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Serum ferritin is associated with non-alcoholic fatty liver disease and decreased B-cell function in non-diabetic men and women ☆,☆☆

Kristina M. Utzschneider [a,b,*], Anna Largajolli [c], Alessandra Bertoldo [c], Santica Marcovina [g], James E. Nelson [d], Matthew M. Yeh [e], Kris V. Kowdley [d,f], Steven E. Kahn [a,b]

[a] VA Puget Sound Health Care System, Department of Medicine, Division of Endocrinology and Metabolism, Seattle, WA
[b] University of Washington, Department of Medicine, Division of Metabolism, Endocrinology and Nutrition, Seattle, WA
[c] University of Padua, Padua, Italy
[d] Digestive Disease Institute, Virginia Mason Medical Center and Benaroya Research Institute, Seattle, WA
[e] University of Washington, Department of Pathology, Seattle, WA
[f] University of Washington, Department of Medicine, Division of Gastroenterology/Hepatology, Seattle, WA
[g] Department of Medicine, University of Washington and the Northwest Lipid Metabolism and Diabetes Research Laboratory, Seattle, WA

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ABSTRACT

Aims: We sought to determine whether NAFLD is associated with poorer β-cell function and if any β-cell dysfunction is associated with abnormal markers of iron or inflammation.
Methods: This was a cross-sectional study of 15 non-diabetic adults with NAFLD and 15 non-diabetic age and BMI-matched controls. Insulin sensitivity was measured by isotope-labeled hyperinsulinemic–euglycemic clamps and β-cell function by both oral (OGTT) and intravenous glucose tolerance tests. Liver and abdominal fat composition was evaluated by CT scan. Fasting serum levels of ferritin, transferrin-iron saturation, IL-6, TNFα and hsCRP were measured.
Results: Compared to controls, subjects with NAFLD had lower hepatic and systemic insulin sensitivity and β-cell function was decreased as measured by the oral disposition index. Fasting serum ferritin and transferrin-iron saturation were higher in NAFLD and were positively associated with liver fat. Serum ferritin was negatively associated with β-cell function measured by both oral and intravenous tests, but was not associated with insulin sensitivity. IL-6, TNFα and hsCRP did not differ between groups and did not correlate with serum ferritin, liver fat or measures of β-cell function.
Conclusions: These findings support a potential pathophysiological link between iron metabolism, liver fat and diabetes risk.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD), defined as fat accumulation in the liver in the absence of excessive alcohol intake, is strongly associated with insulin resistance, obesity and type 2 diabetes (Utzschneider & Kahn, 2006). NAFLD has also been shown to be a risk factor for the development of type 2 diabetes (Chon et al., 2012; Sung, Jeong, Wild, & Byrne, 2012). Factors underlying this increased risk to develop type 2 diabetes have not been fully elucidated. Obesity and insulin resistance are certainly factors that may contribute, but β-cell dysfunction is a key feature that contributes to the development of type 2 diabetes (Kahn, Zraika, Utzschneider, & Hull, 2009). However, studies to date have not shown an association between liver fat and β-cell function (Bedogni et al., 2012; Rijkelijkhuizen et al., 2009; Tushuizen et al., 2008). These studies included subjects with normal or impaired glucose tolerance and diabetes using oral glucose tolerance tests to assess β-cell function.

Factors that have been associated with increased diabetes risk in this population include markers of iron metabolism, specifically ferritin which has been found to be increased in NAFLD (Hsiao, Chen, & Wang, 2004; Licata et al., 2009; Zelber-Sagi, Nitzan-Kaluski, Halpern, & Oren, 2007). Iron overload conditions are well known to be associated with β-cell dysfunction and can lead to diabetes (Dmochowski, Finegood, Francombe, Tyler, & Zinman, 1993; Fargion et al., 1992; McClain et al., 2006). Serum ferritin levels are higher in patients with diabetes (Ford & Cogswell, 1999) and the metabolic syndrome (Jehn, Clark, & Guallar, 2004; Vari et al., 2007), suggesting

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* Corresponding author at: VA Puget Sound Health Care System (151), Seattle, WA 98108. Tel.: +1 206 277 3568; fax: +1 206 764 2164.
E-mail address: kutzschn@u.washington.edu (K.M. Utzschneider).

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that body iron stores may play a detrimental role in glucose metabolism even in the absence of overt iron overload (Jehn et al., 2004). Further, higher ferritin levels have been shown to predict the development of type 2 diabetes (Forouhi et al., 2007). The ability of iron depletion to improve insulin sensitivity and β-cell function in healthy individuals (Fernandez-Real, Lopez-Bermejo, & Ricart, 2005) and those with type 2 diabetes (Fernandez-Real et al., 2002) provides additional support for a role of iron in glucose metabolism. Thus, hyperferritinemia in NAFLD may be a necessary cofactor in the NAFLD/diabetes connection by contributing to insulin resistance and/or β-cell dysfunction.

Elevated ferritin levels observed in NAFLD may also reflect the inflammatory milieu within the steatotic liver. The evidence suggests that inflammatory cytokines themselves may play an important role in β-cell dysfunction and β-cell apoptosis, both key features in the pathogenesis of type 2 diabetes. Inflammatory markers such as C-reactive protein, TNFα (Hui et al., 2004; Kugelmas, Hill, Vivian, Marsano, & McClain, 2003) and IL-6 (Kugelmas et al., 2003) have been shown to be elevated in NAFLD, with higher levels in those with steatohepatitis and fibrosis (Yoneda et al., 2007).

We hypothesized that subjects with NAFLD would have lower insulin sensitivity and poorer β-cell function and that higher ferritin levels and inflammatory cytokines in NAFLD may contribute to diabetes risk by being associated with lower insulin sensitivity and/or poorer β-cell function. To examine this hypothesis we studied non-diabetic subjects diagnosed with NAFLD and compared them to age- and BMI-matched control subjects without liver disease. First, we carefully characterized study subjects by performing isotope labeled, hyperinsulinemic–euglycemic clamps to directly measure both hepatic and peripheral insulin sensitivity, oral and intravenous (IV) glucose tolerance tests to measure β-cell function, and fasting iron and inflammatory markers. We then determined associations between liver fat, serum ferritin and transferrin-iron saturation, markers of inflammation and measures of insulin sensitivity and β-cell function.

2. Research design and methods

2.1. Subjects

This cross-sectional study compared subjects with NAFLD to age- and BMI-matched control subjects. All subjects gave written informed consent to participate and the study was approved by the Human Subjects Review Committees at the VA Puget Sound Health Care System and the University of Washington.

Subjects underwent an initial screening visit which included a history, physical exam and fasting blood tests. Case subjects were recruited from local area gastroenterologists and were defined as having NAFLD based on either a liver biopsy within the past 3 years meeting criteria for >5% fatty infiltration or the presence of elevated liver enzymes in conjunction with imaging suggestive of fatty liver. Biopsy samples were available for review from 12/15 subjects. These were reviewed by a single pathologist and scored using the NASH Clinical Research Network criteria (Kleiner et al., 2005). Exclusion criteria for case subjects included cirrhosis on liver biopsy, significant weight loss (>5%) since the liver biopsy, other known causes of elevated liver enzymes or a serum alanine aminotransferase (ALT) >5 times the upper limit of normal (lab normal range: 0–39 U/L). Control subjects were recruited by advertisement and fliers from the Seattle area. They were required to have normal liver enzymes and no history of liver disease. Additional exclusion criteria for all subjects included: self-reported alcohol intake >20 g per day, positive hepatitis C antibody or hepatitis B surface antigen, transferrin-iron saturation >55%, serum creatinine >1.4 mg/dl in men and >1.3 mg/dl in women, hematocrit <33%, pregnancy or lactation, any serious medical condition, or use of any of the following medications: corticosteroids, estrogens at doses higher than standard replacement therapy, tamoxifen, amiodarone, accutane, sertraline, atypical antipsychotics, anti-retroviral medications, niacin, gemfibrozil, fenofibrate, glucose-lowering agents, ursodeoxycholic acid, betaine or milk thistle. HFE gene mutation analysis was not routinely performed.

A total of 34 subjects were studied, and data on 30 were eligible for analysis. Three subjects (two cases and one control) were excluded based on oral glucose tolerance test (OGTT) results in the diabetic range. One subject was determined to have <5% fat on his liver biopsy upon review by the study pathologist and was therefore excluded.

2.2. Study Procedures

All study procedures were performed after an overnight fast of 10–12 h on separate days within two weeks. Plasma samples were placed immediately on ice and processed in a refrigerated centrifuge at 4 °C and aliquots frozen at −70 °C until assayed.

2.2.1. Oral glucose tolerance test (OGTT)

Seventy-five grams of glucose was consumed within 5 min and blood samples drawn at −10, −5, −1, 10, 20, 30, 60, 90 and 120 min. The three basal samples were averaged for the 0 time point. Glucose tolerance status was determined according to American Diabetes Association guidelines (Anonymous, 2011).

2.2.2. Intravenous glucose tolerance test (IVGTT)

Two peripheral intravenous lines were established. The sampling arm was wrapped in a heating pad to "arterialize" the blood samples. Glucose (11.4 g/m^2) was injected over 60 s and blood samples for glucose, insulin and C-peptide were drawn at −10, −5, −1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25 and 30 min relative to the start of the glucose injection.

2.2.3. Hyperinsulinemic–euglycemic clamp

Subjects were admitted the night before and fed a standardized dinner from 7 to 8 pm consisting of 7 kcal/kg (50% calories from carbohydrate, 30% fat and 20% protein). An intravenous catheter was placed in each arm. The sampling arm was wrapped in a heating pad to "arterialize" the blood. At 5 a.m. a primed (200 mg/m^2 × glucose/100 given over 5 min), continuous (2 mg/m^2/min) infusion of 6,6 2d glucose was started and continued throughout the clamp procedure. The two-step hyperinsulinemic–euglycemic clamp procedure started at 8 am and included a low dose insulin infusion (20 mU/m^2/min) for 3 h followed by a primed high dose insulin infusion (160 mU/m^2/min × 5 min then 80 mU/m^2/min) for two hours. Blood glucose was measured every 5 min using an iStat machine and a variable rate infusion of 20% dextrose enriched with 2% 6,6 2d glucose was titrated to maintain the blood glucose concentration at 5 mmol/L (90 mg/dl). Samples were drawn for glucose and insulin every 30 min throughout the clamp. Samples for glucose, insulin and 6,6 2d glucose were drawn every 15 min during the final half hour of the basal, low dose and high dose insulin periods. Samples for free fatty acids (FFAs) were drawn into tubes containing the lipolysis inhibitor orlistat at −30, −15, −1, 10, 20, 30 and 60 min relative to the start of the low dose insulin infusion and placed immediately on ice. FFA samples were processed within 30 min and the plasma flash frozen.

2.2.4. Body composition analyses

Body fat mass (FM) and lean mass (LM) were determined using dual-energy x-ray absorptiometry (Lunar, GE Medical Systems). Unenhanced CT scan images were obtained on a General Electric Discovery HD750 CT scanner. Intra-abdominal (IAF) and abdominal subcutaneous fat (SQF) areas were measured at the top of the iliac crest and quantified using the Tomovision program (SliceOMatic V4.3) by one trained technologist with an intra-observer CV of <7% for IAF and <3% for SQF.

Liver fat was estimated by CT scan by measuring the density ratio between the liver and spleen in Hounsfield units (liver/spleen ratio),