

Serum Ferritin Is Differentially Associated with Anti-oxidative Status and Insulin Resistance in Healthy Obese and Non-obese Women

Original
Article

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Background: Ferritin is known to be associated with insulin resistance (IR) and oxidative stress; however, recent studies have shown that there is an association between ferritin and anti-oxidative status. To date, the biphasic response of ferritin to oxidative stress has not been fully evaluated. Thus, we investigated the association between ferritin and IR and anti-oxidative status in obese and non-obese women.

Methods: We evaluated the homeostasis model assessment of insulin resistance (HOMA-IR) and total anti-oxidant status (TAS) in a total of 111 healthy women between the ages of 32 and 68 years.

Results: In all of the study subjects, ferritin levels were positively correlated with age ($r = 0.38$, $P < 0.001$), body mass index ($r = 0.24$, $P = 0.01$), TAS ($r = 0.38$, $P < 0.001$) and HOMA-IR ($r = 0.20$, $P = 0.04$). In the subgroup analysis, ferritin levels were correlated with age ($r = 0.39$, $P < 0.001$) and TAS ($r = 0.43$, $P < 0.001$) in the non-obese group and with insulin ($r = 0.50$, $P = 0.02$) and HOMA-IR ($r = 0.52$, $P = 0.01$) levels in the obese group. On stepwise multiple linear regression analysis, ferritin was found to be independently associated with TAS ($B = 177.16$, $P < 0.0001$) in the non-obese group and independently associated with HOMA-IR ($B = 30.36$, $P = 0.01$) in the obese group.

Conclusion: Our findings suggest ferritin is associated with IR in obese women and with anti-oxidative status in non-obese women. Further studies are warranted to elucidate the precise role of ferritin in obesity.

Keywords: Ferritins; Obesity; Oxidative Stress

INTRODUCTION

Serum ferritin concentration is an indicator of the total amount of stored iron in the body,¹⁾ which is necessary to maintain the appropriate supply of oxygen and enzymes to cells and tissues.²⁾ Ferritin is also related to oxidative stress and excessive activation of reactive oxygen species, which contributes to the development of insulin resistance (IR), atherosclerosis and cardiovascular disease.^{3,4)} Several clinical studies have shown that elevated ferritin levels are associated with IR, diabetes mellitus

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and metabolic syndrome.⁵⁻⁷⁾

However, Schulpis et al.⁸⁾ showed conflicting data with regard to the relationship between ferritin and anti-oxidant status. The total anti-oxidant status increased as ferritin levels increased in a group of phenylketonuria patients on a high antioxidant diet. In addition, the protective role of ferritin against oxidative stress was confirmed by several *in vivo* studies. Cells enriched by ferritin showed an enhanced resistance to oxidative stress, whereas cells in which ferritin was downregulated had reduced resistance to oxidative stress.⁹⁾ Although the role of ferritin as an anti-oxidant has remained largely hypothetical, ferritin is known to play an anti-oxidative role by sequestering iron, which damages cells through oxidative reactions.¹⁰⁾

The reason for this discrepancy is unclear at present, but could possibly be attributed to differences in metabolic conditions and ethnicity. Currently, few studies have considered the role of obesity in the relationship between ferritin and metabolic parameters. Therefore, the aim of this study was to evaluate the association between serum ferritin levels and cardio-metabolic risk factors and the anti-oxidative status in healthy obese and non-obese women.

METHODS

1. Study Population

Study subjects were recruited through an advertisement at a health promotion center of a women's hospital in Seoul, South Korea from 2009 to 2010. A total of 87 non-obese and 24 obese women between the ages of 32 and 68 years were included in the study. Participants were current non-smokers, had a low alcohol consumption (<2 drinks per week) and were not using medications or supplements that could affect cardio-metabolic function. The study complied with the Declaration of Helsinki, and the review board of Yonsei University College of Medicine approved this study. The institutional review board approval number is 4-2011-0483.

All participants were healthy and had no previous diagnosis or evidence of cardiovascular disease, diabetes, moderate to severe hypertension (resting blood pressure [BP] > 170/100 mm Hg), kidney disease, or liver disease on physical examination. We also excluded any patients with anemia (ferritin < 10 µg/L,

hemoglobin < 12 g/dL).

2. Methods

Height, to the nearest 0.1 cm, and weight, to the nearest 0.1 kg, were measured with an automatic height-weight scale, and body mass index (BMI, kg/m²) was calculated as weight divided by height squared. Obesity was defined as a BMI greater than 25 kg/m², according to the Western Pacific Region of the World Health Organization criteria for obesity.¹¹⁾

Biochemical testing was performed on blood samples collected after study participants had fasted for at least eight hours. Serum levels of fasting glucose, ferritin, total cholesterol, high density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were assayed using an ADIVA 1650 chemistry system (Bayer, Tarrytown, NY, USA). Low density lipoprotein cholesterol was calculated using Friedwald's formula. Fasting insulin was assayed by electrochemiluminescence immunoassay using an Elecsys 2010 (Roche, Indianapolis, IN, USA). High sensitivity C-reactive protein (hsCRP) was measured by a latex-enhanced immunoturbidimetric assay using an ADIVA 1650 chemistry system. IR was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) index: (insulin [µIU/mL] × fasting blood glucose [mg/dL] / 18) / 22.5.

Plasma total anti-oxidant status (TAS) was measured via the colorimetric method using a Radox total anti-oxidant status kit (Radox Laboratories Ltd., San Francisco, CA, USA). The intra- and inter-assay coefficients of variation were 3.08% and 3.75%, respectively.

3. Statistical Analysis

Data are expressed as the mean ± SD for normally distributed data and as the median and interquartile range for non-normally distributed data. After systolic BP, diastolic BP, HDL-C, TG, hsCRP, ferritin, insulin, HOMA-IR, and uric acid levels were logarithmically transformed to eliminate the skewness of the distribution, Pearson's correlation coefficients were calculated to evaluate the relationship between serum ferritin and other metabolic parameters. Additionally, a stepwise multiple linear regression analysis was performed to identify factors contributing to serum ferritin levels in the non-obese and obese groups. Statistical significance was defined as a P-value < 0.05. All calculations were performed using the SPSS ver. 18.0 (SPSS Inc.,

Chicago, IL, USA).

RESULTS

1. Clinical Characteristics

The clinical characteristics of the two study groups are shown in Table 1. The obese group had a significantly higher age, systolic BP, diastolic BP, BMI, TG, and fasting blood glucose levels (all $P < 0.05$). The median ferritin concentration was 57.10 (range, 34.17 to 94.04) $\mu\text{g/L}$ in the non-obese group and 73.05 (range, 41.23 to 130.60) $\mu\text{g/L}$ in the obese group.

2. Correlation between Ferritin Levels and Clinical Variables

Ferritin levels were positively correlated with age ($r = 0.38$, $P < 0.001$), BMI ($r = 0.24$, $P = 0.01$), HOMA-IR ($r = 0.20$, $P = 0.04$) and TAS ($r = 0.38$, $P < 0.001$), as shown in Table 2. In the subgroup analysis, ferritin levels were positively correlated with

age ($r = 0.39$, $P < 0.001$) and TAS ($r = 0.43$, $P < 0.001$) in the non-obese group, and positively correlated with fasting insulin ($r = 0.50$, $P = 0.02$) and HOMA-IR ($r = 0.52$, $P = 0.01$) in the obese group, as shown in Table 2.

3. Multiple Linear Regression Assessment for Independent Relationships between Ferritin and Clinical Variables

In the stepwise multiple regression analysis, TAS level was identified as the explanatory variable accounting for serum ferritin levels in the non-obese group, and HOMA-IR was determined to be the explanatory variable accounting for serum ferritin levels in the obese group (Table 3).

DISCUSSION

Ferritin is known to have biphasic roles in the body's response to oxidative stress; nevertheless, the role of ferritin in humans has

Table 1. Clinical and metabolic variables of the non-obese versus obese study group.

Variables	Non-obese (n = 87)	Obese (n = 24)	P-value
Age (y)	53.26 \pm 11.12	60.75 \pm 3.73	<0.001
Body mass index (kg/m^2)	22.23 \pm 1.70	26.97 \pm 1.41	<0.001
Systolic blood pressure (mm Hg)	122.00 (110.00–135.00)	131.00 (120.75–137.75)	0.02
Diastolic blood pressure (mm Hg)	73.00 (63.00–80.00)	77.00 (72.25–83.75)	0.03
Total cholesterol (mg/dL)	185.15 \pm 33.42	194.25 \pm 30.82	0.23
High density lipoprotein cholesterol (mg/dL)	53.00 (49.00–60.00)	55.50 (51.00–61.75)	0.66
Low density lipoprotein cholesterol (mg/dL)	108.97 \pm 29.66	106.73 \pm 28.93	0.74
Triglyceride (mg/dL)	95.00 (66.00–120.00)	125.50 (98.75–200.00)	0.01
Fasting glucose (mg/dL)	86.75 \pm 10.33	92.92 \pm 8.88	0.01
Insulin (mU/L)	4.03 (2.58–6.08)	5.33 (3.30–6.70)	0.22
HOMA-IR	0.81 (0.59–1.35)	1.26 (0.78–1.61)	0.11
High sensitive C-reactive protein (mg/L)	0.01 (0.00–0.04)	0.04 (0.01–0.17)	0.32
Uric acid (mg/dL)	4.00 (3.40–4.75)	4.05 (3.28–5.48)	0.76
Total anti-oxidant status (nmol/L)	1.28 \pm 0.10	1.32 \pm 0.07	0.13
Ferritin ($\mu\text{g/L}$)	57.10 (34.17–94.04)	73.05 (41.23–130.60)	0.11

Values are presented as mean \pm SD. Skewed data are presented as medians (range).

P-values are calculated by the t-test (normal distribution), Wilcoxon's rank sum test (non-normal distribution).

HOMA-IR: homeostasis model assessment of insulin resistance.

Table 2. Correlations between ferritin levels and cardio-metabolic risk factors and anti-oxidative status.

	Total (n = 111)		Non-obese (n = 87)		Obese (n = 24)	
	r	P-value	r	P-value	r	P-value
Age (y)	0.38	<0.001	0.39	<0.001	0.28	0.19
Body mass index (kg/m ²)	0.24	0.01	0.09	0.43	0.39	0.06
Systolic BP (mm Hg)	0.11	0.25	0.13	0.25	-0.12	0.59
Diastolic BP (mm Hg)	0.10	0.29	0.13	0.24	-0.14	0.50
Total cholesterol (mg/dL)	0.12	0.20	0.17	0.11	-0.10	0.65
Triglyceride (mg/dL)	0.11	0.25	0.16	0.14	-0.11	0.60
LDL-C (mg/dL)	0.07	0.44	0.11	0.30	0.00	0.98
HDL-C (mg/dL)	0.05	0.64	0.08	0.46	-0.11	0.61
Fasting glucose (mg/dL)	0.17	0.07	0.11	0.32	0.23	0.27
Insulin (mU/L)	0.17	0.08	-0.01	0.92	0.50	0.02
HOMA-IR	0.20	0.04	0.00	0.99	0.52	0.01
TAS (nmol/L)	0.38	<0.001	0.43	<0.001	0.19	0.38
hsCRP (mg/L)	0.09	0.35	0.03	0.79	0.10	0.64
Uric acid (mg/dL)	0.05	0.60	0.10	0.38	-0.06	0.80

Coefficient (r) and P-values were calculated by Pearson's correlation analysis. Systolic BP, diastolic BP, HDL-C, triglyceride, hsCRP, ferritin, insulin, HOMA-IR, and uric acid levels were logarithmically transformed before statistical analysis to approximate a normal distribution. BP: blood pressure, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, HOMA-IR: homeostasis model assessment of insulin resistance, TAS: total anti-oxidant status, hsCRP: high sensitive C-reactive protein.

Table 3. Multiple regression analysis to identify independent relationships between ferritin and clinical variables.

Variables	β coefficient	SE	Model r ²	P-value
Non-obese*				
TAS	177.16	50.73	0.18	<0.0001
Obese†				
HOMA-IR	30.36	10.98	0.27	0.01

All variables left in the model were significant at the 0.15 level. No other variables met the 0.15 significance criterion for entry into the model. Variables included in the stepwise model: age, body mass index, systolic blood pressure, ferritin, HOMA-IR, hsCRP, glucose, cholesterol, HDL-C, TG, and TAS. Systolic blood pressure, ferritin, HOMA-IR, hsCRP, HDL-C, and TG were logarithmically transformed before statistical analysis to approximate normal distribution.

TAS: total anti-oxidant status, HOMA-IR: homeostasis model assessment of insulin resistance, hsCRP: high sensitive C-reactive protein, HDL-C: high density lipoprotein cholesterol, TG: triglyceride.

*Data from a total of 87 non-obese women. †Data from a total of 24 obese women.

not been fully clarified. As a pro-oxidant, iron plays a key role in converting poorly reactive free radicals into highly reactive free radicals.¹²⁾ It has been hypothesized that iron contributes to IR by damaging pancreatic beta cells with reactive hydroxyl radicals.^{13,14)}

In contrast, the protective role of ferritin against oxidative

stress has been confirmed by several experiments. Balla et al.¹⁵⁾ reported that cells pretreated with ultraviolet A radiation, which up-regulates the synthesis of ferritin, had elevated anti-oxidant activity. Similar results were obtained with human leukemia cells inundated with various types of oxidative stress.⁹⁾ The possible

mechanism underlying the anti-oxidative effects of ferritin is its capacity to bind to nitric oxide molecules,¹⁶⁾ which have anti-oxidative properties, and to decrease lipid peroxidation by sequestering iron in solution.¹⁷⁾

Despite the fact that ferritin's ability to act against oxidative stress is regulated by many proteins that sequester and mobilize iron,¹⁸⁾ the precise factors and pathophysiology that regulate the homeostasis between the anti-oxidant and pro-oxidant properties of ferritin are not clear. In the present study, we found that serum ferritin levels were significantly correlated with IR (as measured by HOMA-IR) in obese women, even after adjusting for potential covariates that are known to be linked to IR. This result is consistent with that of previous studies.¹⁹⁾ However, these relationships were not found in the non-obese group. On the contrary, TAS was positively correlated with serum ferritin levels in the non-obese group. TAS is a valuable method for detecting the actual total anti-oxidant status in humans.²⁰⁾ In previous studies, TAS levels showed negative relationships with coronary atherosclerosis, dyslipidemia, and essential HTN.²¹⁻²³⁾ Our findings suggest that ferritin is associated with the anti-oxidative status of non-obese women. Several possible mechanisms of ferritin against oxidative stress have been proposed, however, the protective roles of ferritin against IR and metabolic syndrome have not been evaluated in previous studies. Our findings suggest that in non-obese women, increased ferritin levels might have protective roles against diseases related to oxidative stress, and further studies are warranted to evaluate these relationships.

The reason for the differing metabolic role of ferritin in obese and non-obese women remains uncertain. However, one possible mechanism for this association is related to adipocytokines derived from fat tissue, which are known to affect oxidative stress and IR.²⁴⁾ Increased fat mass can change adipose biology and alter adipocyte-secreted protein expression.²⁵⁾ Thus, the difference in adiposity between obese and non-obese women may affect the role of adipocytokines associated with oxidative status and IR.

Ikegami et al.²⁶⁾ demonstrated that adiponectin upregulates ferritin heavy chain in murine skeletal muscle tissues. Adiponectin is an adipocyte-derived protein known to reduce IR.^{27,28)} Conversely, in a study of obese adolescents, tumor necrosis factor- α and IL 6 levels, both of which are pro-inflammatory cytokines released by adipocytes, were elevated as ferritin levels increased.²⁹⁾

Therefore, adipocytokines may play a role in regulating the equilibrium between the pro-oxidant and anti-oxidant properties of ferritin in non-obese and obese women. Further studies are needed to explore the pathogenesis of the biphasic role of ferritin in obesity.

The present study has several limitations. The cross-sectional design limits our ability to determine causality. The small sample size is another limitation of this study. Additional longitudinal studies with larger samples are necessary to confirm our findings. Although our study had some limitations, this is, to our knowledge, the first study to assess the association between ferritin and anti-oxidative status and IR in order to evaluate the effect of BMI on this association. In conclusion, the serum ferritin level was independently associated with anti-oxidant status in non-obese women and with IR in the group of obese women. Further studies are warranted to elucidate the precise role of serum ferritin in obesity.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported

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