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Fasting Serum Ferritin and Non-HDL Cholesterol Independently Predict Plasma Glucose Level 120 minutes After Glucose Loading in an Oral Glucose Tolerance Test in Physiologically Healthy Young Japanese Men

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ABSTRACT

Objective: This study intended to clarify whether serum ferritin levels are a marker for the early detection of glucose intolerance and diabetes in young adults.
Methods: 382 young healthy Japanese individuals aged 22-29 years underwent oral glucose tolerance test (OGTT). We analyzed the relationship between serum ferritin levels and insulin secretion ability, insulin sensitivity, lipid metabolism markers, and complete blood count.
Results: Among 233 men, ferritin showed a positive correlation with age, HOMA-β, LDL-C, non-HDL-C, Hb, platelet count, and 120-minute post-load plasma glucose and insulin. Ferritin showed a negative correlation with Matsuda and disposition indices. The multivariate analysis revealed that fasting plasma glucose, ferritin, and non-HDL-C are independent predictors of the 120-minute post-load plasma glucose in young men. Among 149 women, ferritin showed a positive correlation with Hb and CRP, and a negative correlation with HbA1c and HDL-C. Men were divided into groups based on ferritin levels above or below the average of 97.8 ng/mL; women were similarly divided at 24.7 ng/mL. Men with higher levels of ferritin showed decreased levels of Matsuda index and disposition index and increased insulin levels at 60 minutes compared to men with lower levels of ferritin. Women with higher ferritin levels showed lower HbA1c, but higher Hb than those with lower ferritin levels.
Conclusion: Among healthy young Japanese men, serum ferritin levels and non-HDL-C independently predict prolonged glucose elevation in the OGTT.

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Key Words
OGTT, ferritin, insulin sensitivity, young Japanese, sex difference

I. Introduction

According to the 10th edition of the International Diabetes Federation's (IDF) Diabetes Atlas, addressing the increasing number of diabetic patients is an urgent global issue. The cost of diabetes treatment has tripled over the past 15 years, reaching USD 966 billion. 541 million adults exhibited impaired glucose tolerance (IGT), a high-risk condition for type 2 diabetes (T2DM)[1]. Annually, 41 million people die from non-communicable diseases (NCDs), accounting for over 70% of deaths worldwide. Metabolic factors associated with the risk of NCDs include high blood pressure, obesity/overweight, hyperglycemia, and hyperlipidemia[2]. Addressing the growing number of diabetic patients and their prediabetic counterparts, glucose intolerance, is crucial on a global scale[1,2]. To prevent the onset of diabetes, early detection of IGT and lifestyle changes are essential. In IGT, a pre-stage of T2DM, postprandial plasma glucose increases before the rise in fasting plasma glucose levels[3,4]. Early detection of IGT through fasting blood sampling is very challenging; therefore, there is a need to establish a marker that can detect IGT and high-risk groups for T2DM early with a single fasting blood sample.

We report prolonged elevation of plasma glucose in the oral glucose tolerance test (OGTT) in healthy Japanese individuals in their 20s who are within the physiological range for glycemic control. Furthermore, even in young and healthy Japanese individuals, insulin sensitivity and secretion gradually decrease[5]. In these individuals, the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) showed a significant correlation with hematocrit, hemoglobin, red blood cells (RBC), white blood cells (WBC), platelet count, lipid parameters, and body mass index (BMI)[6]. Fasting serum insulin levels and insulin resistance can influence blood rheology by modulating hematological parameters and lipid parameters[7]. These findings suggest that hematological parameters may contribute to the early detection of insulin resistance and IGT. In humans, hemoglobin in red blood cells contains 70% of the body's iron[8]. Iron, a micronutrient and transition metal, is essential for maintaining physiological homeostasis, but in excess, it causes diseases. Excessive iron deposits are associated with hypertension[9], diabetes[10,11], dyslipidemia[12], and metabolic syndrome[13,14], which increase the risk of cardiovascular disease[15,16]. There is a relationship between the rise in serum ferritin and diabetes[10,11]. However, it is unclear whether serum ferritin levels can serve as a marker for the early detection of IGT or diabetes. In this study, we analyzed the clinical significance of serum ferritin levels using OGTT data in young Japanese individuals with normal glucose tolerance.

II. Materials and Methods

2.1. Participants

A total of 382 young adults (233 men and 149 women; aged 22–29 years) with no history of diabetes underwent a 75g OGTT from June 2012 to July 2016. All participants provided informed consent, and the Gunma University Ethical Review Board for Medical Research Involving Human Subjects (No. 12–41) approved the study. We adhered to the Declaration of Helsinki for all ethical and confidentiality issues.

2.2. Study design

We conducted a 75g OGTT after a 12-hour fast, measured plasma glucose (PG0, PG30, PG60, PG120) and insulin levels (IRI0, IRI30, IRI60, IRI120) at 0, 30, 60, and 120 minutes, and determined serum levels of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), hemoglobin A1c (HbA1c), and pre-load ferritin. 233 men and 149 women were split at the median value of fasting serum ferritin. Metabolic parameters were compared using t-tests between genders and between ferritin groups (higher vs. lower) within each gender. The correlations between ferritin levels and metabolic parameters were analyzed separately in men and women groups.

2.3. Laboratory Assays

We measured the heights and weights of the participants to calculate the body mass index (BMI; weight (kg)/height (m^2)). We determined the fasting serum concentrations of HDL-C, LDL-C, TG, Fe (QUICKAUTO NEO Fe "SHINO-TEST") using enzymatic methods with an automatic analyzer (LABOSPECT 008; Hitachi, Tokyo). We measured the serum concentrations of CRP and ferritin (FER-LATEX RX "SEIKEN") using a latex-enhanced turbidimetric immunoassay with the same analyzer. Serum insulin levels were measured by chemiluminescent immunoassay using an automated analyzer (AIA-2000 LA; Tosoh, Tokyo). Plasma glucose levels and HbA1c were measured using the hexokinase method and high-performance liquid chromatography, respectively, with automatic analyzers (ADAMS Glucose GA-1170 and ADAMS A1c HA8180; Arkray, Tokyo, Japan).

2.4. Statistical Methods

We calculated the HOMA-IR, the Homeostatic Model Assessment for beta-cell function (HOMA-β), the Matsuda index[17], and the insulinogenic index[18] as reported. The insulin secretion/insulin resistance (disposition) in-

dex was calculated as the product of the insulinogenic index and the Matsuda index [19]. The HOMA-IR, indicating insulin resistance in the liver, was calculated using fasting plasma glucose [PG0] (mg/dL) × fasting immunoreactive insulin [IRI0] (μU/mL) / 405. HOMA-β, reflecting insulin secretion capacity, was calculated as [IRI0] (μU/mL) × 360 / ([PG0] (mg/dL) -63). The Matsuda index, representing peripheral insulin sensitivity, is defined as 10,000 /square root of ([PG0] × [IRI0] × [mean glucose (mg/dL)] × [mean insulin (μU/mL)]). The insulinogenic index, quantifying the initial insulin secretion, was determined by the ratio of ([IRI30]−[IRI0]) to ([PG30]−[PG0]). Statistical analyses were performed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA). The characteristics of the study participants were presented as median (first quartile-third quartile) The parameters were compared using Welch's t test. Separate Spearman correlation analyses were conducted for young men and young women. The stepwise multivariate linear regression analysis was employed to assess the independent impact of metabolic parameters on PG120. Candidates for independent variables were age, BMI, PG0, HbA1c, IRI0, TG, HDL-C, LDL-C, non-HDL-C, ferritin, Fe, Ht, WBC, and Plt.

III. Results

3.1. Characteristics of 382 young healthy Japanese

Table 1 displays characteristics of 382 healthy Japanese youths, all categorized as having normal glucose tolerance by the OGTT. Significant gender differences were observed in glucose metabolism and lipid markers, hematological markers, and ferritin levels. Men showed

Table 1 Characteristics of young and healthy Japanese.

	men (n=233)	women (n=149)	*P*
Age (years)	23 (23 – 24)	23 (22 – 24)	**0.011***
BMI (kg/m^2)	21.3 (20.1 – 23)	19.5 (18.4 – 20.7)	**<0.001***
PG0 (mg/dL)	92 (88 – 96)	89 (84 – 93)	**<0.001***
PG30 (mg/dL)	137 (123 – 153)	126 (105 – 141)	**<0.001***
PG60 (mg/dL)	115 (96 – 137)	104 (87 – 125)	**0.003***
PG120 (mg/dL)	95 (82 – 106)	95 (82 – 108)	0.992
HbA1c (%)	5.3 (5.2 – 5.4)	5.3 (5.2 – 5.4)	0.694
IRI0 (μU/mL)	5.5 (4.2 – 7.8)	5.9 (4.6 – 8)	0.180
IRI30 (μU/mL)	44.4 (31 – 65)	53.5 (39.6 – 76)	**0.003***
IRI60 (μU/mL)	36.6 (25.5 – 54.3)	41.1 (28.8 – 60.4)	0.121
IRI120 (μU/mL)	25.3 (15.5 – 40)	35.5 (25 – 57.6)	**<0.001***
HOMA-IR	1.26 (0.94 – 1.88)	1.27 (1 – 1.8)	1.000
Matsuda index	7.15 (5.35 – 9.45)	6.7 (5.05 – 8.68)	0.294
HOMA-β	72.0 (53.5 – 94.5)	86.9 (70.88 – 115.2)	**<0.001***
insulinogenic index	0.91 (0.61 – 1.42)	1.37 (0.72 – 2.16)	**0.001**
disposition index	6.28 (4.38 – 9.85)	8.15 (5 – 13.4)	**0.004**
HDL-C (mg/dL)	58 (51 – 65)	69 (62 – 78)	**<0.001***
LDL-C (mg/dL)	97 (77 – 117)	94 (79 – 106)	0.834
TG (mg/dL)	70 (50 – 99)	60 (46 – 78)	**0.009***
non-HDL-C (mg/dL)	113 (95 – 136)	111 (96 – 124)	0.675
Hb (g/dL)	15.6 (15.1 – 16.2)	13.9 (13.3 – 14.5)	**<0.001***
WBC (× 10^3/μL)	5.1 (4.4 – 6.1)	5.4 (4.3 – 6.4)	0.434
Plt (× 10^4/μL)	22.5 (19.6 – 25.3)	24.6 (218 – 27.7)	**0.001***
ferritin (ng/mL)	97.8 (65.6 – 144.3)	24.7 (11.6 – 44.8)	**<0.001***
Fe (μg/dL)	122 (89 – 155)	110 (77 – 149)	**0.002***
CRP (mg/dL)	0.03 (0.02 – 0.06)	0.01 (0.01 – 0.03)	**0.011***

Data are shown as median (first quartile – third quartile) $P < 0.05$ in Welch's t-test considered significant. BMI, body mass index; PG0, fasting plasma glucose; PG30, 30 minutes postload plasma glucose; PG60, 60 minutes postload plasma glucose; PG120, 120 minutes postload plasma glucose; IRI0, preload immunoreactive insulin; IRI30, 30 minutes postload immunoreactive insulin; IRI60, 60 minutes postload immunoreactive insulin; IRI120, 120 minutes postload immunoreactive insulin; HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-β, homeostatic model assessment for beta cell function; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; TG, triglyceride; Hb, hemoglobin; WBC, white blood cells; Plt, platelets; CRP, C-reactive protein.

higher values in age, BMI, PG0, PG30, PG60, TG, hemoglobin, ferritin, Fe, and CRP, but lower in IRI30, IRI120, HOMA-β, insulinogenic index, disposition index, HDL-C, and platelets compared to women. Serum ferritin concentrations, within normal ranges, were more than three times higher in men. Women exhibited greater insulin secretion capacity. Due to distinct differences in ferritin, insulin secretion, and plasma glucose levels during the OGTT between genders, the data were analyzed separately for men and women.

3.2. Characteristics of Young Subjects with Different Ferritin Levels.

Among 233 men, divided by the median level of fasting serum ferritin (≧ 97.8 ng/mL), those with higher levels showed significantly greater IRI60, but lower Matsuda index and disposition index. Serum Fe level was not statistically different between 2 groups (**Table 2**). In contrast, among 149 women, divided by their average ferritin level (≧ 24.7 ng/mL), higher ferritin correlated with increased hemoglobin and Fe, and decreased HbA1c (**Table 3**).

3.3. Correlation of Serum Ferritin with Metabolic Parameters

In 233 men, ferritin positively correlated with age, PG120, IRI60, IRI120, HOMA-β, LDL-C, non-HDL-C, hemoglobin, platelets, and Fe, but negatively with the Matsuda index and disposition index (**Table 4**). Among 149 women, positive correlations with hemoglobin, Fe, and CRP were found, and negative correlations with HbA1c and HDL-C (**Table 4**).

3.4. Predictors of plasma glucose levels 120 minutes after glucose load in the OGTT.

We conducted a stepwise multivariate regression

Table 2 Characteristics of young men with higher (≧ 97.8 ng/mL) and lower ferritin values.

	lower group (n=116)	higher group (n=117)	*P*
Age (years)	23 (22 – 24)	23 (23 – 25)	0.847
BMI (kg/m^2)	21.3 (19.9 – 22.9)	21.3 (20.3 – 23.2)	0.744
PG0 (mg/dL)	92 (88 – 96)	92 (88 – 96)	0.953
PG30 (mg/dL)	136 (123 – 152)	139 (123 – 153)	0.744
PG60 (mg/dL)	113 (92 – 131)	115 (97 – 146)	0.946
PG120 (mg/dL)	94 (82 – 101)	98 (82 – 110)	0.102
HbA1c (%)	5.3 (5.2 – 5.4)	5.3 (5.2 – 5.4)	0.639
IRI0 (μU/mL)	5.4 (4.0 – 7.4)	6.1 (4.4 – 7.8)	0.213
IRI30 (μU/mL)	45.1 (31.4 – 63.4)	43.2 (31.0 – 65.3)	0.948
IRI60 (μU/mL)	34.1 (24.6 – 52.2)	39 (27.3 – 56.6)	**0.042***
IRI120 (μU/mL)	23.0 (14.6 – 35.7)	27.3 (18.6 – 43.2)	0.102
HOMA-IR	1.20 (0.88 – 1.81)	1.34 (1 – 1.95)	0.213
Matsuda index	7.70 (5.97 – 9.62)	6.5 (5.14 – 9.15)	**0.015***
HOMA-β	68.3 (51.1 – 86.05)	74.92 (56.57 – 100.5)	0.132
insulinogenic index	0.97 (0.63 – 1.48)	0.87 (0.61 – 1.37)	0.555
disposition index	6.92 (4.98 – 10.47)	5.61 (3.83 – 9.62)	**0.031***
HDL-C (mg/dL)	59 (52 – 65)	57 (51 – 65)	0.265
LDL-C (mg/dL)	92 (77 – 111)	99 (77 – 123)	0.132
TG (mg/dL)	67 (48 – 99)	73 (50 – 97)	0.213
non HDL-C (mg/dL)	111 (91 – 129)	118 (98 – 144)	0.326
Hb (g/dL)	15.6 (14.9 – 16.2)	15.6 (15.2 – 16.2)	0.943
WBC (× 10^3/μL)	5.2 (4.3 – 6.1)	5.1 (4.6 – 6.0)	0.948
Plt (× 10^4/μL)	21.9 (192.8 – 249.3)	23.0 (20.0 – 25.7)	0.213
Fe (μg/dL)	120 (80 – 155)	125 (93 – 155)	0.534
CRP (mg/dL)	0.03 (0.01 – 0.06)	0.03 (0.02 – 0.06)	0.265

Data are shown as median (first quartile – third quartile) $P < 0.05$ in Welch's t-test considered significant. BMI, body mass index; PG0, fasting plasma glucose; PG30, 30 minutes postload plasma glucose; PG60, 60 minutes postload plasma glucose; PG120, 120 minutes postload plasma glucose; IRI0, preload immunoreactive insulin; IRI30, 30 minutes postload immunoreactive insulin; IRI60, 60 minutes postload immunoreactive insulin; IRI120, 120 minutes postload immunoreactive insulin; HOMA-IR, homeostasis model assessment insulin resistance; HOMA-β, homeostasis model assessment beta cell; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; TG, triglyceride; Hb, hemoglobin; WBC, white blood cells; Plt, platelets; CRP, C-reactive protein.

analysis to determine the independent factors affecting glucose elevation in the OGTT among 233 men, but not among 149 women. We examined the relationship between PG120 and various parameters, including age, BMI, HbA1c, IRI0, TG, HDL-C, LDL-C, non-HDL-C, ferritin, hematocrit, leukocytes, and platelets, which do not require OGTT. Non-HDL cholesterol, PG0, and ferritin were independent factors associated with PG120 among 233 young men with normal glycemic control (**Table 5**). PG120 can be predicted using the following formula: PG120 = 41.787 + 0.082 * (non-HDL-C) + 0.431 * (PG0) + 0.037 * (ferritin)

IV. Discussion

In this study, we found that in healthy Japanese men in their 20s, serum ferritin, non-HDL-C, and PG0 concentrations may be predictive of PG120. Contrary to this, serum ferritin levels, which are generally low in healthy Japanese women in their 20s, can influence hemoglobin levels.

In patients with IGT and T2DM, postprandial hyperglycemia precedes the elevation of fasting plasma glucose [3),4)]. Screening for high risk of IGT and T2DM using random glucose tests is effective, following the diagnostic criteria for diabetes [3)]. The OGTT proves to be useful in identifying risks for IGT and T2DM among individuals with normal glucose tolerance [20),21)]. During the OGTT, high post-load plasma glucose levels indicate an increased risk of T2DM, even without elevated fasting glucose [20)]. Therefore, early identification of individuals at increased risk of developing IGT and T2DM is crucial. Our findings indicate that over 70% of healthy Japanese

Table 3 Characteristics of young women with higher (≧ 24.7 ng/mL) and lower ferritin values.

	lower group (n=74)	higher group (n=75)	*P*
Age (years)	23 (22 – 24)	23 (22 – 24)	0.563
BMI (kg/m^2)	19.5 (18.7 – 20.8)	19.5 (18.3 – 20.6)	0.934
PG0 (mg/dL)	89 (83 – 93)	88.5 (84.3 – 92.8)	0.671
PG30 (mg/dL)	126 (107 – 143)	126 (104.8 – 139.8)	0.685
PG60 (mg/dL)	103 (87 – 127)	106 (84 – 124)	0.806
PG120 (mg/dL)	96 (82 – 105)	93 (81 – 111)	0.936
HbA1c (%)	5.3 (5.2 – 5.4)	5.3 (5.2 – 5.4)	**0.015***
IRI0 (μU/mL)	5.4 (4.3 – 8.3)	6.0 (5.3 – 7.6)	0.369
IRI30 (μU/mL)	51.2 (39.8 – 75.1)	55.4 (39.5 – 78.1)	0.164
IRI60 (μU/mL)	40.0 (26.6 – 59.9)	43.3 (30.9 – 59.6)	0.120
IRI120 (μU/mL)	34.6 (25.0 – 53.0)	36.8 (24.6 – 62.1)	0.219
HOMA-IR	1.21 (0.90 – 1.84)	1.31 (1.11 – 1.75)	0.368
Matsuda index	6.64 (5.30 – 9.11)	6.71 (4.68 – 8.40)	0.682
HOMA-β	86.7 (65.2 – 114.7)	89.0 (73.8 – 113.4)	0.368
insulinogenic index	1.37 (0.65 – 2.14)	1.36 (0.78 – 2.30)	0.934
disposition index	8.55 (4.82 – 13.57)	7.77 (5.10 – 12.39)	0.250
HDL-C (mg/dL)	71 (64 – 79)	66 (57 – 77)	0.085
LDL-C (mg/dL)	93 (79 – 104)	95 (79 – 109)	0.805
TG (mg/dL)	63 (46 – 76)	60 (46 – 79)	0.461
non HDL-C (mg/dL)	108 (94 – 121)	115 (99 – 128)	0.567
Hb (g/dL)	13.5 (13.0 – 14.2)	14.2 (13.6 – 14.7)	**0.001***
WBC (× 10^3/μL)	5.0 (4.0 – 6.0)	5.0 (4.0 – 6.0)	0.663
Plt (× 10^4/μL)	24.6 (21.9 – 27.8)	24.1 (21.5 – 27.7)	0.678
Fe (μg/dL)	60 (95 – 137)	129 (99 – 152)	**0.029***
CRP (mg/dL)	0.01 (0.01 – 0.03)	0.02 (0.01 – 0.04)	0.288

Data are shown as median (first quartile – third quartile) $P < 0.05$ in Welch's t-test considered significant. BMI, body mass index; PG0, fasting plasma glucose; PG30, 30 minutes postload plasma glucose; PG60, 60 minutes postload plasma glucose; PG120, 120 minutes postload plasma glucose; IRI0, preload immunoreactive insulin; IRI30, 30 minutes postload immunoreactive insulin; IRI60, 60 minutes postload immunoreactive insulin; IRI120, 120 minutes postload immunoreactive insulin; HOMA-IR, homeostasis model assessment insulin resistance; HOMA-β, homeostasis model assessment beta cell; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; TG, triglyceride; Hb, hemoglobin; WBC, white blood cells; Plt, platelets; CRP, C-reactive protein.

individuals in their 20s, with normal glucose tolerance, exhibit a prolonged increase in PG120 during the OGTT[5]. However, previous studies have failed to identify markers in fasting blood samples—both in glucose metabolism and lipid metabolism—that predict postprandial glucose PG120[5]. We established a correlation between insulin sensitivity, insulin secretion capacity, and both lipid and hematological metabolic markers in fasting blood samples among healthy Japanese individuals in their 20s[5),6)]. There are significant hematological differences between genders in healthy Japanese individuals in their 20s[7)]. In the OGTT, a prolonged increase in PG120 was notably more prevalent in men than in women within this demographic[7)]. These differences are attributable to the higher

Table 4 Spearman's correlation of serum ferritin with metabolic parameters.

	men (n=233)		women (n=149)	
	ρ	P	ρ	P
Age (years)	0.151	**0.021***	-0.006	0.942
BMI (kg/m^2)	0.113	0.085	-0.006	0.937
PG0 (mg/dL)	-0.015	0.819	-0.018	0.827
PG30 (mg/dL)	0.045	0.496	0.004	0.965
PG60 (mg/dL)	0.109	0.096	-0.003	0.971
PG120 (mg/dL)	0.154	**0.019***	-0.027	0.743
HbA1c (%)	0.044	0.506	-0.209	**0.01***
IRI0 (μU/mL)	0.112	0.088	0.058	0.481
IRI30 (μU/mL)	0.020	0.763	0.033	0.693
IRI60 (μU/mL)	0.147	**0.025***	0.155	0.060
IRI120 (μU/mL)	0.205	**0.002***	0.080	0.330
HOMA-IR	0.084	0.199	0.047	0.567
Matsuda index	-0.150	**0.022***	-0.083	0.312
HOMA-β	0.152	**0.021***	0.061	0.459
insulinogenic index	-0.036	0.584	0.065	0.428
disposition index	-0.172	**0.008***	-0.005	0.954
HDL-C (mg/dL)	-0.099	0.134	-0.182	**0.026***
LDL-C (mg/dL)	0.198	**0.002***	0.038	0.649
TG (mg/dL)	0.085	0.197	-0.020	0.807
non-HDL-C (mg/dL)	0.232	**0.000***	0.079	0.340
Hb (g/dL)	0.135	**0.040***	0.411	**<0.01***
WBC (× 10^3/μL)	0.062	0.345	0.115	0.163
Plt (× 10^4/μL)	0.130	**0.047***	-0.097	0.237
Fe (μg/dL)	0.130	**0.048***	0.380	**0.025***
CRP (mg/dL)	0.070	0.285	0.197	**0.016***

$P < 0.05$ considered significant. BMI, body mass index; PG0, fasting plasma glucose; PG30, 30 minutes postload plasma glucose; PG60, 60 minutes postload plasma glucose; PG120, 120 minutes postload plasma glucose; IRI0, preload immunoreactive insulin; IRI30, 30 minutes postload immunoreactive insulin; IRI60, 60 minutes postload immunoreactive insulin; IRI120, 120 minutes postload immunoreactive insulin; HOMA-IR, homeostasis model assessment insulin resistance; HOMA-β, homeostasis model assessment beta cell; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; TG, triglyceride; Hb, hemoglobin; WBC, white blood cells; Plt, platelets; CRP, C-reactive protein.

Table 5 Multivariate stepwise regression analysis for PG120 and related factors in young men.

Dependent variables	Model	B	SE	β	t	P
PG120	Constant	41.787	15.452		2.704	0.007
	non-HDL-C	0.082	0.36	0.147	2.253	0.025
	PG0	0.431	0.161	0.17	2.679	0.008
	ferritin	0.037	0.017	0.138	2.119	0.035

$P < 0.05$ considered significant. PG0, fasting plasma glucose; PG120, 120 minutes postload plasma glucose: HDL-C, high density lipoprotein-cholesterol.

insulin secretion potential and the lower BMI in young women [7]. To understand the gender disparity in glucose tolerance among young individuals, this study focused particularly on ferritin within the hematological parameters. Our analysis revealed that serum ferritin levels in healthy men in their 20s were about three times higher than in women.

Ferritin is involved in iron storage and protection against oxidative stress. The concentration of serum ferritin is a marker of iron dynamics and inflammation [22]. Serum ferritin levels increase due to cellular damage [23] and are associated with carcinogenesis [24),25]. This study found that, in healthy Japanese women in their 20s, unlike men, serum ferritin levels positively correlated with hemoglobin and CRP, and negatively with HDL-C and HbA1c. These findings suggest that, in healthy Japanese women, serum ferritin levels reflect the dynamics of iron in RBCs and systemic inflammation, aligning with previous studies [22),23]. Previous research has shown that young, healthy Japanese women exhibit greater insulin secretion than men, contributing to their superior glucose tolerance [7]. Elevated serum ferritin levels in healthy and obese women represent a risk of hyperglycemia [26]. However, in healthy young women, serum ferritin did not show a correlation with insulin secretion or sensitivity, aligning with recent findings [26),27]. This can be partially attributed to the fact that healthy young women have lower BMIs, hemoglobin, and ferritin levels compared to men. Estrogen and iron levels in menstruating women negatively correlate with hepcidin and positively with each other. Estrogen increases serum iron while decreasing hepcidin synthesis [29]. Improved glucose tolerance in young women compared to men may be due to differences in 17β-estradiol [30] and muscle properties [31]. Women have higher lipid reserves in the body and muscles than men [31]. Low doses of 17β-estradiol reduce fasting plasma glucose in postmenopausal women with T2DM [30]. The relationship between ferritin and these factors needs to be clarified.

In contrast to young women, serum ferritin levels were correlated with metabolic markers of glucose in men. In young men, serum ferritin levels showed a positive correlation with PG120, IRI60, IRI120, and HOMA-β, and a negative correlation with the Matsuda index and disposition index. These findings suggest that higher levels of serum ferritin indicate increased peripheral insulin resistance, basal insulin secretion, postprandial insulin secretion, and PG120 values. These findings are consistent with elevated serum ferritin levels in T2DM [10),11),26),32),33]. The majority of studies have shown that T2DM and IGT are associated with high levels of serum ferritin [10),11),26),32),33]. Previous reports corroborate these findings. Testosterone was negatively correlated with insulin secretion in men [34]. Androgen administration induced insulin resistance in healthy women [35]. Higher levels of testosterone in women increase the risk of metabolic syndrome and diabetes [36]. Testosterone increases hemoglobin and hematocrit by stimulating the production of erythropoietin and reduces the concentrations of ferritin and hepcidin [37]. There is an inverse correlation between serum ferritin levels and testosterone in young male adolescents [38]. This study demonstrated that serum ferritin levels predict hyperglycemia after glucose loading even in men in their 20s with normal glucose tolerance. The serum ferritin level is useful for assessing the risk of IGT and T2DM in healthy young men with a single blood sample. Serum ferritin levels can assist in screening high-risk individuals for IGT and T2DM.

This study also showed that serum ferritin levels positively correlated with LDL-C and non-HDL-C in healthy young men. Elevated levels of LDL-C and non-HDL-C are risk factors for ischemic heart disease [39),40]. The American Heart Association (AHA) includes non-HDL-C as one of the eight essential metrics (Life's Essential 8) for cardiovascular health [41]. These results are in line with previous research. Testosterone suppresses pro-inflammatory responses and increases immunomodulatory cytokines such as interleukin (IL)-10 [42),43]. Pro-inflammatory cytokines significantly influence the levels of serum ferritin and hepcidin. Hepcidin is crucial for innate and adaptive immunity [44]. Serum ferritin increases pro-inflammatory cytokines such as IL-1β and TNF-α [45),46]. Regarding inflammation, this study showed that CRP was higher in men than women. BMI, TG, and poor self-rated health (smoking, physical activity, and drinking) are related to CRP [47),48]. From that perspective, our data showed BMI and TG were higher in men than women. These factors may relate to higher CRP. In this study, multivariate analysis suggested that ferritin, non-HDL-C, and fasting plasma glucose are predictive factors for PG120 in the OGTT among healthy Japanese men in their 20s. These findings suggest that serum ferritin levels in healthy young men may reflect glucose and lipid metabolism.

In this study, while the correlation between ferritin and Fe was well observed in women, that was not clear in men. Other factors may modify the relationship between ferritin and Fe in men. Although the detailed mechanism is still unknown, possible mechanisms have been proposed that ferritin causes oxidative stress in the liver, in-

terfere insulin signaling in the liver, or iron deposition result muscle damage and decrease glucose uptake [49),50),51)]. Similarly, the specific mechanism by which serum ferritin is related to lipid metabolism disorders is still unknown. One potential pathway is ferritin heavy and light chains can bind apoB [52)].

This study has several limitations. First, excluding the 90 min value may have compromised our estimates of the accuracy of the Matsuda index. Secondly, this study is purely cross-sectional and provides only indirect evidence of an increased risk for the development of T2DM. Third, participants underwent a single 75g OGTT. Therefore, intraindividual variability was not assessed. Fourth, we did not measure the sex hormones that can affect insulin secretion/resistance. Finally, although we confirmed only a 12-hour overnight fast, which may affect insulin sensitivity or secretion, we did not consider anthropometric measurements, dietary intake, and exercise habits, particularly the behavior of the previous day.

V. Conclusion

In this study, we demonstrated that serum ferritin predicts post-glucose load hyperglycemia in normoglycemic men in their 20s. Furthermore, it reflects lipid metabolism, such as TG and non-HDL-C, in healthy young men. Therefore, serum ferritin is a key marker for the early detection of IGT, T2DM, and dyslipidemia in young men. Further large-scale prospective studies including lipid metabolism, inflammation, and testosterone are required to validate these findings.

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Institutional Review Board Statement: The Gunma University Ethical Review Board for Medical Research Involving Human Subjects (No. 12–41) approved the study. We adhered to the Declaration of Helsinki for all ethical and confidentiality issues.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. All data generated or analyzed during this study are included in this published article.

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