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Increased Serum Ferritin Concentrations and Liver Enzyme Activities in Patients with Metabolic Syndrome

VASILIS TSIMIHODIMOS, M.D.,[1] IRENE GAZI, M.D.,[1] RIGAS KALAITZIDIS, M.D.,[2] MOSES ELISAF, M.D.,[1] and KOSTANTINOS C. SIAMOPOULOS, M.Sc., M.D.[2]

ABSTRACT

Emerging scientific evidence suggests that increases in body iron represent a risk factor for the development of metabolic syndrome and diabetes. The aim of our study was to determine the body iron stores in patients with metabolic syndrome, and to evaluate the potential relationship of iron overload with specific features of the metabolic syndrome, such as fatty liver. A total of 490 individuals were enrolled. The diagnosis of metabolic syndrome was based on National Cholesterol Education Program–Adult Treatment Panel III (ATPIII) criteria. The metabolic syndrome group was consisted of 185 patients having three or more criteria, whereas individuals with less than three criteria constituted the control group. Metabolic syndrome patients displayed higher ferritin concentration as compared to control individuals. Ferritin levels were positively correlated with insulin concentration, as well as with Homeostasis Model Assessment (HOMA) index values. Multiple regression analysis revealed that ferritin was the most important independent determinant of insulin resistance indices. Patients with metabolic syndrome also exhibited increased concentrations of alanine aminotransferase and γ-glutamyltranspeptidase compared to controls. Multiple regression analysis revealed that ferritin concentration was the most important determinant of γ-glutamyltranspeptidase levels. Patients with the metabolic syndrome exhibit an increase in body iron stores as well as elevated concentrations of liver enzymes compared to the individuals who do not fulfill the criteria for the diagnosis of this syndrome. Our data support a direct role of increased body iron in the pathogenesis of insulin resistance, whereas iron overload may also contribute to the development of specific features of the metabolic syndrome, such as fatty liver.

INTRODUCTION

THE RELATIONSHIP BETWEEN BODY iron stores and carbohydrate metabolism has been well established during previous years. The most representative example of this association is probably hemochromatosis. It is well known that this disorder, which is characterized by extreme body iron overload, represents the most important cause of secondary diabetes mellitus.[1] However, apart from hemochromatosis, it has been proposed that even milder increases in body iron content may adversely affect carbohydrate metabolism and may predispose to the development of diabetes mellitus.[2–4] Although the pathophysiological basis of this association

Departments of [1]Internal Medicine and [2]Nephrology, Medical School, University of Ioannina, Ioannina, Greece.

is not clear, it has been suggested that iron overload, by inducing the formation of reactive oxygen species, may initially interfere with the peripheral utilization of glucose, whereas in a subsequent step it may reduce the production of insulin by the pancreatic beta cells.[5-8]

Metabolic syndrome represents a cluster of cardiovascular risk factors that recently has become a public health problem of epidemic proportions. Individuals with this syndrome are at an increased risk for the subsequent development of cardiovascular events and diabetes mellitus and recently published guidelines stress the importance of targeting preventive and therapeutic strategies for this patient population.[9] Although the pathogenesis of the metabolic syndrome remains indeterminate, it is widely accepted that insulin resistance represents a key feature of this syndrome.[10] Based on the assumption that iron overload may induce or exacerbate insulin resistance, previous studies tried to delineate the relationship between body iron stores and the risk for the development of metabolic syndrome.[11,12] These studies clearly showed that the indices of insulin resistance are significantly correlated with the serum concentrations of ferritin and that individuals with iron overload may exhibit an increased risk for the subsequent development of metabolic syndrome.[11,12] However, other studies failed to find an association between iron stores and glycaemic control or the presence of type 2 diabetes.[13,14]

Non-alcoholic fatty liver disease (NAFLD) comprises a wide spectrum of liver disorders that range from simple hepatic steatosis to non-alcoholic steatohepatitis and cirrhosis.[15] Although the pathogenesis of this disorder has not been clearly determined, its strong association with the features of metabolic syndrome supports the conception that NAFLD is probably the hepatic manifestation of this syndrome.[16] Clinical and epidemiological studies revealed that insulin resistance is the major determinant of the incidence and the severity of NAFLD.[15] In addition, since iron is toxic for hepatocytes, one could expect that the increased body iron content observed in patients with disturbed carbohydrate metabolism may directly or indirectly (via the induction of insulin resistance) contribute to liver dysfunction. However, so far, the relevance of serum iron levels in the pathogenesis of NAFLD remains a subject of discussion.[17-20]

The aim of our study was to determine the serum concentrations of ferritin (one of the most reliable markers of body iron stores) in Greek patients with metabolic syndrome defined according to the criteria proposed by the National Cholesterol Education Program (Adult Treatment Panel III) guidelines. In addition, in an effort to determine the relative contribution of body iron levels in the pathogenesis of NAFLD, the correlation of ferritin levels with certain biochemical surrogate markers of NAFLD (serum concentrations of aminotransferases and gamma-glutamyl transpeptidase [GGT]) was also examined.

METHODS

Patients

Five hundred twenty unrelated patients examined at the Outpatient Clinics of the University Hospital of Ioannina for suspected metabolic disorders were included in the study. The diagnosis of metabolic syndrome was made according to the National Cholesterol Education Program–Adult Treatment Panel III criteria.[9] Thus, the metabolic syndrome group was consisted of patients having three or more criteria, whereas participants with less than three criteria were served as controls. None of the study individuals had any clinical or electrocardiographic evidence of coronary heart disease. Patients who were found to be diabetic (fasting glucose values greater than 126 mg/dL) or receiving medications known to interfere with lipids, glucose, or iron metabolism were excluded from the study. In addition, participants with a history of blood donation or a diagnosis of anemia during the previous 6 months as well as women of child bearing potential who had a positive pregnancy test were also excluded. The presence of hereditary hemochromatosis was excluded by appropriate genetic analyses. To avoid the potential confounding effect of

the presence of subclinical inflammatory or infectious diseases on iron metabolism indices, data from patients with C-reactive protein (CRP) values greater than 10 mg/dL or elevated white blood cells counts (greater than 11,000/μL) as well as from individuals with a history of a febrile illness during the previous 3 months were not included in the analysis. Study participants were also excluded if they had a history of liver disease of if they were positive for viral hepatitis tests (A, B, and C) as well as for anti-mitochondrial, anti-nuclear or anti-smooth muscle antibodies. Finally, subjects consuming more than 30 g of alcohol per week were also excluded from the study. Blood pressure was measured in duplicate after 5 min of rest with the patients in the sitting position, and the mean values were used in the analysis. Waist circumference was measured midway between the last rib and iliac crest by an experienced nurse. Blood and urine samples were taken after a 12-h overnight fast, while study participants were on their regular diet. All study participants gave their written informed consent prior to their enrolment in the study. The study protocol was approved by the scientific committee of the University Hospital of Ioannina.

Procedures

Blood samples were obtained in the morning after an overnight fast. Serum was isolated by centrifugation at 1500*g* for 15 min. Serum parameters were measured on an Olympus AU600 Clinical Chemistry analyzer (Olympus Diagnostica, Hamburg, Germany). Concentrations of total cholesterol and triglycerides were determined enzymatically and high-density lipoprotein (HDL)–cholesterol was determined by a direct assay (Olympus Diagnostica). Serum low-density lipoprotein (LDL)–cholesterol was calculated using the Friedewald formula, provided that the concentration of triglycerides was lower than 400 mg/dL. In patients with serum triglyceride values greater than 400 mg/dL (n = 12) LDL-cholesterol concentrations were not determined. Ferritin levels were determined by Immunoassay on an ARCHITECT analyzer (Abbott GmbH Diagnostika, Wiesbaden-Delkenheim, Germany). Insulin levels were determined by a microparticle enzyme immunoassay on an AXSYM analyzer (Abbott GmbH Diagnostika). Homeostasis Model Assessment (HOMA) index was calculated using the formula: serum insulin (mU/mL) × plasma glucose (mmol/l)/ 22.5. High sensitivity–CRP (hs-CRP) levels were determined as previously described.[21] The two most common HFE gene mutations (C282Y and H63D) were excluded by appropriate genetic analyses.[22]

Statistical analysis

Data represent mean ± SD. An unpaired t-test was used for comparisons between study groups, while differences in proportions were assessed with χ^2 test. The distribution of ferritin values was checked for normality with Kolmogorov-Smirnov test. Since ferritin values in our study population were found to follow a normal distribution no log-transformation was applied. Correlations between ferritin and γ-glutamyltranspeptidase concentrations and other metabolic parameters were estimated using linear regression analysis, whereas multiple regression analysis was used for the multivariate assessment of the correlations between those variables. Finally, one-way analysis of variance (ANOVA) as well analysis of covariance (ANCOVA) were used for the comparisons of ferritin and γ-glutamyltranspeptidase concentrations between individuals with different numbers of the components of metabolic syndrome.

RESULTS

The clinical characteristics of the study participants are shown in Table 1. There were no differences in the age and sex distribution between study groups. By contrast, patients with metabolic syndrome had significantly higher waist circumference values, Body Mass Index (BMI), and waist-to-hip ratio as well as elevated blood pressure readings (for both systolic and diastolic blood pressure) compared to control individuals.

The biochemical characteristics of the study population are displayed in Table 2. Patients

TABLE 1. CLINICAL CHARACTERISTICS OF THE STUDY POPULATION

	Metabolic syndrome	*Controls*	*p*
Number	185	305	
Sex (male/female)	78/107	146/159	NS
Age (years)	52.6 ± 11.1	51.5 ± 10.5	NS
Waist circumference (cm)	107.5 ± 13.2	90.2 ± 12.3	<0.001
BMI (kg/m^2)	29.1 ± 3.4	25.3 ± 3.5	<0.001
Waist-to-hip ratio	0.94 ± 0.07	0.87 ± 0.09	<0.001
SBP (mm Hg)	149.6 ± 18.2	131.5 ± 23.1	<0.001
DBP (mm Hg)	93.1 ± 9.6	82.5 ± 13.3	<0.001

Values represent mean ± SD. An unpaired *t*-test was used for comparisons between groups. Differences in proportions were assessed by χ^2 test. A *p* value less than 0.05 was considered significant.

BMI, Body Mass Index; SBP, systolic blood pressure; DBP, diastolic blood pressure; NS, nonsignificant.

TABLE 2. BIOCHEMICAL CHARACTERISTICS OF THE STUDY POPULATION

	Metabolic syndrome	*Controls*	*p*
Glucose (mg/dL)	106 ± 24	94 ± 17	<0.001
Insulin (μU/mL)	14.5 ± 7.7	9.1 ± 5.4	<0.001
HOMA index	3.9 ± 2.6	2.1 ± 1.4	<0.001
Total cholesterol (mg/dL)	238 ± 47	225 ± 39	<0.001
Triglycerides (mg/dL)	196 ± 93	112 ± 66	<0.001
HDL-cholesterol (mg/dL)	44 ± 11	53 ± 12	<0.001
LDL-cholesterol (mg/dL)	156 ± 42	150 ± 34	NS
Ferritin (ng/mL)	84.1 ± 76.9	63.3 ± 58.6	<0.001
GGT (IU/L)	29.3 ± 24.6	22.1 ± 16.3	<0.001
AST (IU/L)	22.5 ± 7.8	21.8 ± 8.1	NS
ALT (IU/L)	28.5 ± 16.5	23.9 ± 12.7	<0.001
AST/ALT	0.89 ± 0.29	1.04 ± 0.41	<0.001
hs-CRP (mg/L)	3.12 ± 1.62	1.9 ± 1.2	<0.001

Values represent mean ± SD. An unpaired *t*-test was used for comparisons between groups, and a *p* value of less than 0.05 was considered significant.

HOMA, Homeostasis Model Assessment; HDL, high-density lipoprotein; LDL, low-density lipoprotein; GGT, γ-glutamyltranspeptidase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; hs-CRP, high sensitivity–C reactive protein; NS, nonsignificant.

TABLE 3. UNIVARIATE ASSESSMENT OF THE CORRELATIONS BETWEEN SERUM FERRITIN CONCENTRATIONS AND VARIOUS METABOLIC PARAMETERS

	Ferritin	
	r	*p*
BMI	0.19	<0.001
Waist circumference	0.35	<0001
Insulin	0.20	<0.005
HOMA index	0.22	<0.001
Triglycerides	0.36	<0.001
HDL-cholesterol	−0.30	<0.001
hs-CRP	0.03	NS

BMI, body mass index; HOMA, Homeostasis Model Assessment; HDL, high-density lipoprotein; hs-CRP, high sensitivity–C reactive protein; NS, nonsignificant.

with metabolic syndrome had higher fasting glucose and insulin concentration as well as elevated values of the HOMA index compared to individuals who do not fulfil the criteria for the diagnosis of this syndrome. In addition, patients with metabolic syndrome exhibited an adverse lipid profile characterised by elevated concentrations of total cholesterol and triglycerides as well as by decreased concentrations of HDL-cholesterol. On the contrary, the concentrations of LDL-cholesterol were similar in both study groups. In addition, the metabolic syndrome group displayed higher concentrations of serum ferritin as well as ele-

vated values of the γ-glutamyltranspeptidase and alanine aminotransferase compared to control population (Table 2). On the contrary, the concentrations of aspartate aminotransferase did not differ significantly between study groups. As a consequence, the ratio of aspartate to alanine aminotransferase was significantly lower in patients with metabolic syndrome. Finally, patients with metabolic syndrome displayed higher hs-CRP values compared to individuals who do not fulfill the criteria for the diagnosis of this syndrome.

Linear regression analysis showed that ferritin levels were positively correlated with waist circumference, fasting insulin and HOMA index values as well as with fasting triglycerides. In addition a significant negative correlation between ferritin and HDL-cholesterol levels was also observed. It must be noted that no significant correlation was observed between serum ferritin and hs-CRP values (Table 3). The same correlations were also observed after subdividing the study individuals into the control and metabolic syndrome groups (data not shown). Figure 1 displays the serum concentrations of ferritin after study participants were subdivided according to their total number of the components (criteria) of the metabolic syndrome. As shown there was a strong linear increase in serum ferritin levels as the number of the components of the metabolic syndrome increased. The same results were also obtained after adjustment for potential confounders, such as age and sex (data not shown). Multivariate analysis revealed that serum ferritin concentrations were predictive of fasting insulin concentrations and HOMA index values after adjustment for other variables, such as age, sex, blood pressure readings and lipid values (beta values 0.15 and 0.18, respectively; $p < 0.001$ for both correlations; Table 4). Serum γ-glutamyltranspeptidase concentrations were also positively correlated with the number of the components of metabolic syndrome (p for trend was <0.001; data not shown). Furthermore, γ-glutamyltranspeptidase was positively correlated with blood pressure values (both systolic and diastolic), fasting insulin and waist circumference values, total cholesterol and triglyceride concentrations as well as with serum ferritin. In addition, a negative correlation with HDL-cholesterol levels was also observed (Table 5). The same correlations were also observed after subdividing the study individuals into the control and metabolic syndrome groups (data not shown). Multiple regression analysis included all the previously mentioned variables revealed that serum ferritin was the most important determinant of serum γ-glutamyltranspeptidase concentrations (beta 0.359; $p < 0.001$), whereas HOMA index values was also a significant predictor (beta 0.143; $p < 0.01$; (Table 6).

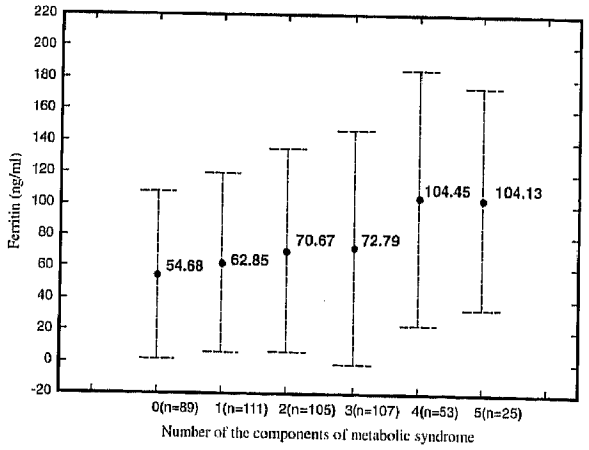

FIG. 1. Serum ferritin concentrations in individuals with different numbers of the components of metabolic syndrome. Values represent mean ± standard deviation. Differences between groups were assessed by one-way analysis of variance (ANOVA). The p value for trend was <0.001.

TABLE 4. MULTIVARIATE ASSESSMENT OF THE CORRELATIONS BETWEEN HOMA INDEX VALUES AND VARIOUS METABOLIC PARAMETERS

	HOMA	
	Beta	*p*
Ferritin	0.18	<0.001
Age	0.04	NS
Sex	0.09	NS
Waist circumference	0.20	<0.001
SBP	0.02	NS
DBP	0.10	NS
Triglycerides	0.12	NS
HDL-cholesterol	−0.14	<0.05

HOMA, Homeostasis Model Assessment; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; NS, nonsignificant.

TABLE 5. UNIVARIATE ASSESSMENT OF THE CORRELATIONS BETWEEN γ-GLUTAMYLTRANSPEPTIDASE (GGT) CONCENTRATIONS AND VARIOUS METABOLIC PARAMETERS

	GGT	
	r	*p*
BMI	0.20	<0.001
Waist circumference	0.28	<0001
Insulin	0.39	<0.005
HOMA index	0.35	<0.001
Triglycerides	0.30	<0.001
HDL-cholesterol	−0.29	<0.001
SBP	0.19	<0.001
DBP	0.26	<0.001
Ferritin	0.47	<0.001

BMI, body mass index; HOMA, Homeostasis Model Assessment; HDL, high-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure.

DISCUSSION

Previous studies have shown that body iron stores are closely related to carbohydrate metabolism and that individuals with elevated body iron content exhibit an increased risk for the subsequent development of diabetes mellitus.[2,4] The results of our study show that serum ferritin concentrations are significantly correlated with the surrogate markers of insulin resistance (such as fasting insulin levels and HOMA index values) and thus they support those previous studies. In addition, our data indicate that patients with metabolic syndrome display indications of liver dysfunction that is probably related to the presence of NAFLD. Multivariate analysis confirmed the results of previously published studies since it showed that insulin resistance is one of the most important determinants of the development of this disorder.[19,23] However, in our patients serum ferritin levers were also significantly associated with the serum concentrations of liver enzymes, thus indicating that body iron stores may also be implicated in the pathogenesis of NAFLD. Alternatively, serum ferritin may represent a marker of the clustering of multiple metabolic ablormalities in patients with metabolic syndrome, thus supporting the role of altered metabolic status in the pathogenesis of NAFLD.

TABLE 6. MULTIVARIATE ASSESSMENT OF THE CORRELATIONS BETWEEN γ-GLUTAMYLTRANSPEPTIDASE (GGT) VALUES AND VARIOUS METABOLIC PARAMETERS

	GGT	
	Beta	*p*
Ferritin	0.36	<0.001
HOMA index	0.14	<0.010
Age	0.01	NS
Sex	0.02	NS
Waist circumference	0.01	NS
SBP	0.03	NS
DBP	0.08	NS
Triglycerides	0.12	NS
HDL-cholesterol	−0.12	NS

HOMA, homeostasis model assessment; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; NS, nonsignificant.

One of the most important limitations of our study as well as of the previous studies that tested the impact of serum iron stores[11] on the risk for the development of metabolic syndrome is their cross-sectional design which does not allow the assessment of causality. Thus, it is not known whether the elevations of the body iron stores precede and contribute to the development of insulin resistance or if they merely represent a consequence of the clustering of multiple metabolic abnormalities, since previous studies have shown that hyperinsulinemia may boost intestinal iron absorption.[24] Although it is clear that prospective studies are needed to delineate this issue, the results of previously published research are suggestive of the former mechanism. Indeed, prospective studies have shown that iron overload is associated with an increased risk of diabetes.[2,4] Since the metabolic syndrome usually precedes the development of diabetes in most cases, it could be assumed that the increased body iron burden may increase the risk of diabetes mellitus by inducing the development of metabolic syndrome. Once insulin resistance has been established, hyperinsulinemia may induce the absorption of iron thus leading to the development of a vicious circle that ultimately results in diabetes mellitus. Another piece of evidence supporting the above mechanism comes from studies showing that blood donation, which resulted in a significant iron removal, substantially improved insulin sensitivity in diabetic patients[25] and healthy individuals.[26] The same results were also obtained after the administration of iron chelators, which

resulted in an improvement of the metabolic control in patients with diabetes mellitus.[27]

Serum ferritin is a widely used marker of body iron stores.[28] Nevertheless, it is well known that ferritin concentrations may also be increased in inflammatory conditions. Thus, in an effort to minimize the potential confounding effect of inflammation on body iron indices, we excluded from the current study the individuals with elevated C-reactive protein and white blood cell values. Previous studies have shown that patients with metabolic syndrome exhibit a subclinical inflammation characterized by low level increments of C-reactive protein that can not be detected with the conventional methods.[29] However, the lack of any correlation between high sensitivity CRP and serum ferritin levels in our patients argues against subclinical inflammation as an important source of the elevation of ferritin values in our study. Recently published studies in patients with established NAFLD indicate that serum ferritin may not accurately reflect the body and liver iron stores in this patient group.[12] Thus the use of ferritin as a surrogate marker of body iron status possibly represents a potential limitation of our study. In addition, the assessment of insulin resistance in our patients with the use of surrogate markers (such as fasting insulin and HOMA index values) and not by a clamp technique may also represent an important limitation.

Our data indicates that patients with metabolic syndrome also exhibit increased concentrations of alanine aminotransferase and γ-glutamyltranspeptidase compared to individuals who do not fulfill the criteria for the diagnosis of this syndrome. Since the most common causes of liver dysfunction had been excluded during the initial screening, it can be assumed that the elevated concentrations of liver enzymes were due to NAFLD, a condition that is very common in this patient population.[15] Although the importance of insulin resistance in the development and progression of this disease has been clearly established,[15] the role of increased body iron stores in the pathogenesis of NAFLD remains controversial.[17–20] However, our results clearly show that, at least in our patients, ferritin levels were the most important determinant of γ-glutamyltranspeptidase concentration, thus indirectly supporting the potential implication of increased iron stores in the pathophysiology of fatty liver disease. Our findings are also in line with previous studies showing that γ-glutamyltransferase levels were associated with red meat consumption and dietary heme iron.[30] Previous studies in patients with established NAFLD suggested that serum ferritin is not a reliable marker of body iron status nor it accurately reflects the liver iron content.[17] In this case, the increased ferritin concentrations in patients with metabolic syndrome may reflect the clustering of multiple metabolic abnormalities which, in turn, may predispose to the development of NAFLD.

Recently published studies have shown that except for its value as a surrogate marker of NAFLD, γ-glutamyltranspeptidase may also represent an important risk factor for cardiovascular disease.[31] Thus, the increased γ-glutamyltranspeptidase levels in patients with the metabolic syndrome may also explain, at least in part, the increased cardiovascular morbidity and mortality that has been observed in this patient population.

In conclusion, patients with metabolic syndrome defined according to NCEP criteria display increased iron stores and elevated liver enzyme concentrations as compared to individuals that do not fulfill the criteria for the diagnosis of this syndrome. Our data revealed a significant association between serum ferritin concentrations and insulin resistance indices thus indirectly supporting the potential implication of increased body iron in the pathogenesis of this condition. In addition, the disturbances in iron metabolism observed in patients with this syndrome may also contribute to the development of NAFLD. Further prospective studies are needed to delineate the role of the increased iron burden in the pathophysiology of the metabolic syndrome as well as to determine the dietary factors that may predispose to this increase.

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Address reprint requests to:
Dr. Moses Elisaf
Department of Internal Medicine
Medical School
University of Ioannina
45110 Ioannina, Greece

E-mail: egepi@cc.uoi.gr