CLINICAL STUDY

# Association between serum ferritin and the insulin resistance syndrome in a representative population

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## Abstract

*Objective*: Unexplained hepatic iron overload with increased serum ferritin (SF) values has been associated with the insulin resistance syndrome (IRS), defined by the presence of one or more of the following criteria: increased body mass index (BMI), diabetes, hyperlipidemia or hypertension. However, as yet the association between IRS and SF in a representative population has not been investigated.

*Methods*: The study subjects participated in a nationwide epidemiological survey on metabolic disorders in the adult German population. The 1200 probands included in this study are representative of the German population. To eliminate major causes of secondary iron overload, 114 (9.5%) subjects with excessive alcohol consumption and 16 (1.5%) subjects with serological evidence for hepatitis B or C were excluded. For all remaining 1070 probands, complete clinical data of SF, HbA1c, known diabetes, BMI, cholesterol, high-density lipoprotein-cholesterol and blood pressure were available.

*Results*: SF values were significantly increased in men and women with high BMI ($>25\,\mathrm{kg/m^2}$), increased cholesterol ($>200\,\mathrm{mg/dl}$), and increased systolic ($>160\,\mathrm{mmHg}$) blood pressure, in women with diabetes, and in men with increased diastolic ($>95\,\mathrm{mmHg}$) blood pressure. Furthermore, there was a significant correlation between the number of IRS criteria and SF.

*Conclusions*: This study shows a significant correlation between SF and the presence of IRS criteria in a large representative population. Interestingly, the severity of the IRS seems to be associated with increased SF levels suggesting a causal connection. Further studies are required to investigate the pathophysiological mechanism and consequences of increased SF levels in patients with IRS.

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## Introduction

The insulin resistance syndrome (IRS) is a condition of increasing incidence in western countries (1). The metabolic syndrome is closely linked to insulin resistance and numerous studies indicate a link to hepatic iron overload. Increased serum ferritin, reflecting hepatic iron overload due to hemochromatosis or blood transfusions, is often associated with insulin resistance (2, 3). Also, patients with nonalcoholic steatohepatitis (NASH) are known to have an increased prevalence of the insulin resistance syndrome. Often these patients are obese and suffer from type 2 diabetes and hyperlipidemia (4–6). In 1997, Moirand *et al.* described a new syndrome with otherwise unexplained hepatic iron overload, increased serum ferritin and normal transferrin saturation (7). Ninety-five percent of these patients had additional metabolic disorders. Most of the above-mentioned studies focused on patients with proven hepatic iron overload, thereby using small numbers of patients and controls. However, population-based studies are needed to estimate the association between serum iron parameters and the IRS. Some population-based studies have been performed, showing an association between increased serum ferritin and serum alanine transaminase (8), essential hypertension (9) and myocardial infarction (10). Overall, data concerning the prevalence of potentially relevant iron overload in subjects selected for having metabolic syndrome are scanty. In a recent study, Bozzini *et al.* (11) reported increased prevalence of body iron excess in patients with the metabolic syndrome identified within the Verona Heart Project according to criteria defined by the Adult Treatment Panel III (ATP III) report (12). The aim of the present study was to determine the prevalence of the IRS using the criteria defined by Mendler *et al.* (13) and to elucidate the association between IRS and serum ferritin levels in a representative German population.

## Subjects and methods

### Study population

The subjects in this study had originally participated in the Diabetomobil study, a nationwide epidemiological survey on metabolic disorders in the adult German population. Streets and house numbers in cities as well as rural communities in five of the sixteen German states were randomly chosen, and all inhabitants were asked to participate in the survey. One thousand three hundred and six participants were investigated by a physician in a mobile survey unit. All participants filled out a questionnaire including medical history, current health status and personal data (marital status, education, occupation, household size). Sex, age, body mass index (BMI) and personal data were compared with statistical data on the total German population provided by the German Federal Statistics Office (Statistisches Bundesamt, Wiesbaden, Germany). The cohort revealed no significant difference with regards to these parameters as compared with the total German population, and was therefore considered representative (14, 15). All study subjects gave informed consent for the Diabetomobil study. The participants remained anonymous throughout the study.

### Physical examination

Body weight and height were measured, and BMI was calculated. Systolic and diastolic blood pressure (BP syst, BP diast) were measured using a sphygomanometer.

### Biochemical measurements

Blood samples were taken by venipuncture. Ferritin was measured in frozen serum samples by immunonephelometry (N Latex Ferritin; Dade Behring, Marburg, Germany). Cholesterol and high-density lipoprotein (HDL)-cholesterol were determined by dry chemistry (DT 60; Kodak, Stuttgart, Germany), and HbA1c was measured by an on-site immunoassay (DCA 2000; Bayer AG, Leverkusen, Germany), as previously described (16). Anti-hepatitis B core antigen (anti-HBc), hepatitis B surface (HBs)-antigen and anti-hepatitis C virus (anti-HCV) were tested by a fully automated microparticle enzyme immunoassay (Abbot GmbH, Wiesbaden-Delkenheim, Germany), as previously described (14, 15).

### Exclusion criteria

Complete data and laboratory analyses were available from 1200 of the 1306 participants. Subjects with excessive alcohol intake or hepatitis B or C were excluded from further analysis to eliminate major forms of hepatic iron overload. Participants were asked for their daily consumption of beer, wine or liquor in ml, and gram alcohol intake/day was calculated. From 1200 subjects, 46/571 women (8.1%) with a daily alcohol consumption >40 g/day and 68/629 (10.8%) men with a daily alcohol consumption >60 g/day were excluded, similar to previous, comparable studies (7). Blood samples of all participants were tested for anti-HBc (15); 76/1086 subjects (7.0%) were positive for anti-HBc and further tested for HBs-antigen. Four of these were positive (0.4%) and were excluded. In addition, all blood samples were tested for anti-HCV (14); 12/1086 samples were positive or borderline reactive (1.1%) and were excluded from further analysis.

### Diabetes prevalence

Participants were considered to have diabetes if they either reported a previous diagnosis of diabetes or had HbA1c values $\geq 6.5\%$. The latter was based on the Third National Health and Nutrition Examination Survey (NHANES III) with a sample of 23 258 subjects representative for the US population, showing that an HbA1c value above 3 standard deviations (6.5%) has a specificity of 99.6% for detecting undiagnosed diabetes (17). Diagnosis according to the WHO guidelines with repeated fasting glucose values >7 mmol/l (18) was not possible due to the study design. In our study, 57 of the 1070 (5.3%) probands reported previously diagnosed diabetes. Four of these had diabetes type 1 and 53 had diabetes type 2; 13 of the remaining 1013 probands had an HbA1c $\geq 6.5\%$ and were therefore considered as having diabetes. All of these were over the age of 30 and considered to have diabetes type 2. In total, the prevalence of diabetes was 6.5% (70/1070).

### Definition of insulin resistance syndrome

In accordance with previous reports (7, 13), elements of the insulin resistance syndrome were defined by the following conditions: BMI $>25\,\text{kg/m}^2$, diabetes, serum cholesterol >200 mg/dl, HDL-cholesterol $\leq 40$ mg/dl, BP syst >160 mmHg or BP diast >95 mmHg.

### Statistics

All statistical tests were calculated with SPSS software (SPSS for Windows, rel. 12.0.1 2003; SPSS Inc., Chicago, IL, USA). A *P* value of less than 0.05 was considered statistically significant. Descriptive data were presented as means and standard deviations for continuous data and percentages for categorical data. Differences in baseline characteristics were examined by the Mann-Whitney U-test for non-normal distributed data. Serum ferritin levels in different subgroups were depicted as boxplots. The top and bottom of

each box indicate the 25th and the 75th percentiles. The line through the box is the median, and the error bars are the 5th and 95th percentiles respectively. Significance levels determined by the Mann-Whitney U-test were indicated in the figures. In order to characterize the risk of probands with elevated serum ferritin values to have certain elements of the IRS, subjects with serum ferritin values higher than the 67. percentile (serum ferritin 126.2 µg/l) or the 95. percentile (serum ferritin 371.9 µg/l) were compared with those having ferritin values lower than the 67. percentile, and unadjusted as well as adjusted odds ratios were calculated by binary logistic regression with 95% confidence intervals. To assess the relationship between the number of IRS criteria and serum ferritin levels, a Kruskal-Wallis test was used.

## Results

Of the 1306 participants in the study, 1070 were eligible for further analysis. To eliminate major forms of hepatic iron overload, subjects with excessive alcohol intake or chronic viral hepatitis were excluded. The baseline characteristics of the study cohort are shown in Table 1. Age was distributed equally, and there were no significant differences in serum cholesterol, systolic blood pressure or diabetes prevalence due to gender. Women had a lower BMI, lower diastolic blood pressure, and higher HDL-cholesterol. As expected, serum ferritin was significantly lower in women. The distribution of serum ferritin in men and women was analyzed according to the previously defined components of the IRS, and graphically depicted with boxplots (Fig. 1A–F). Participants with a BMI $> 25 \, \text{kg/m}^2$ had significantly higher serum ferritin values, regardless of gender ($P < 0.001$ for women and men; Fig. 1A). Furthermore, women with diabetes had significantly higher ferritin values ($P < 0.001$), whereas no significant difference was observed for men ($P = 0.25$; Fig. 1B). Moreover, serum ferritin levels were significantly higher in probands with elevated serum cholesterol above 200 mg/dl than in those individuals with cholesterol under or equal to 200 mg/dl ($P < 0.001$ for women and men; Fig. 1C).

No significant changes in serum ferritin values in women or men with low HDL-cholesterol under or equal to 40 mg/dl compared with those with normal HDL-cholesterol were seen (Fig. 1D). Men and women with high BP syst ($> 160 \, \text{mmHg}$) had significantly higher serum ferritin values (women: $P = 0.018$; men: $P = 0.001$; Fig. 1E). Men with high BP diast ($> 95 \, \text{mmHg}$) had significantly higher serum ferritin values ($P = 0.011$), whereas there was only a trend towards higher serum ferritin values in women with high BP diast ($P = 0.087$; Fig. 1F).

Interestingly, probands already diagnosed with hypertension but with normal blood pressure at the physical examination also had significantly elevated serum ferritin levels (data not shown).

Logistic regression analyses were performed to determine the odds ratios for probands with serum ferritin values higher than the 67. percentile (353 probands) and 95. percentile (53 probands) respectively. The odds ratios for diabetes were 2.6 (67. percentile; $P < 0.001$) and 4.4 (95. percentile; $P < 0.001$). For BMI $> 25 \, \text{kg/m}^2$, odds ratios were 2.5 for the 67. percentile and 4.0 for the 95. percentile ($P < 0.001$ each). Cholesterol values $> 200 \, \text{mg/dl}$ had odds ratios of 2.0 (67. percentile; $P < 0.001$) and 1.9 (95. percentile; $P < 0.05$). HDL cholesterol $> 40 \, \text{mg/dl}$ showed odds ratios of 0.5 for the 67. percentile and 0.2 for the 95. percentile ($P < 0.001$ each). Hypertension with increased systolic BP $> 160 \, \text{mmHg}$ was associated with an odds ratio of 2.5 ($P < 0.001$) and 3.9 ($P < 0.005$) for the 67. and 95. percentile respectively. Increased diastolic BP $> 95 \, \text{mmHg}$ had odds ratios of 2.0 (67. percentile) and 3.7 (95. percentile) ($P < 0.001$ each). After adjustment for all other parameters tested, odds ratios for diabetes, BMI and HDL-cholesterol remained significant, indicating independent risk factors for elevated serum ferritin values (Fig. 2). The adjusted odds ratio for hypercholesterolemia was also significantly increased in the group with serum ferritin values higher than the 67. percentile, but lost significance in the group higher than the 95. percentile. Conversely, the adjusted odds ratio for increased diastolic BP was only significant in the group with serum ferritin values higher than the 95. percentile (Fig. 2).

**Table 1** Characteristics of the study cohort (mean values±standard deviation).

|  | Women | Men | Total | *P* value |
|---|---|---|---|---|
| Number | 516 (48.2%) | 554 (51.8%) | 1070 (100%) | |
| Age (years) | 44.5±14.6 | 45.1±14.1 | 44.8±14.4 | 0.49 |
| BMI (kg/m²) | 24.2±4.4 | 25.6±3.4 | 24.9±4.0 | <0.01 |
| Cholesterol (mg/dl) | 208.8±46.1 | 204.8±46.4 | 206.8±46.2 | 0.28 |
| HDL-cholesterol (mg/dl) | 67.2±16.7 | 53.0±15.9 | 59.9±17.8 | <0.01 |
| BP syst (mmHg) | 129.5±23.3 | 134.5±20.8 | 132.9±19.4 | 0.28 |
| BP diast (mmHg) | 84.0±12.7 | 86.5±12.1 | 85.8±10.4 | <0.05 |
| Diabetes prevalence | 5.6% | 7.4% | 6.5% | 0.24 |
| Ferritin (µg/l) | 70.3±72.2 | 166.6±142.9 | 120.2±124.0 | <0.01 |
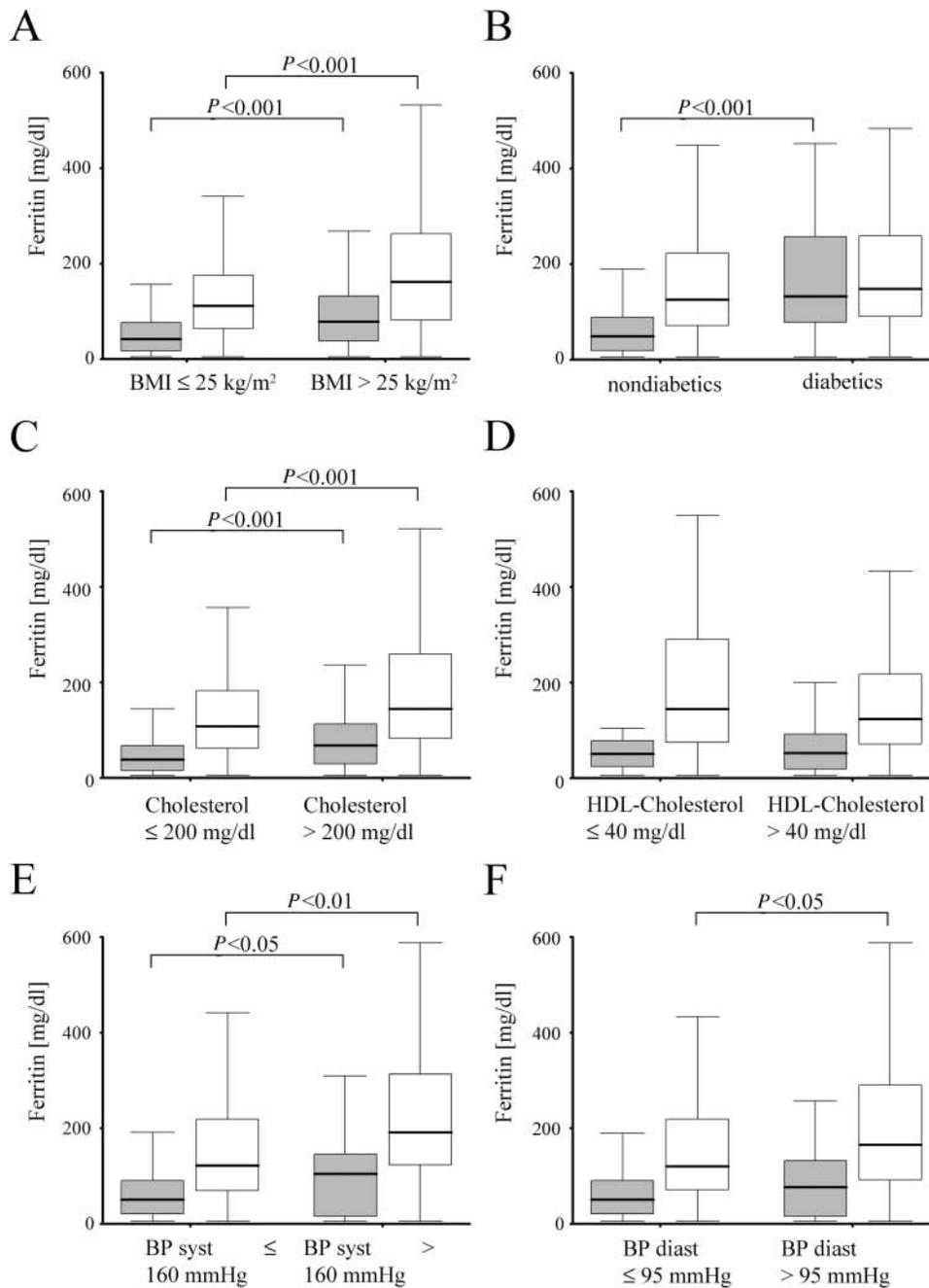
**Figure 1** Serum ferritin levels in male (white boxes) and female (grey boxes) individuals discriminated according to the presence or absence of defining criteria of the insulin resistance syndrome (A–F). Results are depicted as boxplots. The top and bottom of each box indicate the 25th and the 75th percentiles. The line through the box is the median, and the error bars are the 5th and 95th percentiles. Significance levels were determined by the Mann–Whitney U-test and are indicated in the figure.

Usually, the metabolic syndrome is characterized by the combination of several elements of the IRS. In our study, 302 probands (28.2%) had no IRS criteria (median serum ferritin 52.5 µg/l, 25. and 75. percentiles 23.8 µg/l and 100.0 µg/l respectively), 382 probands (35.7%) had 1 IRS criterion (median ferritin: 78.0 µg/l, 25. percentile: 37.0 µg/l, 75. percentile: 143.0 µg/l), 250 subjects (23.4%) had 2 IRS criteria (median serum ferritin 113.5 µg/l, 25. and 75. percentiles 59.8 µg/l and 211.3 µg/l respectively), 91 subjects (8.5%) had 3 IRS criteria (median ferritin 142.0 µg/l;

63.0 µg/l and 253.0 µg/l for the 25. and 75. percentiles respectively), and 45 probands (4.2%) had 4 or more IRS criteria (median serum ferritin 147.0 µg/l, 25. and 75. percentiles 92.5 µg/l and 316.5 µg/l respectively) (Fig. 3). The number of IRS elements present correlated significantly with serum ferritin values ($P < 0.001$; Kruskal-Wallis test). Notably, 76% of the probands had at least two elements of the IRS. The correlation between the number of IRS elements and serum ferritin remained significant if women and men were analyzed separately ($P < 0.001$ for women and
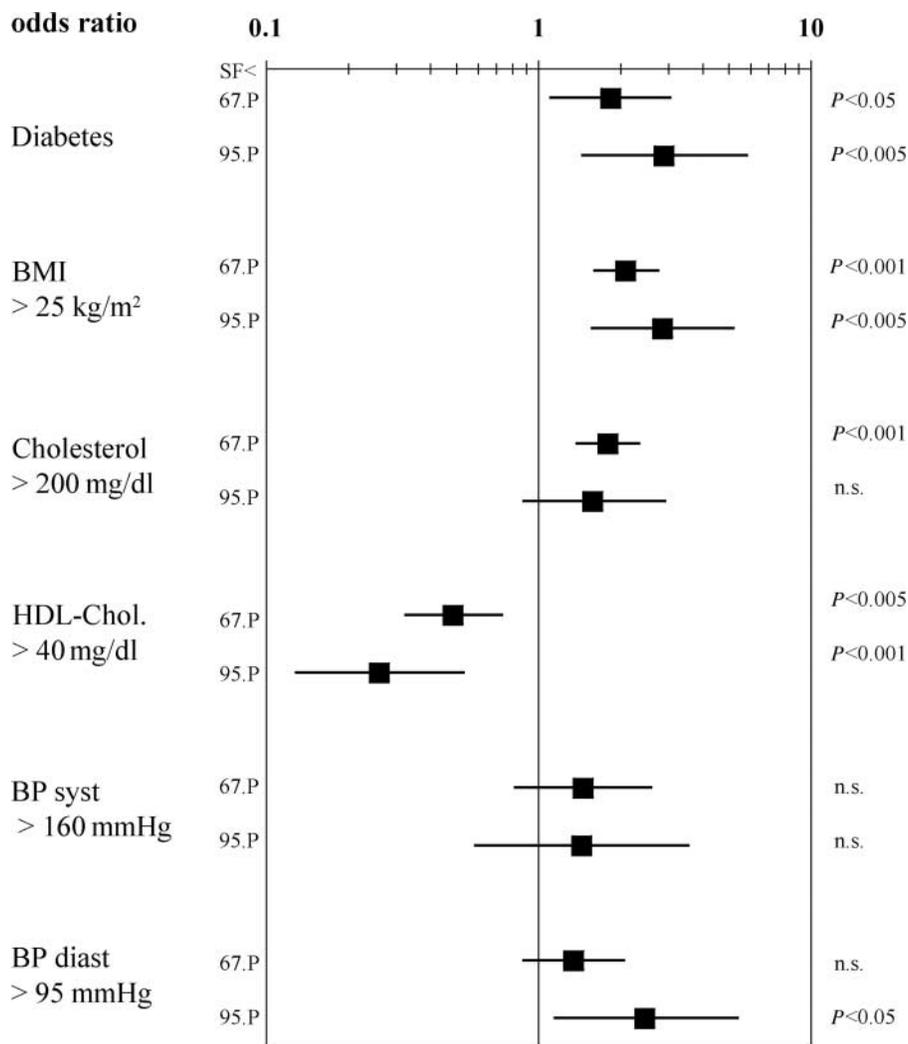
**Figure 2** Odds ratios for the components of the insulin resistance syndrome for serum ferritin values higher than the 67. percentile (67.P) and the 95. percentile (95.P) respectively. The odds ratios were adjusted for diabetes, BMI, cholesterol, HDL-cholesterol (HDL-Chol.), systolic blood pressure (BP syst) and diastolic blood pressure (BP diast) respectively. Significance levels are shown on the right; n.s., not significant.

men respectively; Table 2). Menopausal status cannot be excluded as a confounding variable and might have weakened the association between the components of the IRS and serum ferritin levels in the group of older women. However, the trend that the number of IRS elements was increasing with age did not differ between women and men (data not shown).

## Discussion

Increased serum ferritin has been associated with a variety of conditions that contribute to the metabolic syndrome. In this study representing the adult German population, we found significant associations between increased serum ferritin and diabetes, BMI, hypercholesterolemia and hypertension respectively. Using these parameters to define the insulin resistance syndrome, Mendler *et al.* (13) found that 94% of their patients with hepatic iron overload fulfilled at least

one of these criteria. In our study, 72% met at least one and 36% at least two of these criteria. Assuming a pathophysiological link between insulin resistance and iron overload, the high prevalence of the IRS defined in this way suggests the existence of additional manifestation factors because otherwise the majority of the population would be at risk for hepatic iron overload. Recently, criteria for diagnosing the IRS have been published in the Adult Treatment Panel III (ATP III) report (12) and by the WHO (18). The ATP III defined IRS by the existence of at least 3 of the following: increased waist circumference, increased triglycerides, low HDL-cholesterol, increased blood pressure, and increased blood glucose (1). In our study, only 12.7% of the population fulfilled 3 or more criteria of the IRS. This is most likely due to the differences in the definition of the criteria. For example, it has to be considered that the higher cut-offs of arterial blood pressure used as IRS criteria in the present study may account for the lower prevalence found. However, a lower prevalence of the IRS in Germany compared
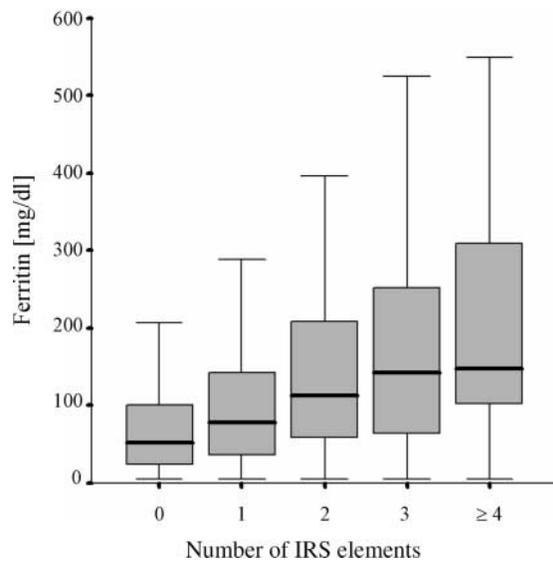
**Figure 3** Correlation between serum ferritin values and the number of defining criteria of the insulin resistance syndrome. Results are depicted as boxplots. The top and bottom of each box indicate the 25th and the 75th percentiles. The line through the box is the median, and the error bars are the 5th and 95th percentiles. The number of IRS elements increased significantly with serum ferritin values ($P < 0.01$; Kruskal–Wallis test).

with the US cannot be excluded. The association between serum ferritin and diabetes has previously been addressed by population-based studies. In 1013 middle-aged Finnish men (19), increased serum ferritin was associated with elevated fasting blood glucose and serum insulin. In the NHANES III survey, increased serum ferritin was associated with newly and previously diagnosed diabetes (8). In the study by Tuomainen, the percentage of men and women with diabetes and elevated serum ferritin was about 40%, compared with 13% without diabetes (19). Interestingly, our study revealed quite similar numbers for women with elevated serum ferritin values (41% with and 8% without diabetes), but different percentages for men (22% with and 14% without diabetes). In our study, increased blood pressure was also significantly associated with increased serum ferritin. This finding supports previous data from Piperno *et al.*, who found increased serum ferritin values in patients

with essential hypertension (9). Hypertension, together with other elements of the insulin resistance syndrome, may be responsible for the higher risk of patients with lower transferrin receptor/serum ferritin ratios for acute myocardial infarction (20, 21). However, since most of the odds ratios of hypertensive probands for increased serum ferritin values did not remain significant in our study after correction for all other features of the IRS, the effect of hypertension on serum ferritin may be secondary. Similarly, a recently published study revealed an association between serum ferritin and the metabolic syndrome in US adults, whereas the association between serum ferritin and hypertension did not remain significant after correction for other factors of the metabolic syndrome (22). The pathophysiological mechanism of increased serum ferritin in patients with IRS is unclear. In our study, the severity of the IRS was associated with increased ferritin levels, thereby indicating a causal connection. Iron removal by phlebotomy has been shown to alleviate fatigue and arthralgias in patients with insulin resistance-associated iron overload (23), and to improve insulin sensitivity in patients with diabetes (24), thereby pointing towards causality. However, studies investigating histological liver iron overload in patients with NASH failed to show consistent results (25–27). Despite the fact that serum ferritin values did not differ significantly between patients reporting an infection within the last 12 months and those who did not (data not shown), we cannot rule out the possibility that increased serum ferritin is secondary to subclinical infections or other causes of inflammation, especially since C-reactive protein (CRP) has been shown to be associated with diabetes, increased BMI (28), fasting glucose and insulin (29). However, in recent studies investigating healthy probands, serum ferritin remained positively correlated with blood glucose and insulin resistance (30) or with the metabolic syndrome (22) after adjustment for CRP.

To eliminate major forms of hepatic iron overload, subjects with excessive alcohol consumption were excluded, similar to previous, comparable studies (7). Women reporting moderate alcohol consumption had similar serum ferritin levels as those reporting no alcohol intake, while men reporting moderate alcohol consumption had slightly higher serum ferritin levels than

**Table 2** Insulin resistance syndrome and ferritin values for women and men.

| | Women | | | Men | | |
|---|---|---|---|---|---|---|
| No. of IRS elements | Number | % | Median ferritin μg/l (interquartile range) | Number | % | Median ferritin μg/l (interquartile range) |
| 0 | 172 | 33.3 | 33.0 (41.5) | 130 | 23.5 | 102.5 (86.3) |
| 1 | 204 | 39.5 | 56.0 (67.8) | 178 | 32.1 | 115.0 (161.5) |
| 2 | 89 | 17.2 | 79.0 (98.5) | 161 | 29.1 | 142.0 (179.5) |
| 3 | 31 | 6.0 | 102.0 (96.0) | 60 | 10.8 | 192.0 (188.3) |
| ≥4 | 20 | 3.9 | 103.5 (192.0) | 25 | 4.5 | 213.0 (261.0) |

those reporting no alcohol consumption. However, these slight differences seen in men did not affect the results found in the current study (data not shown). Furthermore, it has to be considered that hereditary hemochromatosis was the cause of hyperferritinemia in some subjects analyzed, since it is predisposing also to diabetes. However, the hemochromatosis genotype was available in a subgroup of the study population (124 of 1070 subjects) and here, serum ferritin levels did not differ significantly between C282Y heterozygous and wild-type patients (data not shown). Further, based on the published prevalence of C282Y homozygosity in Germany (less than 1%) and in accordance with the results of genotyping in the above mentioned subgroup of patients (only 1 of 124 subjects was homozygous for the C282Y mutation), it can be assumed that the results obtained in our study are not significantly influenced by the prevalence of hereditary hemochromatosis.

In conclusion, we have shown that serum ferritin is positively correlated with elements of the insulin resistance syndrome in a representative German population. Further studies are required to investigate the pathophysiological mechanism of increased ferritin levels in patients with IRS. Eventually, increased ferritin levels reflecting hepatic iron overload may provide both a potential pathophysiological link and a prognostic marker for hepatic steatosis and steatohepatitis, histological findings frequently seen in patients with IRS.

## Acknowledgements

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