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Serum Ferritin Level is an Independent Predictor of Insulin Resistance in Non-diabetic Men Aged Between 30-69 Years: Korean National Health and Nutrition Examination Survey 2008-2010

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Objective: We investigated predictors of insulin resistance in Korean population from the Korean National Health and Nutrition Examination Surveys.

Methods: Adults aged between 30-69 years without anemia were selected for the analysis. Fat mass was measured by dual-energy X-ray absorptiometry, and 24-hour nutritional information was obtained by interview from study subjects. Insulin resistance and beta cell function were calculated from homeostatic model assessment.

Results: 6,256 men and 8,003 women were included in the the study. Among participants, 9.8 (standard error [SE] 0.4) % of men and 6.8 (SE 0.4) % of women had diabetes mellitus (DM); moreover, serum ferritin level was higher in the DM patients compared to the non-DM subjects both in men and women ($p < 0.001$). Serum ferritin levels correlated significantly with insulin resistance (age and body mass index adjusted $p < 0.001$) in men, but not in women. Serum ferritin levels also correlated with total body fat ($p = 0.009$), triglyceride levels ($p < 0.001$), alanine aminotransferase (ALT) levels ($p < 0.001$) and leukocyte count ($p < 0.001$), but not with iron or fat intake. Multivariate analysis showed that serum ferritin level was an independent predictor of insulin resistance even after adjusting body fat, ALT levels, triglyceride levels and leukocyte count ($\beta = 0.027$, $p = 0.027$). Beta cell function was not associated with ferritin levels both in men and women.

Conclusion: Elevated serum ferritin was a risk factor for DM by increasing insulin resistance rather than affecting beta cell function. Serum ferritin level was an independent predictor of insulin resistance even after adjusting body fat or systemic inflammation in men.

Key Words: Ferritin, Insulin resistance, Diabetes mellitus

INTRODUCTION

Several cross sectional studies showed that elevated serum ferritin concentrations were associated with cardio-vascular disease.[1,2] essential hypertension,[3] and diabetes.[4,5] As serum ferritin concentration reflects systemic infla-mmation,[6] it is not clear whether elevated ferritin level is cause or result in developing metabolic disease which is also known to accompany systemic inflammation.[7,8] However, recent longitudinal study showed that elevated serum ferritin levels were independently associated with future development of metabolic syndrome during the

5-year follow-up period,[9] which suggests that elevated ferritin level might be a risk factor of metabolic syndrome. Ferritin is strongly associated with insulin resistance[5,10] and insulin resistance is one of important beneath mechanism of metabolic diseases.[11,12] However, it is difficult to investigate the lone effect of ferritin on insulin resistance. Serum ferritin concentration is marker of iron stores in the body[13] as well as systemic inflammation[6] and is associated with the amount of heme-iron intake: heme-iron intake[14] and anemia[14-16] also have been reported to be independently associated with insulin resistance or metabolic disease. Furthermore, central adiposity, an important risk factor of insulin resistance, is also associated with ferritin level.[17] Such factors above make it difficult to differentiate the lone effect of ferritin on insulin resistance.

Therefore, we performed this study to assess the associated factor of ferritin level with body fat mass measurement, the personal dietary information and other metabolic parameters and to investigate the lone effect of ferritin on insulin resistance adjusting possible confounders in a large-sized national survey in the Korean population.

MATERIALS AND METHODS

1. Subjects

The Korean Ministry of Health and Welfare has conducted a series of four Korean National Health and Nutrition Examination Survey (KNHANES) in non-institutionalized Korean civilians since 1998 using a stratified, multistage, probability-sampling design to be representative of the Korean population. To ensure the results represented the entire Korean population weights were assigned to each respondent. This weighting method guarantees unbiased point estimates of population parameters for the entire population and its subsets.[18]

We selected and compared the data in 2008 to 2010

for our study as body fat mass was measured by dual-energy X-ray absorptiometry since 2008. The number of the entire participants of health examination was 9,308 (response rate, 74.3%) in KNHANES 2008, 10,078 (79.2%) in KNHANES 2009 and 8,473 (77.5%) in KNHANES 2010. Among them, adults aged between 30-69 years with hemoglobin level 10-18 g/dL were included in our study.

2. Measurement of metabolic parameters

Anthropometric measurements were conducted by well-trained examiners in the same manner in each study. Weight was measured to the nearest 0.1 kg using a calibrated balance-beam scale (Giant-150N; Hana, Seoul, Korea) and waist circumference was measured to the nearest 0.1 cm from the narrowest point between the lower borders of the rib cage and the iliac crest at the end of normal expiration.

Fat mass and lean body mass were measured by dual-energy X-ray absorptiometry (Discovery QDR 4500 W, Hologic Inc., Denver, CO, USA) and percent of body fat was calculated from fat mass divided by (fat mass + lean body mass).

Venous blood samples were drawn after a 12-hour (h) overnight fast and plasma was separated immediately by centrifugation. Plasma concentrations of glucose and lipids were measured enzymatically in a central laboratory; Advia 1650/2400 (Siemens, New York, NY, USA) in 2008 and Hitachi Automatic Analyzer 7600 (Hitachi, Tokyo, Japan) in 2009 and 2010 hemoglobin A1c (HbA1c) was measured by high-performance liquid chromatography assay using BIO-RAD Variant II (Bio-Rad, Carlsbad, CA, USA) in 2008 and HLC-723G7 (Tosoh, Japan) in 2009 and 2010.

To confirm and compare accuracy and consistency in each survey, all biochemical measurements were double-checked on another day and at least 40 samples were selected to be measured using the standard method according to Clinical and Laboratory Standards Institute

Table 1. Characteristics of study participants from Korean National Health and Nutrition Examination Surveys 2008–2010

	Men (n=6,256)	Women (n=8,003)	p value
Age (years)	46.3±0.2	47.0±0.2	<0.001
Body mass index (kg/m^2)	24.3±0.1	23.5±0.1	<0.001
Hemoglobin (g/dL)	15.3±0.0	13.1±0.0	<0.001
AST (IU/L)	25.2±0.2	20.2±0.1	<0.001
ALT (IU/L)	28.2±0.3	18.2±0.2	<0.001
Leukocyte count (10^3/μL)	6.6±0.3	5.7±0.2	<0.001
Prevalence of DM (%)	9.8(0.4)	6.8(0.4)	<0.001
Ferritin (ng/mL)	131.6±2.2	46.0±0.6	<0.001
Subjects with DM	177.8±9.2	66.4±3.0	
Subjects without DM	127.1±2.3	44.7±0.6	
Subjects with MetS	165.9±5.8	61.9±1.6	
Subjects without MetS	121.4±3.3	40.9±0.7	

Data was presented as mean±standard error or percent.
AST; aspartate aminotransferase, ALT; alanine aminotransferase, DM; diabetes mellitus, MetS; metabolic syndrome

guideline. Details of measurement of the metabolic parameters have been described previously.[19,20]

Insulin resistance (IR) and pancreatic beta cell function (B) were assessed by the homeostasis model assessment (HOMA)- IR and HOMA-B respectively.[21] Cases of diabetes mellitus (DM) were defined as subjects who were users of anti-diabetic medication including insulin at the point of the survey, had HbA1C of ≥6.5%, or had 8h-fasting plasma glucose levels ≥126 mg/dL. The working definition of the National Cholesterol Education Program-Adult Treatment Panel III[22] was used to define metabolic syndrome (MetS): three or more of the following criteria- 1) waist circumference >90 cm in men and >80 cm in women using the International Obesity Task Force criteria for the Asian-Pacific population to determine waist circumference; 2) triglycerides ≥150 mg/dL or medication use; 3) HDL cholesterol <40 mg/dL in men and <50 mg/dL in women or medication use; 4) blood pressure ≥130/85 mmHg or antihypertensive medication use; and 5) fasting glucose ≥100 mg/dL or medication.

3. Assessment of dietary indices

Nutritional information was collected by 24-h recall administered by a trained dietary interviewer at the Korea Health Industry Development Institute. This 24-h recall was based on food intake on 1 weekday and the nutrients were quantified using the Database Management System (Korea Health Industry Development Institute, Seoul, Korea). To assist subjects with recall, supplementary tools such as food models and 2-dimensional food volumes and containers were used. Detailed information has been published elsewhere.[23]

4. Statistical analyses

All data were analyzed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). The results are presented as means (standard deviation, SD) or as prevalence (% and standard error [SE]). In the case of variables not normally distributed, logarithmic transformation was performed before analysis. Sampling weights were used to account for the complex sampling, and all analyses were conducted independently for each sex. Weight for time-series analysis was used for multivariate analysis of combined data from KNHANES 2008-2010 as Korea Centers for Disease Control and Prevention recommended.[23] A p value of <0.05 was considered statistically significant.

Table 2. Association between ferritin and metabolic parameters

	Men (n=5,394)				Women (n=7,826)			
	β^*	p^*	$\beta^\dagger$	$p^\dagger$	β^*	p^*	$\beta^\dagger$	$p^\dagger$
Age	-0.002	<0.001	-0.002	<0.001	0.014	<0.001	0.014	<0.001
Hemoglobin	2.055	<0.001	1.723	<0.001	5.048	<0.001	4.595	<0.001
AST	0.657	<0.001	0.647	<0.001	0.883	<0.001	0.534	<0.001
ALT	0.456	<0.001	0.433	<0.001	0.589	<0.001	0.427	<0.001
Leukocyte count	0.218	<0.001	0.139	0.001	0.187	<0.001	0.203	<0.001
Triglyceride	0.228	<0.001	0.196	<0.001	0.260	<0.001	0.084	<0.001
HDL cholesterol	-0.215	0.001	-0.083	0.242	-0.429	<0.001	-0.168	0.004
LDL cholesterol	-0.120	0.003	-0.166	<0.001	0.276	<0.001	-0.077	0.085
BMI	0.851	<0.001	0.834	<0.001	0.882	<0.001	0.262	0.003
Total body fat†	0.296	<0.001	0.151	0.009	0.351	<0.001	0.155	0.065
Fat percentage of total body†	0.354	<0.001	0.169	0.007	0.676	<0.001	0.200	0.042
Lean mass†	0.599	<0.001	-0.220	0.156	-0.125	0.277	-0.199	0.161
HOMA-IR	0.267	<0.001	0.188	<0.001	0.143	<0.001	0.046	0.138
HOMA-B	0.066	0.010	-0.018	0.498	-0.113	<0.001	0.005	0.857
Daily fat intake	0.025	0.153	-0.013	0.476	-0.152	<0.001	-0.017	0.352
Daily protein intake	0.060	0.027	0.020	0.444	-0.094	0.002	-0.002	0.931
Daily CHO intake	-0.107	0.004	-0.125	0.001	0.007	0.851	-0.042	0.214
Daily iron intake	-0.006	0.816	-0.024	0.322	0.010	0.690	-0.008	0.722
Daily energy intake	0.060	0.083	0.013	0.714	-0.092	0.014	-0.028	0.413

*Monovariate linear regression analysis, †Linear regression analysis adjusting age and BMI, †Measured by dual-energy X-ray absorptiometry
AST; aspartate aminotransferase, ALT; alanine aminotransferase, BMI; body mass index, HOMA-IR; homeostasis model assessment of insulin resistance, HOMA-B; homeostasis model assessment of beta cell function, CHO; carbohydrate

RESULTS

1. Serum ferritin level according to DM or MetS

Among 15,059 adults aged between 30-69 years in KNHANES 2008-2010, only the subjects with blood hemoglobin level within 10-18 g/dL were selected to exclude the confounding factors for ferritin level. Finally 6,256 men and 8,003 women were included in our study. Mean age was 46.7 (SE, 0.2) years and mean body mass index (BMI) was 23.91 (SE, 0.03) kg/m^2 (Table 1). As there were significant differences in age, BMI and blood ferritin level according to gender, the further analysis to investigate the associated factors of ferritin was performed in each gender respectively. Among entire study population, 9.8 (SE 0.4)% of men and 6.8 (SE 0.4)% of women

had DM. Serum ferritin level was significantly higher in DM patients compared to non-DM subjects both in men and women (177.8 [SE 9.2] ng/mL vs. 127.1 [SE 2.3] ng/mL in men; 66.4 [SE 3.0] ng/mL vs. 44.7 [SE 0.6] ng/mL in women; $p<0.001$ in both; Table 1). As age and BMI was significantly associated in serum ferritin level ($p<0.001$ in both sex), we subsequently adjusted age and BMI. Even after adjusting age and BMI, serum ferritin level was significantly higher in DM patients in men ($p<0.001$) but not in women ($p=0.276$).

Subjects with MetS had also significantly higher ferritin level compared to those without MetS in both sex (Table 1): after adjusting age and BMI, serum ferritin level was significantly higher in the subjects with MetS ($p<0.001$ in men and $p=0.003$ in women).

2. Predictor of serum ferritin level

For further analysis of the predictor of ferritin level, only non-DM subjects were included for excluding confounding factors such as medication, and age and BMI were adjusted in all the analyses. Both in men and women, alanine aminotransferase (ALT), aspartate amino-transferase (AST), hemoglobin level and leukocyte count were significantly associated with serum ferritin level (Table 2). Percent of total body fat (β=0.169 [95% confidence interval, 0.046-0.292], p=0.007 in men and β=0.200 [0.007-0.393], p=0.042 in women) and serum triglyceride level (β=0.196 [0.148-0.243], p<0.001 in men and β=0.084 [0.039-0.129], p<0.001 in women) were also positively correlated with ferritin level. In men, ferritin level was significantly associated with HOMA-IR (β=0.188 [0.127-0.249], p<0.001) but not with HOMA-B (p= 0.498). By contrast, in the case of women, the statistically significant association between HOMA-IR and ferritin level was lost after adjusting age and BMI (Table 2): multiple linear regression analysis with age, BMI and ferritin level showed that HOMA-IR was determined by age (β=-0.001 [-0.001-0.000], p=0.009) and BMI (β=1.274 [1.191-1.358], p<0.001) but not by ferritin level (p=0.138).

Among dietary component, total daily energy intake or the amount of daily protein, fat or iron intake was not associated with serum ferritin level both in men and women (Table 2). In men, the amount of carbohydrate intake was negatively associated with ferritin level (β= -0.125 [-0.198 - -0.053], p<0.001).

3. The association between insulin resistance and serum ferritin level in men

To clarify the association between insulin resistance and ferritin level in men, we performed the further analysis adjusting variables which might affect both insulin resistance and ferritin. Multivariate linear regression analysis showed that ferritin level, BMI, ALT and triglyceride were significantly associated with HOMA-IR (Table 3). In men, ferritin level was independently associated with HOMA-IR in the entire model analyzed (p<0.05; Table 3).

Table 3. Predictor of insulin resistance (HOMA-IR) in men without diabetes mellitus

Model	β*	p* value
Ferritin (unadjusted)	0.106	<0.001
Ferritin	0.060	<0.001
BMI	1.567	<0.001
Age	-	0.633
Ferritin	0.031	0.002
BMI	1.359	<0.001
ALT	0.149	<0.001
Age	-	0.184
Ferritin	0.030	0.003
BMI	1.340	<0.001
ALT	0.148	<0.001
WBC	0.069	0.005
Age	-	0.144
Ferritin	0.027	0.007
BMI	1.321	<0.001
ALT	0.144	<0.001
WBC	0.057	0.024
Hb	0.273	0.016
Age	-	0.062
Ferritin	0.021	0.027
BMI	1.237	<0.001
ALT	0.121	<0.001
WBC	0.027	0.290
Triglyceride	0.112	<0.001
Age	-	0.495
Ferritin	0.048	<0.001
Total body fat mass[†]	0.522	<0.001
Age	-	0.108
Ferritin	0.059	<0.001
Fat percent of total body[†]	0.627	<0.001
Age	-	0.423

*Multivariate linear regression analysis
Measured by dual-energy X-ray absorptiometry
BMI; body mass index, ALT; alanine aminotransferase, HOMA-IR; homeostasis model assessment of insulin resistance

DISCUSSION

We extensively assessed the associated factors of serum ferritin level including lipid profile, liver function, hemoglobin, inflammatory marker, body composition and dietary information and confirmed the results of the previous studies: serum ferritin level was significantly associated with triglyceride, body fat content, AST and ALT level, and leukocyte count as well as hemoglobin level. Interestingly, in men, ferritin level was significantly correlated with insulin resistance even after adjusting body fat, inflammatory markers, ALT level or lipid concentration. Many previous studies suggested that the association between ferritin and insulin resistance is mediated by body fat content,[17] systemic inflammation[6] or nonalcoholic fatty liver disease.[24] However, our study suggested that elevated ferritin level might be an independent risk factor of insulin resistance aside from the mediators suggested in previous studies. Reduction of iron stores by blood donation or phlebotomy have been reported to improve insulin sensitivity,[25,26] which also supports that iron overload might be a primary cause of insulin resistance.

The molecular mechanism that ferritin induces insulin resistance has not been clearly defined yet. However, recent reports showed that iron status influences insulin signaling and glucose uptake in the liver[27] and adipose tissue.[28] Iron overloading decreased insulin signaling evaluated by the phospho-Akt/Akt ratio and up-regulated adipokines favoring insulin resistance such as resistin,[28] which can explain the independent association between ferritin level and insulin resistance in our study.

We also found the significant difference in ferritin level between the subjects with or without DM. Our study showed that the elevated ferritin level might increase the risk of DM via increased insulin resistance rather than beta cell dysfunction, which was corresponded to previous study.[5] Iron rather increases beta cell mass and improves beta cell function.[25,29]

In the case of women, the statistically significant association between insulin resistance and ferritin level was lost after adjusting age and BMI as previous reports.[5,30] Multivariate analysis showed that HOMA-IR in women was determined by age and BMI but not by ferritin level. Such a weak correlation between insulin resistance and ferritin level in women might be resulted from that there are more confounding factors in serum ferritin level in women compared to men and menstrual iron loss might be one of most important confounders.[31] Significantly lower serum level of ferritin in women compared to men in our study might be caused by the menstrual iron loss in women.

Interestingly, there was no association between the amount of iron intake and ferritin level in KNHANES. Heme-iron intake was known to be independently associated with insulin resistance,[14] and very recently, only heme-iron from red meat was reported to be associated with greater risk of metabolic syndrome and cardiovascular disease and heme-iron from other sources was not.[32] Although we did not differentiate the iron source according to heme-containing, monovariate analysis in our study showed that protein intake rather than iron intake was significantly associated with ferritin level in men, which also supports that the amount of red meat intake might be more important than the amount of total iron intake in ferritin level or insulin resistance. In men, the amount of carbohydrate intake showed a significant negative correlation with ferritin level. Although we cannot explain the exact cause of the association, non-carbohydrate source of energy intake, such as red meat, might positively affect ferritin level.

The limitation of our study was we did not consider menopausal state in women. And relative wide range of hemoglobin level for study inclusion might also play a confounding factor for analysis on ferritin level. However, we excluded the subjects with hemoglobin less than 10 g/dL and performed analysis after adjusting hemoglobin

level to minimize the effect of anemia. We analyzed large-sized national surveys in Korea and extensively assessed the associated factors of serum ferritin level including lipid profile, liver function, hemoglobin, inflammatory marker, body composition and dietary information. Furthermore, we excluded the subjects with DM in the analysis on the associated factors of ferritin level, which can reduce the probability of confounders in the association between insulin resistance and ferritin level.

In conclusion, elevated serum ferritin was a risk factor of DM via increasing insulin resistance rather than affecting beta cell function in the Korean population. Serum ferritin level was an independent determining factor of insulin resistance even after adjusting body fat or systemic inflammation in men.

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