	34.8 ± 28.2	30.3 ± 22.9	26.7 ± 14.8	22.6 ± 25.3**	24.2 ± 26.1**	0.022	0.790	0.724
R2* (s^{-1})[2]	28.7 ± 0.6	36.4 ± 7.0	49.5 ± 7.2**	46.5 ± 15.2**	48.4 ± 10.5**	46.9 ± 11.9**	0.006	0.356	0.324
M-HIC (μmol/g)[2]	26.7 ± 5.8	28.1 ± 9.9	40.6 ± 7.3**	40.6 ± 10.1**	40.0 ± 6.8**	39.4 ± 10.9**	0.007	0.660	0.249

[1] All values are means ± SDs. P values were derived by using general linear model ANOVA after adjustment for age and BMI. Tukey's test was performed for post hoc analysis. The chi-square test was used to compare the sex ratio in each group (P = 0.602). *,**Compared with the NGT group: *$P < 0.05$, **$P < 0.01$; †,‡compared with the prediabetes group: †$P < 0.05$, ‡$P < 0.01$. Comparisons between groups were made for overall subjects in each group (including women and men). ACR, albumin-to-creatinine ratio; FIN, fasting insulin; FPG, fasting plasma glucose; Hb A_{1c}, glycated hemoglobin; HA, hyaluronic acid; HOMA-IR, homeostatic model assessment of insulin resistance; IV-C, collagen IV; M-HIC, magnetic resonance imaging–estimated hepatic iron concentration; NGT, normal glucose tolerance; PCIII, type III procollagen; PPG, 2-h plasma glucose in an oral-glucose-tolerance test; SI, serum iron; sTfR, soluble transferrin receptor; TC, total cholesterol; T2D, type 2 diabetes; TIBC, total-iron-binding capacity; TS, transferrin saturation.

[2] Subjects who underwent a magnetic resonance imaging examination (n = 88) had values of R2* and M-HIC. Numbers of women and men in NGT, prediabetes, and T2D groups were 13 and 3, 9 and 8, and 41 and 14, respectively. No significance was observed in the sex ratio between groups in this subset by the chi-square test (P = 0.098).

TABLE 2
Medication for and duration of type 2 diabetes

Index and category	*n* (%)
Medication	
Oral hypoglycemic agents	95 (56)
Oral hypoglycemic agents + insulin therapy	43 (26)
Insulin therapy	30 (18)
Duration	
Newly diagnosed diabetes (<1 y)	33 (20)
1–3 y	26 (15)
3–5 y	23 (13)
5–10 y	38 (23)
>10 y	48 (29)

M-HIC and R2* values in prediabetes and T2D groups without fatty liver remained significantly higher than those in the NGT group even after adjustment for age and BMI [M-HIC: 40.7 ± 9.7 and 38.4 ± 9.6 μmol/g compared with 27.7 ± 9.4 μmol/g, respectively ($P < 0.01$); R2* value: 48.0 ± 12.7 and 47.7 ± 12.2 compared with 34.7 ± 7.2 s^{-1}, respectively; ($P < 0.01$)]. There was no significant difference in M-HIC or MRI R2* values between T2D patients with or without fatty liver [M-HIC: 41.9 ± 10.6 compared with 38.38 ± 9.6 μmol/g, respectively ($P = 0.23$); R2* value: 46.4 ± 10.0 compared with 47.72 ± 12.2 s^{-1}, respectively ($P = 0.15$)]. Correlations between biomarkers of blood lipids and M-HICs were analyzed by Spearman's coefficient analysis, and no significance was observed between TC, triglycerides, LDL cholesterol, and the M-HIC (*see* supplemental Table 1 under "Supplemental data" in the online issue). In addition, the proportion of subjects with a mild hepatic iron overload (defined as an M-HIC of 36–85 μmol/g) in NGT, prediabetes, and T2D groups was 12.5%, 70.6%, and 63.6%, respectively.

Factors associated with circulating sTfR

By univariate analysis for all 298 subjects, factors associated with sTfR were TC, triglycerides, LDL cholesterol, and hepcidin (**Table 3**). The closest association was observed with serum hepcidin and sTfR ($r = 0.658$, $P < 0.001$). Multiple linear regression analysis with stepwise-variable selection was performed to predict circulating sTfR for all subjects. Hepcidin ($P < 0.001$), Hb A_{1c} ($P < 0.001$), and ferritin ($P = 0.031$) contributed independently to 83% of the sTfR variance after effects of age, BMI, TC, triglycerides, LDL cholesterol, TIBC, and serum iron were controlled for.

Correlations between hepatic iron stores and Hb A_{1c}

Spearman's partial correlation analysis was performed for the 88 subjects who completed MRI examination; factors associated with the M-HIC were age, BMI, ferritin, and MRI R2* values (Table 3). No significant correlation was observed between the

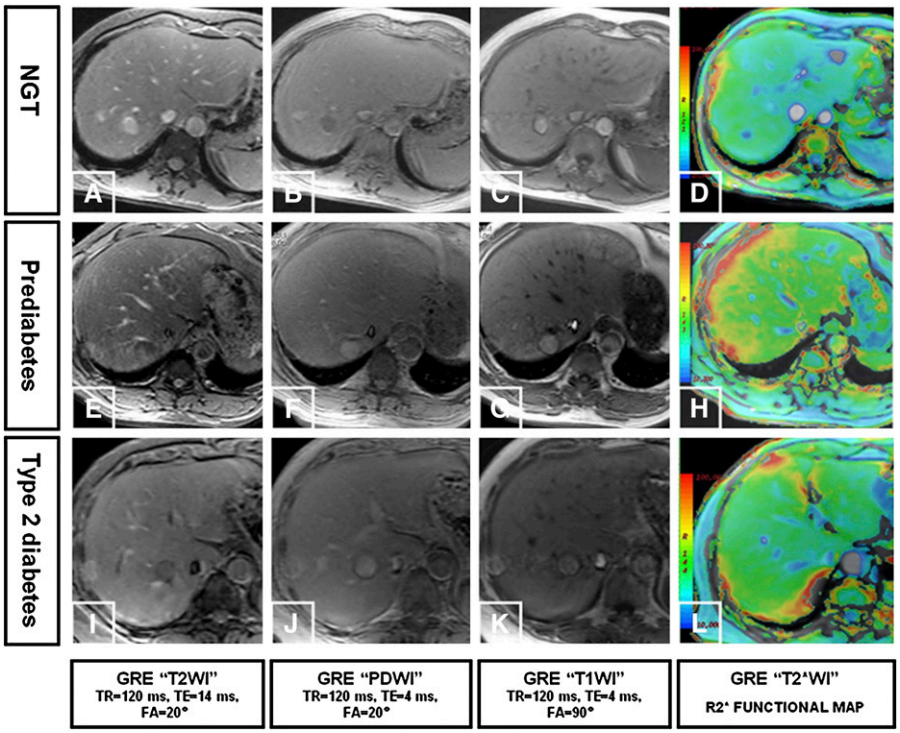

FIGURE 1. Representative hepatic iron MRI imaging in the 3 groups. Magnetic resonance raw images were acquired from axial gradient echo breath-hold sequences for M-HIC calculation, and R2* functional maps were reconstructed by the T2*WI acquisition from an NGT subject (A–D) [age: 56 y; BMI (in kg/m^2): 23.8; M-HIC: 25 ± 20 μmol/g; R2*: 28 ± 4 s^{-1}], a prediabetic subject (E–H) (age: 58 y; BMI: 23.6; M-HIC: 45 ± 20 μmol/g; R2*: 48 ± 5 s^{-1}), and a patient with type 2 diabetes (I–L) (age: 58 y; BMI: 23.9; M-HIC: 40 ± 20 μmol/g; R2*: 40 ± 5 s^{-1}). A, E, and I: Axial slice of GRE T2-weighted imaging sequence. B, F, and J: Axial slice of GRE PDWI sequence. C, G, and K: Axial slice of GRE T1WI sequence. D, H, and L: Axial slice of the R2* functional map performed to obtain comparable color maps as varying shades of blue, green, yellow, and red in order of increasing R2* values (color range: 10–100). FA, flip angles; GRE, gradient-recalled-echo; M-HIC, magnetic resonance imaging–estimated hepatic iron concentration; MRI, magnetic resonance imaging; NGT, normal glucose tolerance; PDWI, proton density–weighted imaging; T2*WI, T2*-weighted imaging; TE, echo time; TR, repetition time.

TABLE 3
Correlations between M-HIC, sTfR, and characteristics and blood biomarkers of subjects[1]

	sTfR (n = 298)		M-HIC (n = 88)	
	Coefficient	P	Coefficient	P
Age	−0.08	0.16	0.26	0.01
BMI	−0.04	0.53	0.27	0.01
TC	-0.21	<0.01	0.01	0.92
Triglycerides	-0.19	<0.01	0.13	0.22
HDL cholesterol	0.03	0.64	−0.06	0.61
LDL cholesterol	−0.15	0.01	−0.04	0.71
TIBC	−0.04	0.49	−0.08	0.45
Hepcidin	0.66	<0.01	0.21	0.06
Ferritin	−0.07	0.27	0.29	0.01
sTfR	—	—	−0.09	0.43
Serum iron	0.06	0.35	−0.04	0.68
M-HIC	−0.09	0.43	—	—
R2*	−0.21	0.05	0.44	<0.01
Hb A_{1c}	-0.29	<0.01	0.18	0.10

[1] Spearman's partial correlation was performed for the analysis. Hb A_{1c}, glycated hemoglobin; M-HIC, magnetic resonance imaging–estimated hepatic iron concentration; sTfR, soluble transferrin receptor; TC, total cholesterol; TIBC, total-iron-binding capacity.

M-HIC and Hb A_{1c} in all subjects who completed MRI (P = 0.097). However, the M-HIC was significantly associated with Hb A_{1c} in nondiabetic subjects (r = 0.44, $P < 0.01$). We further performed a stepwise multivariate regression analysis for nondiabetic subjects (NGT + prediabetics). When Hb A_{1c} was considered as a dependent variable, the M-HIC contributed independently to 43.3% of the Hb A_{1c} variance after effects of age, BMI, TC, triglycerides, LDL cholesterol, TIBC, hepcidin, ferritin, and serum iron were controlled for ($P < 0.001$). A significant correlation was only observed between the M-HIC and the insulin sensitivity index in T2D subjects ($r = -0.46$, P = 0.01) but not in nondiabetic subjects (r = 0.16, P = 0.38).

DISCUSSION

To our knowledge, our findings provided novel and robust evidence to support the hypothesis of a mild hepatic iron overload in a Chinese population with altered glucose homeostasis. As an indicator of total body iron, the HIC evaluated by using the MRI T2* GRE technique in T2D patients was ~40% higher than that in NGT subjects after adjustment for age and BMI. In particular, the HIC of the prediabetes group was significantly higher than that of the NGT group. However, the HIC in the T2D group did not increase to a higher concentration in parallel with the aggravation of insulin resistance compared with in the prediabetes group. To the best of our knowledge, there are no other studies that reported the assessment of hepatic iron stores by using MRI techniques in Chinese subjects with prediabetes and T2D.

The strengths of this study were in 2 aspects. First, the noninvasive methodologies of the MRI GRE technique and MRI T2* functional imaging were used to evaluate intravital hepatic iron stores, and both of these techniques showed increased hepatic iron stores in patients with prediabetes and T2D. Because of the unavoidable pain and potential risk of complications, a liver biopsy as a traditional gold-standard for the evaluation of the HIC is particularly unpractical when applied to healthy subjects of patients with prediabetes (23). Moreover, an evaluation of biopsy specimens suffers from a sampling bias because hepatic iron stores are heterogeneous, which was shown in the MRI T2* map in our study. These measures usually underestimate or overestimate the degree of hepatic iron stores according to the location from which the biopsy is taken (16). MRI techniques can examine transverse sections of the entire liver and measure all collected ROI signals, which avoids the sampling bias as much as possible. The MRI T2* GRE technique offers us a noninvasive way to measure the HIC in contrast to a liver biopsy, whereas laboratory tests (eg, serum ferritin, serum iron, and transferrin saturation) have been somewhat unreliable and confounded by different factors (22). Previous studies proved that the M-HIC is a reliable HIC indicator with linear dependence on the iron concentration in vivo (5–230 μmol/g) (20). Although the M-HIC were normal or with minor hepatic iron overload (15–70 μmol/g) in all of our subjects, the linear correlation ensured comparisons between groups in our study.

Second, the iron status of NGT, prediabetes, and T2D subjects were evaluated together in this study. Although many studies compared the iron status of T2D patients with healthy subjects, studies on prediabetes were limited. In our study, the serum ferritin concentration in the prediabetes group was higher than in the NGT group, which was similar to the results of previous reports (24, 25). Moreover, a significantly increased HIC was also observed in subjects with prediabetes in our study. Both of these variables indicated a mild-elevated iron storage in subjects with altered glucose homeostasis. In previous studies, a higher intake of total dietary iron or heme iron was reported with a higher risk of T2D in China because of economic developments and lifestyle changes (3, 4). In our study, the MRI T2* GRE technique and R2* functional mapping provided us with visual and robust evidence to offset the limits of serum ferritin shown in previous studies. Moreover, a close correlation between the M-HIC and Hb A_{1c} was shown in nondiabetic subjects (NGT + prediabetics). These results supplemented a necessary logistical link to prove the potential important role of iron in the pathogenesis of T2D. With consideration of these results, more attention should particularly be paid to iron metabolism of prediabetic and high-risk populations.

HICs in Chinese subjects with altered glucose homeostasis ranged from 20 to 70 μmol/g (median: 40 μmol/g), which were remarkably lower than in Western patients with insulin resistance–associated hepatic iron overload whose HICs ranged from 38 to 332 μmol/g (median: 90 μmol/g) (26). The result of our study suggested a mild hepatic iron overload, which was even significantly lower than the Western criteria for insulin resistance–associated hepatic iron overload, may play a role in the insulin resistance of the Chinese population. Previous studies have proved that frequent blood donations or iron-chelation therapy that lead to decreasing iron stores could constitute a protective effect on the development of diabetes (27, 28). With consideration of the results of the current study, whether the iron intake for prediabetes or high-risk groups should be the same as the normal standard of Dietary Reference Intakes warrants further investigations.

Moreover, a similar HIC but significantly higher serum ferritin concentration were observed in the T2D group than in the

prediabetes group. Combined with decreased serum iron and transferrin saturation in T2D patients compared with subjects with prediabetes, this phenomenon implied a decreasing circulating iron in T2D patients even though hepatic iron stores remained higher. A diet control with a decreased dietary intake of iron after a diagnosis of T2D may attenuate hepatic iron accumulation. Furthermore, an increased amount of renal and urinary iron was reported in patients even in the early stages of renal dysfunction (29). Although patients with a definite diagnosis of macroalbuminurine (albumin-to-creatinine ratio >300 mg/g Cr) were excluded, it is possible that an early impairment of renal function in T2D patients, especially in patients with microalbuminuria, may have been accompanied by an increased excretion of iron and may have attenuated the continuous accumulation of hepatic iron.

Our study had a few limitations. First, the study could not be performed for perfectly matched age, BMI, and blood lipids concentrations across the 3 groups. However, correlations between biomarkers of blood lipids and M-HIC were also analyzed by Spearman's partial correlation analysis, and no significance was observed. Furthermore, because potential influences of age and BMI were adjusted in the general linear model ANOVA and possible confounders were taken into account in stepwise multiple linear regression, the effect from these confounders was minimized. Second, because mild intrahepatic iron deposition is a frequent finding in nonalcoholic fatty liver disease (18, 30), the potential influence of fatty liver on the evaluation of the HIC was also considered in our study. Subjects without fatty liver were analyzed separately, and M-HICs in this subgroup was also significantly higher than in the NGT group. Moreover, fatty liver was not shown to be an independent predictor of M-HIC by multiple linear regression. Therefore, increased hepatic iron stores in T2D patients were not false-positive results confounded by fatty liver in our study. Third, because of the expensive cost, a complex post-analysis of the T2* GRE technique, and limited MRI-related resources, the number of subjects who completed an MRI examination was significantly less than the number of subjects who received blood sampling. However, subjects who completed MRI were randomly assigned from each group of subjects who received blood sampling. Therefore, we deduced that this subset could reflect characteristics of the larger sample. Furthermore, the validity of our results was 0.89 as calculated by PASS software (NCSS). Thus, we ensured the reliability and power of the study while taking into account the effect of limited resources.

In conclusion, to our knowledge, our findings provided the first data on the HIC in Chinese population with altered glucose homeostasis and novel evidence to support the hypothesis of a mild hepatic iron overload in this population. However, hepatic iron stores in the T2D group did not increase to a higher concentration in parallel with the aggravation of insulin resistance compared with in the prediabetes group. A cohort study concerned with the effect of the attenuation of excess iron on the improvement of glucose metabolism in a prediabetic population is warranted.

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REFERENCES

1. Yang W, Lu J, Weng J, Jia W, Ji L, Xiao J, Shan Z, Liu J, Tian H, Ji Q, et al. Prevalence of diabetes among men and women in China. N Engl J Med 2010;362:1090–101.
2. Fernández-Real JM, López-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. Diabetes 2002;51:2348–54.
3. Luan de C, Li H, Li SJ, Zhao Z, Li X, Liu ZM. Body iron stores and dietary iron intake in relation to diabetes in adults in North China. Diabetes Care 2008;31:285–6.
4. Shi ZM, Hu XS, Yuan BJ, Gibson R, Dai Y, Garg M. Association between serum ferritin, hemoglobin, iron intake, and diabetes in adults in Jiangsu, China. Diabetes Care 2006;29:1878–83.
5. Jehn ML, Guallar E, Clark JM, Couper D, Duncan BB, Ballantyne CM, Hoogeveen RC, Harris ZL, Pankow JS. A prospective study of plasma ferritin level and incident diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol 2007;165:1047–54.
6. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. JAMA 2004;291:711–7.
7. Rajpathak SN, Wylie-Rosett J, Gunter MJ, Negassa A, Kabat GC, Rohan TE, Crandall J; Diabetes Prevention Program (DPP) Research Group. Biomarkers of body iron stores and risk of developing type 2 diabetes. Diabetes Obes Metab 2009;11:472–9.
8. Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. Clin Chim Acta 2003;329:9–22.
9. Fernández-Real JM, Moreno JM, López-Bermejo A, Chico B, Vendrell J, Ricart W. Circulating soluble transferrin receptor according to glucose tolerance status and insulin sensitivity. Diabetes Care 2007;30:604–8.
10. Hernández C, Lecube A, Carrera A, Simó R. Soluble transferrin receptors and ferritin in Type 2 diabetic patients. Diabet Med 2005;22:97–101.
11. Freixenet N, Vilardell C, Llauradó G, Giménez-Palop O, Berlanga E, Gutiérrez C, Caixàs A, Vendrell J, González-Clemente JM. Men with hyperferritinemia and diabetes in the Mediterranean area do not have a higher iron overload than those without diabetes. Diabetes Res Clin Pract 2011;91:e33–6.
12. Angelucci E, Brittenham GM, McLaren CE, Ripalti M, Baronciani D, Giardini C, Galimberti M, Polchi P, Lucarelli G. Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med 2000;343:327–31.
13. Ferrannini E. Insulin resistance, iron, and the liver. Lancet 2000;355:2181–2.
14. Dinneen SF, Silverberg JD, Batts KP, O'Brien PC, Ballard DJ, Rizza RA. Liver iron stores in patients with non-insulin-dependent diabetes mellitus. Mayo Clin Proc 1994;69:13–5.
15. Gandon Y, Olivié D, Guyader D, Aubé C, Oberti F, Sebille V, Deugnier Y. Non-invasive assessment of hepatic iron stores by MRI. Lancet 2004;363:357–62.
16. Emond MJ, Bronner MP, Carlson TH, Lin M, Labbe RF, Kowdley KV. Quantitative study of the variability of hepatic iron concentrations. Clin Chem 1999;45:340–6.
17. Virtanen JM, Komu ME, Parkkola RK. Quantitative liver iron measurement by magnetic resonance imaging: in vitro and in vivo assessment of the liver to muscle signal intensity and the R2* methods. Magn Reson Imaging 2008;26:1175–82.
18. Xie P, Zhou XX, Zhang Q. [Pathogenesis and treatment of non-alcoholic fatty liver disease.] Zhong Xi Yi Jie He Xue Bao 2010;8:201–9 (in Chinese).
19. Stern SE, Williams K, Ferrannini E, DeFronzo RA, Bogardus C, Stern MP. Identification of individuals with insulin resistance using routine clinical measurements. Diabetes 2005;54:333–9.
20. St Pierre TG, Clark PR, Chua-Anusorn W. Measurement and mapping of liver iron concentrations using magnetic resonance imaging. Ann N Y Acad Sci 2005;1054:379–85.
21. Alústiza JM, Artetxe J, Castiella A, Agirre C, Emparanza JI, Otazua P, García-Bengoechea M, Barrio J, Mújica F, Recondo JA, et al. MR quantification of hepatic iron concentration. Radiology 2004;230:479–84.
22. Salo S, Alanen A, Leino R, Bondestam S, Komu M. The effect of haemosiderosis and blood transfusions on the T2 relaxation time and 1/T2 relaxation rate of liver tissue. Br J Radiol 2002;75:24–7.
23. Caldwell S, Northup PG. Bleeding complication with liver biopsy: is it predictable? Clin Gastroenterol Hepatol 2010;8:826–9.

24. Sharifi F, Nasab NM, Zadeh HJ. Elevated serum ferritin concentrations in prediabetic subjects. Diab Vasc Dis Res 2008;5:15–8.
25. Mascitelli L, Pezzetta F. High iron stores and impaired endothelial function in prediabetic subjects. Am J Cardiol 2006;97:1550.
26. Mendler MH, Turlin B, Moirand R, Jouanolle AM, Sapey T, Guyader D, Le Gall JY, Brissot P, David V, Deugnier Y. Insulin resistance-associated hepatic iron overload. Gastroenterology 1999;117:1155–63.
27. Facchini FS. Effect of phlebotomy on plasma glucose and insulin concentrations. Diabetes Care 1998;21:2190.
28. Hua NW, Stoohs RA, Facchini FS. Low iron status and enhanced insulin sensitivity in lacto-ovo vegetarians. Br J Nutr 2001;86:515–9.
29. Nankivell BJ, Boadle RA, Harris DCH. Iron accumulation in human chronic renal disease. Am J Kidney Dis 1992;20:580–4.
30. Sharp PA. New insights into the role of iron in the development of nonalcoholic fatty liver disease. Hepatology 2010;52:408–10.

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