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Circulating myokine levels in different stages of glucose intolerance

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Abstract

Type 2 diabetes is the fastest growing metabolic disease in the world. Recently, muscle is considered an endocrine organ which secretes various peptides that play an important role in insulin resistance and metabolic syndrome. We assessed 4 different myokines, irisin, interleukin-13 (IL-13), follistatin-related protein-1 (FSTL-1), and fractalkine, in normal, prediabetes, and diabetes patients.

A total of 126 participants who visited Gangnam Severance Hospital were enrolled and divided into normal, prediabetes, and diabetes groups based on oral glucose tolerance test and hemoglobin a1c. A cross-sectional study was conducted to measure and compare serum levels of irisin, IL-13, FSTL-1, and fractalkine among the groups.

Irisin level showed a tendency to increase in prediabetes group compared to normal group ($P < .1$) but showed a significant decrease when comparing diabetes from prediabetes group ($P < .001$). IL-13 decreased in diabetes group compared to prediabetes and normal group ($P < .001$, $P < .05$, respectively). FSTL-1 of diabetes group was lower than that of prediabetes group ($P < .05$), and fractalkine was higher in diabetes group compared to that of prediabetes and normal group ($P < .01$, $P < .01$, respectively).

Irisin, IL-13, and FSTL-1 levels were reduced in diabetes group compared to normal or prediabetes group while fractalkine showed a progressive increase from normal to diabetes group. Further studies are warranted to study the roles of various myokine in diabetes through a larger prospective study.

Abbreviations: BMI = body mass index, DBP = diastolic blood pressure, DM = type 2 diabetes, FPG = fasting plasma glucose, FSTL = follistatin-related protein, HbA1c = glycated hemoglobin, HDL-C = high-density lipoprotein cholesterol, HOMA-IR = homeostasis model assessment of insulin resistance, IL-13 = interleukin-13, LDL-C = low-density lipoprotein cholesterol, NGT = normal glucose tolerance, OGTT = oral glucose tolerance test, PPG = postprandial glucose, preDM = prediabetes, SBP = systolic blood pressure, TC = total cholesterol, TG = triglyceride.

Keywords: irisin, myokine, type 2 diabetes

1. Introduction

Type 2 diabetes is the fastest growing metabolic disease in the world which involves various organs. There are about 4.8 million (13.7%) Korean adults diagnosed with diabetes mellitus, and about 8.3 million (24.8%) Korean adults with impaired fasting glucose. With this trend, it is estimated that the number of diabetes patients will increase to 5.9 million by 2050 in Korea.[1,2]

Various studies have been conducted on the importance of muscle in the development of diabetes and insulin resistance. Similar to adipokines secreted from adipose tissue, myokines secreted from muscle are involved in insulin resistance and metabolic syndrome.[3,4] Myokine is a hundreds of proteins secreted by myocytes following muscular contraction, and myokine receptors are found in muscle, liver, fat, and pancreas which play an important role in glucose metabolism. Irisin is one of the earliest and most studied myokines, but its relation to blood glucose, lipid, or metabolic syndrome is still controversial.[5–12] After irisin, a variety of new myokine has been discovered, but not many studies have been conducted yet.

The purpose of this study is to compare the myokine levels in normal, prediabetes, and diabetes patients and to analyze their clinical significance.

2. Methods

2.1. Subjects

A total of 126 participants who visited the Gangnam Severance Hospital were enrolled. Eligible patients were men and women between the ages of 20 and 75 who underwent 75 g oral glucose tolerance test (OGTT) and glycated hemoglobin (HbA1c) test. Participants were divided into 3 groups by American Diabetes

Association diagnostic guidelines: normal, prediabetes, and diabetes group. Patients with estimated glomerular filtration rate $<30 \text{ mL/min}/1.73 \text{ m}^2$, aspartate aminotransferase/alanine aminotransferase >3 times greater the upper normal limit, taking glucocorticoids or any herbal medicine within the past 3 months, chronic inflammatory disease in the active phase or acute infection status, and pregnant or lactating women were excluded. This study was approved by the Institutional Review Board of Gangnam Severance Hospital (3-2006-0005).

2.2. Clinical characteristics

Height and weight were measured, and body mass index (BMI) was calculated by dividing the weight (kg) by the square of the height (m^2). Systolic and diastolic blood pressures were measured by trained nurses with an automatic blood pressure monitor (HEM-7080IC; Omron Healthcare, Lake Forest, IL). Lifestyle, personal medical history of acute or chronic illness, and medication history were assessed using a standard questionnaire.

2.3. Biochemical parameters

Blood samples were taken from the antecubital vein after at least 8 hours of fasting. The fasting plasma glucose concentrations were measured by a standard glucose oxidase method (747 Automatic Analyzer, Hitachi, Tokyo, Japan). Fasting serum insulin was determined by chemiluminescence (RIA Kit, Daiichi, Japan), and HbA1c was measured by high performance liquid chromatography (Variant II, Bio-Rad, Hercules, CA). Triglyceride, total cholesterol, and high-density lipoprotein cholesterol were assessed using Hitachi 7600-120 automated chemistry analyzer (Hitachi 747, Tokyo, Japan). Calculation of low-density lipoprotein cholesterol was done using the Friedewald formula.

A standard 75 g OGTT was also performed, and plasma glucose and insulin levels were measured in venous blood collected at 0, 60, and 120 minutes after ingestion. Insulin resistance was estimated using HOMA equations as follows: homeostasis model assessment of insulin resistance (HOMA-IR) = (fasting glucose [mg/dL] × fasting insulin [mcIU/mL]/405).

Blood samples for myokine tests were taken after 8 hours of fasting and were immediately centrifuged, and serum samples were analyzed for interleukin-13 (IL-13, abcam [ab46038]), fractalkine (R&D [DCX310]), follistatin-related protein-1 (FSTL-1, Boster Immuoleaders [EK0965]), and irisin (Phoenix [EK-067-16]) using enzyme-linked immunosorbent assay.

2.4. Statistical analysis

Statistical analysis was performed using SPSS version 23.0 for Windows (SPSS Inc, Chicago, IL). All data were presented as mean ± standard deviation. The intergroup comparisons were performed using analysis of variance tests. The relationships between myokine and various clinical parameters were examined using Pearson correlation. A value of $P < .05$ was considered statistically significant.

3. Results

3.1. Baseline characteristics

The mean age of subjects was 54 ± 13 years, and 62.7% were men (n = 79). Subjects were stratified into 3 groups according to their glycemic status. The biochemical parameters and clinical

Table 1

Baseline characteristics of participants according to diabetes stages.

	Normal	Prediabetes	Diabetes	P
N	19	36	71	
Age (yrs)	43.2 ± 11.8	52.6 ± 11.7[*]	57.8 ± 12.9[*]	<.01
Sex (M/F)	9/10	22/14	48/23	
SBP (mm Hg)	126.5 ± 16.0	121.3 ± 25.6	122.7 ± 12.0	NS
DBP (mm Hg)	77.8 ± 8.5	78.2 ± 16.0	77.2 ± 8.6	NS
BMI (kg/m²)	24.1 ± 2.7	23.8 ± 5.5	24.4 ± 2.9	NS
FPG (mg/dL)	93.44 ± 6.04	105.50 ± 10.43[*]	140.83 ± 40.71[*,†]	<.01
1 hr PPG (mg/dL)	130.22 ± 41.24	170.25 ± 61.22[*]	277.72 ± 75.23[*,†]	<.01
2 hr PPG (mg/dL)	106.22 ± 19.20	140.02 ± 33.93[*]	266.42 ± 86.64[*,†]	<.01
HbA1c (%)	5.33 ± 0.35	5.96 ± 0.79[*]	7.34 ± 1.55[*,†]	<.01
TC (mg/dL)	183.81 ± 31.28	181.22 ± 36.87	181.40 ± 34.97	NS
TG (mg/dL)	109.07 ± 46.36	118.11 ± 68.05	132.64 ± 129.80	NS
HDL-C (mg/dL)	50.42 ± 10.63	48.00 ± 12.07	47.38 ± 13.36	NS
LDL-C (mg/dL)	118.61 ± 26.21	112.11 ± 30.14	110.44 ± 32.37	NS
HOMA-IR	1.70 ± 1.36	2.26 ± 2.02	3.11 ± 2.40	NS

Data are mean ± standard deviation.
BMI = body mass index, DBP = diastolic blood pressure, FPG = fasting plasma glucose, HbA1c = glycated hemoglobin, HDL-C = high-density lipoprotein cholesterol, HOMA-IR = homeostasis model assessment of insulin resistance, LDL-C = low-density lipoprotein cholesterol, NS = not significant, PPG = postprandial glucose, SBP = systolic blood pressure, TC = total cholesterol, TG = triglyceride.
[*] $P < .05$ compared to normal.
[†] $P < .05$ compared to prediabetes.

characteristics are presented in Table 1. Age, fasting plasma glucose (FPG), postprandial glucose (PPG), and HbA1c were elevated in prediabetes and diabetes groups.

3.2. Myokine at different stages of diabetes

Circulating myokine levels according to glycemic status are presented in Table 2 and Figure 1. Serum irisin level showed a tendency to increase in prediabetes group compared to normal group ($P < .1$), but it was reduced in diabetes group compared to prediabetes group ($P < .001$). Ultimately, irisin was significantly lower in diabetes group compared to both the prediabetes and normal group ($P < .001$, $P < .001$, respectively). IL-13 was reduced in diabetes group compared to prediabetes and normal glucose group ($P < .001$, $P < .05$, respectively), and FSTL-1 was lower in diabetes group compared to prediabetes group ($P < .05$). Fractalkine was higher in diabetes group compared to prediabetes and normal glucose group ($P < .01$, $P < .01$, respectively), and it showed a progressive increase with the deterioration of glucose control.

Also, irisin and IL-13 showed negative correlations with FPG, PPG, and HbA1c (all $P < .001$, all $P < .05$, respectively). Fractalkine showed positive correlation with FPG, PPG, and HbA1c (all $P < .001$).

Table 2

Myokine levels according to diabetes stages.

	Normal	Prediabetes	Diabetes	P
N	19	36	71	
Irisin (ng/mL)	175.61 ± 46.28	202.50 ± 54.67[*]	128.43 ± 38.24[†,‡]	<.01
IL-13 (pg/mL)	29.66 ± 7.43	33.46 ± 18.35	20.04 ± 14.09[†,‡]	<.01
FSTL-1 (ng/mL)	400.62 ± 159.59	497.56 ± 340.0	350.86 ± 220.63[‡]	<.05
Fractalkine (ng/dL)	360.05 ± 107.89	379.28 ± 186.60	522.79 ± 210.86[†,‡]	<.01

Data are mean ± standard deviation.
FSTL = follistatin-related protein, IL = interleukin.
[*] $P < .1$ compared to normal.
[†] $P < .05$ compared to normal.
[‡] $P < .05$ compared to prediabetes.
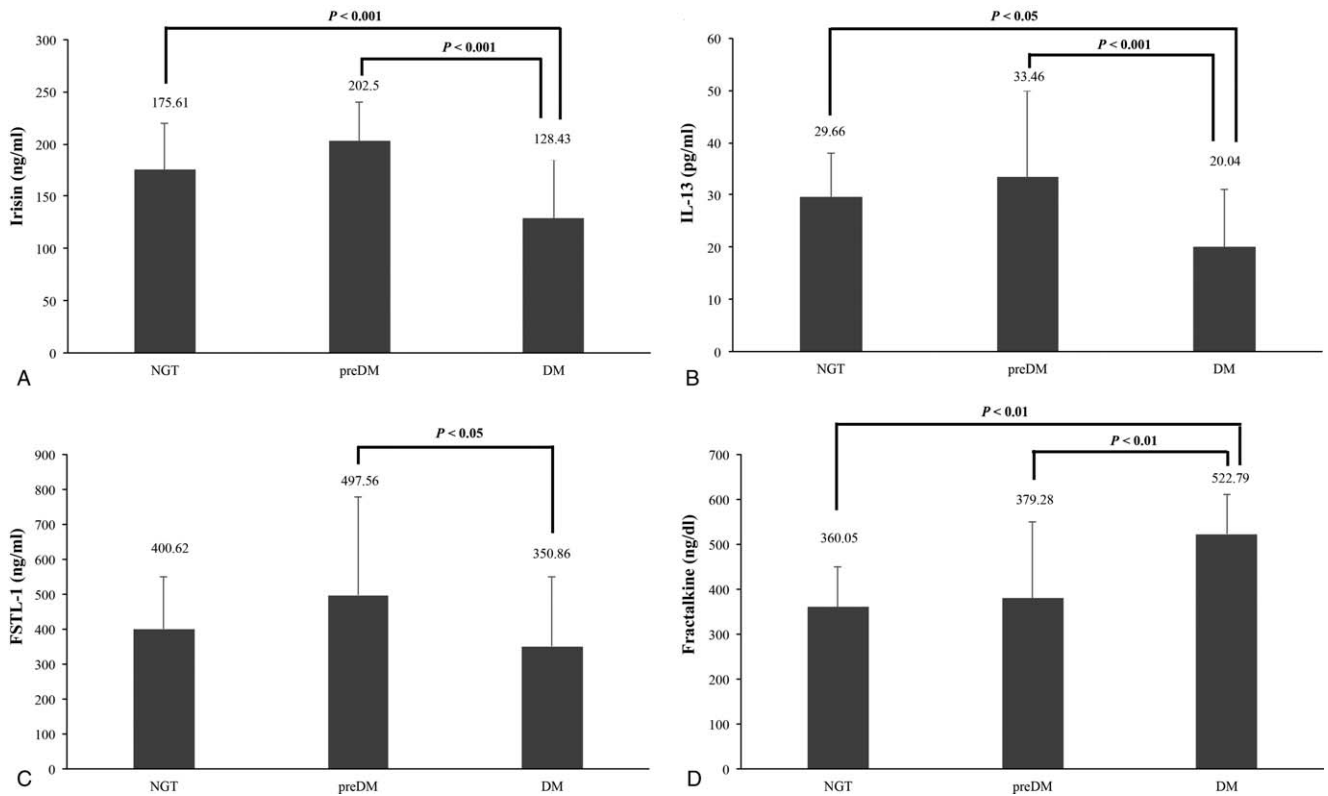
Figure 1. (A) Irisin, (B) IL-13, (C) FSTL-1, and (D) fractalkine levels according to diabetes stages. DM=type 2 diabetes, FSTL=follistatin-related protein, IL=interleukin, NGT=normal glucose tolerance, preDM=prediabetes.

3.3. Association between changes of myokine and glycemic status

All 4 myokines, irisin, IL-13, FSTL-1, and fractalkine consistently showed statistically significant differences between subject with prediabetes and diabetes. Multivariate logistic regression analysis revealed that diabetes group had lower irisin and IL-13 compared to prediabetes group after adjusting for sex, age, BMI, HbA1c, and HOMA-IR (Table 3).

4. Discussion

In the present study, we noticed a significant association between myokine levels and glycemic status in Korean adults. Even after adjusting for various metabolic parameters, there were independent associations between diabetes status and irisin and IL-13. To

the best of our knowledge, this is probably the first human study to reveal the association of diabetes status and various myokine other than irisin.

Muscle tissue has been recently recognized as another endocrine regulator of metabolism. Irisin, whose expression is induced by peroxisome proliferator-activated receptor-γ coactivator 1α and exercise, is known to act on white adipocytes and conversion to brown adipocytes, resulting in increase of thermogenesis and energy expenditure, improvement of insulin sensitivity and glucose tolerance, and reduction in body weights in animal study and in vitro study.[13] Although a lot of human researches on irisin have been done in recent years, the results are still controversial. Stengel et al[14] reported positive correlation between irisin levels and BMI in 40 participants whose BMI were between 12.6 and 36.9 kg/m². Park et al[11] also reported positive

Table 3

Odds ratios and 95% confidence intervals for myokine levels of diabetes compared with prediabetes.

	Irisin		IL-13		FSTL-1		Fractalkine	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Model 1	0.969 (0.957–0.981)	<.001	0.951 (0.925–0.978)	<.001	0.998 (0.997–1.000)	.014	1.004 (0.001–1.006)	.002
Model 2	0.968 (0.955–0.980)	<.001	0.955 (0.929–0.982)	.001	0.998 (0.997–1.000)	.043	1.004 (0.001–1.006)	.002
Model 3	0.974 (0.960–0.989)	.001	0.965 (0.935–0.997)	.030	0.998 (0.996–1.000)	NS	1.002 (0.999–1.004)	NS
Model 4	0.971 (0.955–0.988)	.001	0.961 (0.925–0.998)	.042	0.998 (0.996–1.000)	NS	1.002 (0.999–1.005)	NS

Model 1: Unadjusted.
Model 2: Adjusted for age and sex.
Model 3: Model 2 + HbA1c.
Model 4: Model 3 + BMI, HOMA-IR.
BMI=body mass index, CI=confidence interval, FSTL=follistatin-related protein, HbA1c=glycated hemoglobin, HOMA-IR=homeostasis model assessment of insulin resistance, IL=interleukin, NS=not significant, OR=odds ratio.

correlation between irisin levels and BMI, blood pressure, fasting plasma glucose, triglyceride, and HOMA-IR, and showed that irisin was an independent marker of metabolic syndrome in 151 participants. On the other hand, there are several studies reporting that irisin showed low levels in diabetes or metabolic syndrome patients, and Li et al[15] reported decreased irisin in diabetes was independently associated with elevated advanced glycation end products.[5,6,9]

In our study, irisin showed increasing tendency from normal to prediabetes group $(P < .1)$, then decreased significantly as diabetes progressed further $(P < .001)$, despite of no significant difference of BMI and HOMA-IR among the 3 groups. Chen et al[16] reported obese acanthosis nigricans patients, characterizing hyperinsulinemia, showed higher irisin levels compared to obese patients without acanthosis nigricans. Huerta et al[17] researched irisin levels in 73 healthy overweight/obese women without diabetes, and reported their high irisin levels decreased with weight loss. Both studies suggested that the increase of irisin may be due to the compensatory protective response against early glucose impairment and impaired beta cell function. Initial increasing tendency of irisin in our study can be thought of as a result of these compensatory protective effects, and patients who already had diabetes showed significantly lower irisin levels, since these protective effects do not remain. This is the first time that irisin levels have been analyzed with dividing into 3 groups.

IL-13 is known to increase the glucose uptake by skeletal muscle, oxidation, and glycogen synthesis via an Akt-dependent mechanism. However, muscles of diabetes patients express more microRNA let-7a and let-7d, which are direct translational repressors of the IL-13 gene and result in lower serum IL-13.[18] Moreover, Rutti et al[19] conducted ex vivo study using human and rat islets and reported that IL-13 protects beta-cells from IL-1β induced apoptosis, a cytokine known to play an important role in type 2 diabetes. They suggested that IL-13 shows beneficial effect on glucose homeostasis by preserving beta-cell mass.[19] There is little human study about IL-13 and glucose metabolism. One study reported that IL-13 levels were higher in insulin-resistant patients and correlated with hyperglycemia by cross-sectional study with 92 nondiabetes participants.[20] In our study, IL-13 was slightly higher in prediabetes group compared to normal control group (33.46 pg/mL, 29.66 pg/mL, respectively), but it failed to show statistically significance. Instead, we confirmed that IL-13 of diabetes patients decreased strongly and showed negative correlation with FPG, PPG, and HbA1c. In addition, IL-13 also had an independent association with diabetes, as a result of regression analysis. Similar to irisin, IL-13 may show a compensatory increase in prediabetes state where insulin resistance is the main pathophysiology, and then decreases significantly with the insulin secretory defect seen in diabetes.

FSTL-1, another myokine secreted from skeletal muscles, stimulates muscle glucose uptake via AMP-activated protein kinase activation in muscle cells.[21,22] Therefore, FSTL-1 is thought to be related with glucose homeostasis, and expected to help diabetes treatment in the future as insulin-independent glucose uptake regulators, but no human research has been conducted yet. In our study, FSTL-1 of diabetes group was lower than that of prediabetes group.

Fractalkine is known to increase beta cell insulin secretion in ex vivo and mice study by Lee et al.[23] Shah et al reported obesity was related with higher fractalkine levels in vitro study, and showed fractalkine levels were increased in patients with type 2 diabetes compared to nondiabetes.[24] Recently, Baldane et al

also showed that fractalkine levels were higher in newly diagnosed diabetes patients, using 33 diabetes patients and 34 normal glucose tolerance subjects.[25] Our study showed that diabetes group not only had higher fractalkine levels than normal glucose group, but also higher than prediabetes group. In addition, fractalkine showed strong positive correlation with FPG, PPG, and HbA1c (all $P < .001$).

This study has several limitations. First, the number of study participants is small and needs a larger sample size to confirm the relationship. Furthermore, due to especially a small number of normal glucose group subjects, the change between normal glucose group and prediabetes group has not been sufficiently analyzed. In addition, establishing a cause–effect relationship was not possible due to the cross-sectional design of our study. In spite of the above limitations, this study has significant implications that are clinically relevant, as it is the first to investigate various myokine in 3 groups according to the glycemic status.

In conclusion, we found that irisin increased in early glycemic impairment possibly by compensatory protective response and decreased after diabetes progression by analyzing it in 3 groups. IL-13 and FSTL-1, which lacked human research, showed low levels in diabetes patients in this study. In particular, IL-13 showed a negative correlation with glycemic parameters such as FFP, PPG, and HbA1c, and was suggested that it could be used as an independent predictive marker. Fractalkine was in line with previous studies that it showed higher levels in diabetes compared to normal glycemic subjects. In addition to this, we included prediabetes group in our study and found that fractalkine levels increased progressively among 3 groups. It also showed strong positive correlation with glycemic parameters such as FFP, PPG, and HbA1c.

This study suggests that each myokine may have various responses and roles in glycemic metabolic impairment, and it is necessary to study the roles of various myokine in diabetes through a larger prospective study.

Author contributions

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