

## Insulin Sensitivity and Liver Fat: Role of Iron Load

Michael Haap, Jürgen Machann, Christine von Friedeburg, Fritz Schick, Norbert Stefan, Nina F. Schwenzer, Andreas Fritsche, Hans Ulrich Häring, and Claus Thamer

Department of Internal Medicine (M.H., C.v.F., N.S., A.F., H.U.H., C.T.), Division of Endocrinology, Diabetology, Angiology, Nephrology, and Clinical Chemistry, and Section on Experimental Radiology (J.M., F.S., N.F.S.), Department of Diagnostic and Interventional Radiology, Eberhard-Karls-University Tübingen, D-72076 Tübingen, Germany

**Context:** Increased liver fat (LF) is associated with insulin resistance. However, a considerable individual variability between LF and insulin sensitivity (IS) is observed, and at equal levels of LF, insulin-resistant as well as insulin-sensitive individuals are found.

**Objective:** Our objective was to study whether hepatic iron load (HIL) explains some of the variation between IS and LF.

**Design:** HIL was measured using a quantitative T2\* magnetic resonance gradient echo imaging technique, and LF was measured by <sup>1</sup>H-magnetic resonance spectroscopy. Low T2\* values indicate high HIL. We studied the association of LF and HIL with anthropometric data and IS. A total of 113 healthy nondiabetic subjects [69 females, 44 males; age 47 ± 1 yr; body mass index (BMI) = 28.9 ± 0.5 kg/m<sup>2</sup>] at increased risk for type 2 diabetes were included in the study.

**Results:** T2\* values adjusted for age negatively associated with serum ferritin levels ( $P < 0.0001$ ) and positively associated with IS ( $P = 0.009$ ). In addition, T2\* values associated with LF ( $P = 0.008$ ) but not with BMI ( $P = 0.6$ ). In a multivariate model, IS adjusted for gender, age, and BMI was associated with T2\* values ( $P = 0.015$ ). IS adjusted for gender and age was independently associated with LF ( $P = 0.033$ ) and T2\* values ( $P = 0.004$ ). In a stepwise regression analysis, LF explained 13.5% ( $P < 0.01$ ) of the variation in IS, and HIL explained an additional 4.1% ( $P = 0.03$ ).

**Conclusions:** HIL explains part of the variation between LF and IS. The mechanism by which iron load induces insulin resistance is possibly independent of the pathways involved in insulin resistance induced by fatty liver disease. (*J Clin Endocrinol Metab* 96: E958–E961, 2011)

The role of fatty liver in the development and prevention of type 2 diabetes and related disorders is well established. Although an increase in hepatic fat is in most cases associated with decreased insulin sensitivity, a considerable variability in the relationship between liver fat and insulin sensitivity exists (1). This phenomenon may partly be explained by interindividual differences in the susceptibility to the negative effects of hepatic fat storage. In accordance with this concept, our group has shown that variations in genes related to hepatic lipid metabolism results in a dissociation of the close relationship of hepatic

fat storage and insulin resistance (1, 2). However, there is also the possibility that hepatic accumulation of metabolites or trace elements other than fat may impact on hepatic glucose metabolism. In this context, it is of interest that increased whole-body iron load has been associated with insulin resistance, glucose intolerance, and type 2 diabetes mellitus (3–5). The mechanism underlying the association between body iron stores and diabetes-related traits is unclear and has not yet been finally identified (4, 6). Changes in liver metabolism represent a plausible link between iron stores and insulin resistance (7). In healthy subjects, high

serum ferritin concentrations are associated with insulin resistance rather than defects in insulin secretion (8). Interestingly, iron load and hepatic iron metabolism have also been proposed to contribute to the development of nonalcoholic fatty liver disease (9, 10). We used a quantitative T2\* magnetic resonance gradient echo imaging technique and <sup>1</sup>H-magnetic resonance spectroscopy (MRS) to simultaneously estimate liver iron stores and liver fat content in a metabolically characterized cohort. We hypothesized that hepatic iron load (HIL) explains part of the variation in insulin sensitivity not explained by the variation in liver fat.

## Subjects and Methods

### Subjects

We studied T2\* values for determination of HIL in 113 subjects at increased risk for type 2 diabetes because of one or more of the following risk factors: being overweight [body mass index (BMI) > 27 kg/m<sup>2</sup>], being a first-degree relative of a patient with type 2 diabetes, or having impaired glucose tolerance or having a history of gestational diabetes. Measurements of liver fat by <sup>1</sup>H-MRS were available in 101 of these subjects. The local ethics committee had approved all protocols. All subjects gave informed written consent.

### Oral glucose tolerance test (OGTT)

A standard 75-g OGTT was performed after a 10-h overnight fast with determination of glucose and insulin at 0, 30, 60, 90, and 120 min. Insulin sensitivity was estimated as proposed by Matsuda and DeFronzo (11). First-phase insulin secretion was estimated using the insulinogenic index. This parameter is defined as the ratio of the increment of serum insulin 30 min after an oral glucose load to blood glucose concentration 30 min after the glucose load [(30-min insulin – fasting insulin)/30-min glucose].

### Analytical procedures

Plasma glucose was measured by the glucose-oxidase method (YSI, Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin and C-peptide were determined by microparticle enzyme immunoassays (ADVIA Centaur; Siemens Healthcare Diagnostics, Eschborn, Germany).

### Magnetic resonance imaging and spectroscopy

All subjects were examined on a 1.5-T whole-body unit (Magnetom Sonata; Siemens Healthcare, Erlangen, Germany). Volunteers were placed on the six-channel spine array coil of the manufacturer in the supine position. An additional body array surface coil with two segments was placed symmetrically on the chest to obtain nearly homogeneous signal sensitivity in the upper abdomen as well as comparable receiver characteristics on the left and right abdominal sides. To assess the topography of the liver, a T1-weighted gradient echo sequence was applied in breath-hold. The parameters were as follows: repetition time (TR) = 3.58 msec; echo time (TE) = 1.79 msec; field of view, 285 × 380 mm<sup>2</sup>; matrix, 144 × 256; bandwidth, 490 Hz/pixel; flip angle, 13°; slices, 40; slice thickness, 5 mm; distance factor,

20%; and time of acquisition, 15 sec. All participants underwent an MRI examination for T2\* evaluation of the liver. To calculate the T2\* relaxation time, a multislice fat-saturated breath-hold two-dimensional multiecho gradient-echo sequence was applied. TR was set to 248 msec. The first echo was acquired at a TE of TE1 = 2.6 msec. The following echoes were acquired in 11 equidistant steps with an increment of ΔTE = 4 msec (12 TE from 2.6–46.6 msec). Field of view was 285 × 380 mm<sup>2</sup>; matrix, 108 × 192 mm<sup>2</sup>; slice thickness, 5 mm; and slices, five. Measuring time was 18 sec, enabling single breath-hold examination. T2\* times were calculated by a monoexponential fit of signal intensities.

Methods are explained in detail in Ref. 12.

Liver fat content was determined by localized stimulated echo acquisition mode <sup>1</sup>H-MRS (TR = 4 sec, TE = 10 msec, 32 scans) in the seventh segment of the liver. The lipid content was quantitatively assessed by analyzing the signal integral (methylene and methyl signals from 0.7–1.5 ppm), using the liver water and lipid signal integral as internal reference (13).

### Statistical analysis

All data are given as Mean ± SEM unless otherwise stated. For variables which were not normally distributed data were logarithmically transformed for further analysis. We used multivariate regression analysis to adjust for the independent effects of covariates. The associations between HIL, metabolic traits and liver fat were adjusted for age. A p-value <0.05 was considered to be statistically significant. All calculations and statistical analyses were performed using the Statistical Package JMP Version 4.0 (SAS Institute Inc., Cary, NC).

## Results

### Associations of HIL with anthropometric measures and laboratory findings

Subject characteristics are shown in Table 1. Mean T2\* values were lower in males compared with females (25.7 ± 1.0 vs. 29.4 ± 0.8, P = 0.001), indicating higher HIL in males. T2\* values were negatively associated with age (P < 0.0001) independent of gender, indicating increased HIL in older subjects. No association of HIL with BMI was observed (P = 0.6). In univariate regression, T2\* values

**TABLE 1.** Characteristics of the study group

Variable	Mean ± SD	Range
n (male/female)	113 (44/69)	
Age	47 ± 11	21–69
BMI (kg/m <sup>2</sup> )	28.9 ± 5.2	19.2–43.5
T2* value (msec)	28.0 ± 7.0	13.6–45.9
Liver fat (% signal) (n = 101)	4.5 ± 5.7	0.1–30.8
Fasting glucose (OGTT, mmol/liter)	5.2 ± 0.5	4.3–6.5
2-h glucose (OGTT, mmol/liter)	6.8 ± 1.5	4.2–11.0
Fasting insulin (OGTT, pM)	56 ± 45	16–373
2-h insulin (OGTT, pM)	430 ± 345	56–1820
Insulin sensitivity (arbitrary units) <sup>a</sup>	14.4 ± 7.6	1.6–33.4

<sup>a</sup> index proposed by Matsuda and de Fronzo (11).

**TABLE 2.** Determinants of insulin sensitivity in multivariate linear regression models

Covariates	Estimate $\pm$ SE	P
Model 1		
Female gender	0.07 $\pm$ 0.05	0.15
Age (yr)	0.23 $\pm$ 0.19	0.21
BMI (kg/m <sup>2</sup> )	−1.63 $\pm$ 0.259	<0.0001
T2* liver (msec)	0.02 $\pm$ 0.007	0.015
Model 2		
Female gender	0.09 $\pm$ 0.05	0.08
Age (yr)	0.25 $\pm$ 0.21	0.22
T2* liver (msec)	0.017 $\pm$ 0.008	0.033
Liver fat (% signal)	−0.14 $\pm$ 0.05	0.004

adjusted for age negatively associated with serum ferritin ( $P < 0.0001$ ) but not with C-reactive protein ( $P = 0.6$ ).

### Associations of HIL with metabolic traits

T2\* values adjusted for age negatively associated with fasting glucose ( $P = 0.0002$ ), fasting insulin levels ( $P = 0.01$ ), and insulin sensitivity estimated from OGTT ( $P = 0.01$ ) but not with 2-h glucose during OGTT ( $P = 0.38$ ). In multivariate linear regression analyses, insulin sensitivity adjusted for gender, age, and BMI was associated with T2\* values ( $P = 0.015$ , see Table 2 for details). We found no effect of T2\* values on insulin secretion ( $P = 0.25$ ) estimated from OGTT adjusted for gender, age, and insulin sensitivity.

### Associations of HIL, liver fat, and insulin sensitivity

T2\* values adjusted for age negatively associated with liver fat ( $P = 0.008$ ), indicating higher HIL in subjects with increased liver fat. Liver fat adjusted for age was strongly associated with insulin sensitivity ( $P = 0.0002$ ). In a multivariate regression analysis, insulin sensitivity adjusted for gender and age was independently associated with liver fat ( $P = 0.033$ ) and HIL ( $P = 0.004$ ) (see Table 2 for details). In a multivariate regression analysis, liver fat explained 13.5% of the variation in insulin sensitivity ( $P < 0.01$ ), and HIL explained an additional 4.1% of the variation in insulin sensitivity ( $P = 0.03$ ).

## Discussion

We studied the association between HIL and glucose metabolism in healthy subjects. Our data suggest that HIL increases systemic insulin resistance and fasting blood glucose levels. Because we have no data on a precise measurement of hepatic insulin resistance, *e.g.* by use of tracer methods, we can only speculate that HIL specifically affects hepatic insulin resistance. This hypothesis is supported by the observation that HIL is related to fasting glucose, fasting insulin, and whole-body insulin sensitivity

but not to insulin secretion. This is in accordance with a previous study from our group showing that serum ferritin levels in healthy subjects are associated with insulin resistance rather than defects in insulin secretion (8). Interestingly, daily meat intake is known to contribute to an increased risk of type 2 diabetes not only in German populations (14). The associations between HIL and insulin sensitivity represent a plausible pathophysiological explanation for this observation.

In our population, HIL was associated with liver fat content. Because hepatic iron content was not a significant determinant of liver fat content independently of age, gender, and insulin sensitivity in our study ( $P = 0.17$ ). Thus, the observed univariate relationship between hepatic iron content and liver fat content may be a result of a co-correlation of the parameters with insulin sensitivity.

Our study is too small to rule out that the associations between HIL, liver fat, and insulin sensitivity are different between groups with different glucose tolerance status or differ in lean, overweight, and obese subjects. We also cannot answer the question whether iron is causally involved in the pathogenesis of hepatic steatosis. The association between liver fat and iron load may simply be caused by the same underlying risk factors, for example nutrient intake and diet. In this context, it is of interest that HIL and liver fat have an impact on insulin sensitivity, independently from each other. In addition, HIL explains a substantial part of the variation in insulin sensitivity that is not explained by liver fat. This finding suggests that both parameters may involve different pathways that are operative in the regulation of hepatic glucose metabolism. The mechanism by which fat accumulation in the liver induces hyperglycemia possibly involves subclinical inflammation and changes in the secretion of hepatokines (1). In addition, hepatic iron overload induces hepatic damage and potentially also insulin resistance by induction of mitochondrial dysfunction (15), increased lipid peroxidation (16), and increased endoplasmic reticulum stress (17). Primary changes in hepatic insulin metabolism and the clearance capacity of the liver may also link hepatic iron overload to insulin resistance (7).

In conclusion, hepatic iron metabolism is an important player in the development of systemic insulin resistance. Because our study lacks a precise measurement of hepatic insulin sensitivity, we can only speculate that HIL induces insulin resistance specifically at the level of the liver. Furthermore, the mechanism by which iron load induces insulin resistance seems to be independent of the pathways involved in insulin resistance induced by fatty liver disease.

## Acknowledgments

Address all correspondence and requests for reprints to: Hans-Ulrich Häring, Internal Medicine IV, Medizinische Universitätsklinik Tübingen, Otfried-Müller-Strasse 10, D-72076 Tübingen, Germany. E-mail: hans-ulrich.haering@med.uni-tuebingen.de.

This study was supported in part by Grant 0315381B from the German Federal Ministry of Education and Research (BMBF).

Disclosure Summary: The authors have nothing to disclose.

## References

1. Stefan N, Kantartzis K, Häring HU 2008 Causes and metabolic consequences of Fatty liver. *Endocr Rev* 29:939–960
2. Stefan N, Staiger H, Häring HU 2011 Dissociation between fatty liver and insulin resistance: the role of adipose triacylglycerol lipase. *Diabetologia* 54:7–9
3. Fernández-Real JM, Ricart-Engel W, Arroyo E, Balançá R, Casamitjana-Abella R, Cabrero D, Fernández-Castañer M, Soler J 1998 Serum ferritin as a component of the insulin resistance syndrome. *Diabetes Care* 21:62–68
4. Ford ES, Cogswell ME 1999 Diabetes and serum ferritin concentration among U.S. adults. *Diabetes Care* 22:1978–1983
5. Jehn M, Clark JM, Guallar E 2004 Serum ferritin and risk of the metabolic syndrome in U.S. adults. *Diabetes Care* 27:2422–2428
6. Rajpathak SN, Crandall JP, Wylie-Rosett J, Kabat GC, Rohan TE, Hu FB 2009 The role of iron in type 2 diabetes in humans. *Biochim Biophys Acta* 1790:671–681
7. Ferrannini E 2000 Insulin resistance, iron, and the liver. *Lancet* 355: 2181–2182
8. Haap M, Fritsche A, Mensing HJ, Häring HU, Stumvoll M 2003 Association of high serum ferritin concentration with glucose intolerance and insulin resistance in healthy people. *Ann Intern Med* 139:869–871
9. Machado M, Cortez-Pinto H 2006 Nash, insulin resistance and iron. *Liver Int* 26:1159–1162
10. Yoneda M, Nozaki Y, Endo H, Mawatari H, Iida H, Fujita K, Yoneda K, Takahashi H, Kirikoshi H, Inamori M, Kobayashi N, Kubota K, Saito S, Maeyama S, Hotta K, Nakajima A 2010 Serum ferritin is a clinical biomarker in Japanese patients with nonalcoholic steatohepatitis (NASH) independent of HFE gene mutation. *Dig Dis Sci* 55:808–814
11. Matsuda M, DeFronzo RA 1999 Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 22:1462–1470
12. Schwenzer NF, Machann J, Haap MM, Martirosian P, Schraml C, Liebig G, Stefan N, Häring HU, Claussen CD, Fritsche A, Schick F 2008 T2\* relaxometry in liver, pancreas, and spleen in a healthy cohort of one hundred twenty-nine subjects—correlation with age, gender, and serum ferritin. *Invest Radiol* 43:854–860
13. Schwenzer NF, Machann J, Martirosian P, Stefan N, Schraml C, Fritsche A, Claussen CD, Schick F 2008 Quantification of pancreatic lipomatosis and liver steatosis by MRI: comparison of in/opposed-phase and spectral-spatial excitation techniques. *Invest Radiol* 43: 330–337
14. Song Y, Manson JE, Buring JE, Liu S 2004 A prospective study of red meat consumption and type 2 diabetes in middle-aged and elderly women: the women's health study. *Diabetes Care* 27:2108–2115
15. Gao X, Campian JL, Qian M, Sun XF, Eaton JW 2009 Mitochondrial DNA damage in iron overload. *J Biol Chem* 284:4767–4775
16. Festa M, Ricciardelli G, Mele G, Pietropaolo C, Ruffo A, Colonna A 2000 Overexpression of H ferritin and up-regulation of iron regulatory protein genes during differentiation of 3T3-L1 pre-adipocytes. *J Biol Chem* 275:36708–36712
17. Lou LX, Geng B, Chen Y, Yu F, Zhao J, Tang CS 2009 Endoplasmic reticulum stress involved in heart and liver injury in iron-loaded rats. *Clin Exp Pharmacol Physiol* 36:612–618