Preptin and Myostatin Independently Increase in Pre-Diabetics and Patients of Type 2 Diabetes Mellitus

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Abstract- The relation between serum preptin, myostatin, insulin, and also homeostatic model assessment-insulin resistance were examined in pre-diabetes persons and newly diagnosed patients with overt type 2 diabetes mellitus. A total of 84 subjects were included in the study and assigned into three groups: normoglycemic participants (group 1=27), pre-diabetes (group 2=30), and T2DM (group 3=29). Serum insulin, preptin, and myostatin levels were measured with immunoradiometric assay (IRMA), enzyme-linked immunosorbent assay (ELISA), and chemiluminescent immunoassay (CLIA), respectively. Patients with T2DM had higher levels of preptin compared to normoglycemic (461.25±53.90 vs. 407.54±54.78, *P*<0.001). Furthermore, these patients had elevated levels of myostatin compared with controls (2710.60±559.09 vs. 2246.37±416.40, *P*<0.001). Preptin and myostatin both positively correlated with serum insulin (r=0.369, *P*=0.01, and r=0.309, *P*=0.04, respectively). However, no significant association was found between serum preptin and myostatin levels. Stepwise multiple regression analysis showed that insulin was affected more by preptin, with only a trivial contribution from myostatin. Serum preptin and myostatin levels increase in pre-diabetic subjects and even further in type 2 diabetic patients. The correlation between preptin and insulin evolves when pre-diabetes or overt type 2 diabetes develops. Moreover, serum myostatin increases in association with insulin and not HOMA-IR in diabetic conditions.

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Keywords: Myostatin; Preptin; Insulin; HOMA-IR; Type 2 diabetes

Introduction

Pancreatic islets secret various hormones along with insulin as the most important one in regulating glucose balance. Other pancreatic peptides including preptin also contribute to the glucose homeostasis (1,2). Preptin is a 34-amino acid peptide derived from proinsulin-like growth factor II (pro-IGF-II), secreted simultaneously with insulin, acting to augment glucose-mediated insulin release (2). Preptin levels in serum are remarkably increased in type 2 diabetes (T2DM), gestational diabetes, and impaired glucose tolerance associated polycystic ovary syndrome (3-6). In addition to its contributions to glucose homeostasis, preptin is involved in bone metabolism and osteoblastogenesis (7).

Myostatin, also called growth/differentiation factor 8 (GDF-8), is a peptide hormone secreted by skeletal muscles, and its production and secretion negatively correlate with muscle mass (8). Declined expression of myostatin is associated with the development of hypertrophic muscles and reduced muscular insulin resistance (9). Moreover, diabetic mice with muscular hypertrophy exhibit elevated levels of myostatin (10). Mechanistically, decreased myostatin expression in animal models of diabetes leads to increased GLUT4 protein expressions and enhanced glucose uptake in muscle tissues (11). Nonetheless, conflicting findings regarding myostatin levels in diabetic patients have been obtained thus far. While one investigation reports elevated levels of serum myostatin in patients with T2DM together with its positive correlation with homeostatic model assessment-insulin resistance (HOMA-IR) (12);

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another study finds no change in serum levels of myostatin between T2DM and normal subjects, stating that only muscular gene expression of the myostatin significantly differs, with T2DM patients having higher levels of muscular myostatin expressions (8).

Based on the above descriptions, the aim of the present investigation was to simultaneously evaluate serum preptin and myostatin levels in T2DM patients as well as pre-diabetes and normal subjects; and to assess their relations together with serum insulin levels and HOMA-IR, the index of insulin resistance.

Materials and Methods

Study design and participants

This case-control study was conducted on eighty-four subjects (56 males and 28 females) from those who referred to Vali-Asr hospital, Tehran, Iran, in 2016. Twenty-nine newly diagnosed T2DM patients, according to the American Diabetes Association (ADA) criteria for the diagnosis of diabetes (13), were assigned to the first group. Thirty subjects with fasting plasma glucose (FPG) of 100 to 126 mg/dl, confirmed by two consecutive measurements, were considered pre-diabetic and allocated to group 2. Group 3 consisted of the progeny of T2DM patients, more than twenty-year-old, and were checked for their FPG with the normoglycemic ones (FGP<100 mg/dl) selected for the study.

Patients with type 1 diabetes, kidney, cardiovascular, and liver, or other known major disease were excluded, and none of the subjects were taking medications affecting glucose metabolism including insulin secretagogues, α-glucosidase inhibitors, biguanides, and thiazolidinediones. The informed consent was obtained from all participants, and the study protocol was approved by the human research ethics committee of the Beheshti University of Medical Sciences, Tehran, Iran.

Measurements

Blood pressure measurements were performed by trained nurses after a resting period of 10 minutes. Fasting blood was drawn to measure serum preptin, myostatin, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). To measure glucose levels fasting plasma samples were utilized. Serum/plasma chemistry parameters including glucose, TG, TC, HDL-C, and LDL-C were measured by enzymatic methods (Pars Azmoon, Tehran, Iran) using an automated chemistry analyzer (Selectra XL, Tehran, Iran). Glycated hemoglobin (HbA1c) measurements were done using whole blood samples with ion exchange chromatography-spectrophotometry method (BioSystems, Barcelona, Spain).

Insulin levels were measured with immunoradiometric assay (IRMA) method using a commercial kit (Izotop, Budapest, Hungary). For Preptin measurements, an enzyme-linked immunosorbent assay (ELISA) method using a commercial kit (Sunlong Biotech., Hangzhou, China) was selected. And finally, myostatin measurements were performed by using a chemiluminescent immunoassay (CLIA) method using a commercial kit (EIAab, Wuhan, China).

Statistical analysis

To analyze data, SPSS statistical software (version 18.0) was utilized. Mean, and standard deviation were the parameters for descriptive statistics. To compare the groups, ANOVA was used, followed by Tukey's post hoc analysis. Finally, to clarify the relationship between the variables, Pearson's correlation coefficient followed by stepwise multiple regression analysis was implemented. Sample size calculations were performed by using the Power and Sample Size Calculation (PS) software, version 3.1.2. The calculation was based on the information obtained from a pilot study for preptin levels. Thus, the sample size was estimated to be nearly 30 subjects per group, in order to achieve an alpha of 5% and a power of 80%.

Results

A total of 84 subjects were dedicated into 3 groups of 1) 29 patients with T2DM in the first group, 2) 30 subjects with impaired fasting glucose (IFG) in the second group, and 3) 27 persons with normoglycemia in the third group of the study.

The mean age of T2DM patients and pre-diabetic subjects were 44.8±7.6 years and 41.6±6.1 years, respectively. The mean age of normoglycemic participants was significantly lower (25.5±4.1) as compared to other two groups (P<0.001), since they were specifically selected from the offspring of the T2DM patients. Clinical features and laboratory characteristics of the study subjects has been shown in table 1.

Table 1. Comparison of clinical and laboratory values of subjects in three study groups

Variables		Group 1 Normoglycemic	Group 2 Pre-DM	Group3 DM	*P*
Clinical variables		**Mean±SD**	**Mean±SD**	**Mean±SD**	
Age (years)		25.5 ± 4.1	41.6 ± 6.1	44.8 ± 7.6	0.0001
Weight (kg)		75 ± 12.3	79 ± 7.9	78 ± 10.4	0.533
BMI (kg/m^2)		27.7 ± 4.6	29.1 ± 4.2	28.9 ± 4.4	0.412
SBP (mmHg)		120.5 ± 14.5	131.3 ± 13.8	130.4 ± 12.9	0.03
DBP (mmHg)		76.5 ± 10.3	79.2 ± 9.9	79.4 ± 11.1	0.232
Laboratory variables	**Myostatin (pg/mL)**	2246.37 ± 416.40	2424.23 ± 449.83	2710.60 ± 559.09	0.0001
	Preptin (pg/mL)	407.54 ± 54.78	444.76 ± 68.61	461.25 ± 53.90	0.0001
	Insulin (µiU/L)	6.23 ± 4.76	7.01 ± 5.13	5.77 ± 3.89	0.0001
	HOMA-IR	1.31 ± 0.28	1.52 ± 0.32	1.61 ± 0.42	0.001
	FPG (mg/dL)	76.5 ± 6.4	115.1 ± 7.2	179.8 ± 35.5	0.0001
	HBA1c (%)	4.6 ± 1.1	5.7 ± 0.8	7.1 ± 1.2	0.0001
	Total cholesterol (mg/dL)	175.7 ± 24.8	186.3 ± 29.2	188.9 ± 31.4	0.195
	Triglyceride (mg/dl)	109.5 ± 42.3	158.4 ± 72.5	185.6 ± 77.8	0.0001
	HDL-C (mg/dL)	53.3 ± 4.4	42.7 ± 5.1	44.5 ± 4.9	0.0001
	LDL-C (mg/dL)	95.8 ± 20.4	108 ± 32.8	101.4 ± 30.6	0.255

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, HOMA-IR: homeostatic model assessment-insulin resistance, FPG: fasting plasma glucose, HBA1c: hemoglobin A1C, HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol

$^*P < 0.05$ is considered statistically significant

As demonstrated in table 1, no significant differences could be noted in clinical variables like body weight, BMI, and diastolic blood pressure between the study groups and only statistically significant elevations in systolic blood pressure observed in pre-diabetic and diabetic subjects as compared to the normoglycemic participants (131.3±13.8 vs. 120.5±14.5, and 130.4±12.9 vs. 120.5±14.5, *P*=0.03, respectively).

Diabetic patients and pre-diabetic persons had significantly elevated levels of serum total cholesterol and triglyceride compared with group 3 normal subjects; furthermore, serum HDL-C levels was demonstrated to be significantly lowered in diabetic and pre-diabetic groups. The only parameter of the lipid profile that turned out to be nearly the same among three study groups was serum LDL-C as shown in table 1. While serum insulin levels were slightly higher in pre-diabetics, no statistically significant differences among serum insulin levels could be observed between the groups. HOMA-IR values, however, were significantly higher in T2DM patients in comparison with normoglycemic subjects (1.61±0.42 vs. 1.31±0.28, *P*<0.01); though no such a difference could be observed for HOMA-IR values either between normoglycemic and pre-diabetic subjects or between pre-diabetic and diabetic participants (Table 1).

Normoglycemic participants had the lowest serum levels of preptin and myostatin (407.54±54.78, and 2246.37±416.40, respectively). Pre-diabetics had higher levels of serum preptin and myostatin (444.76±68.61, 2424.23±449.83, respectively); and the highest concentrations of preptin and myostatin were observed for diabetic patients (461.25±53.90, 2710.60±559.09, respectively); noting that all the differences between groups were statistically significant (*P*<0.001).

Pearson's correlation coefficient analyses for myostatin and preptin in three study groups are presented in tables 2 and 3. Furthermore, the correlation coefficients of pooled data between serum preptin, myostatin, and insulin are illustrated in figure 1. No significant correlation was found between serum myostatin and preptin levels. While serum myostatin had a positive correlation with only serum insulin (r=0.424, *P*=0.02) in pre-diabetic subjects, it positively correlated with both serum insulin (r=0.395, *P*=0.03) and HOMA-IR (r=0.381, *P*=0.04) in type 2 diabetic patients. Serum preptin, in addition to having a stronger positive correlation with serum insulin (r=0.456, *P*=0.01) than serum myostatin in pre-diabetics, demonstrated a positive correlation with serum insulin (r=0.403, *P*=0.03) and HOMA-IR (r=0.370, *P*=0.04) in T2DM patients.

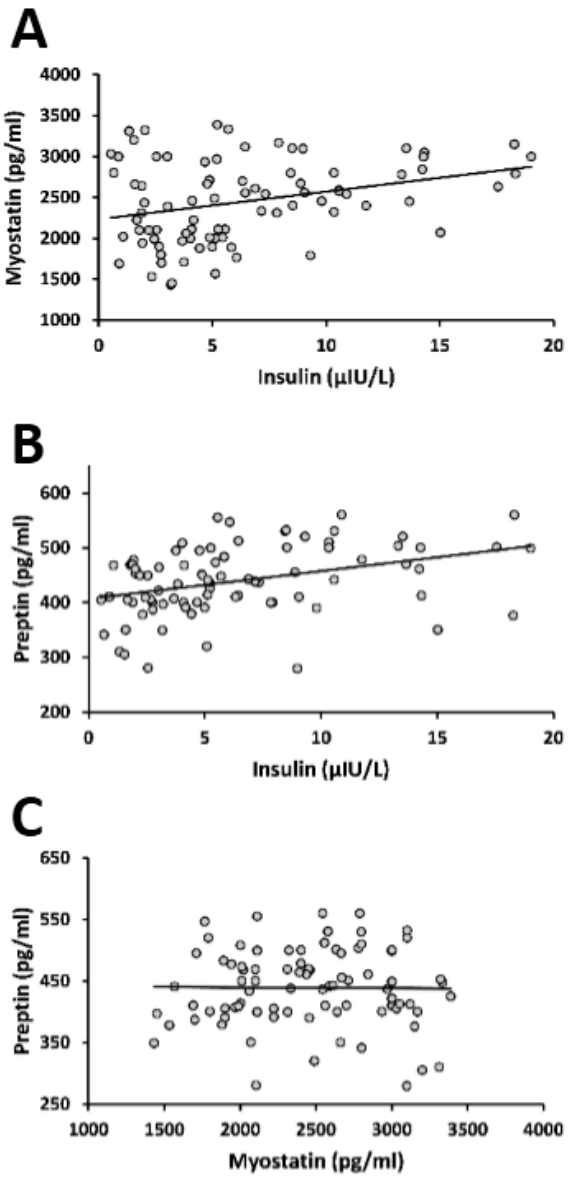

Figure 1. Correlation coefficients of pooled data between serum preptin, myostatin, and insulin.

Table 2. Correlation between myostatin and values of preptin, insulin, and HOMA-IR in the three study groups

Groups	Variables		Preptin	Insulin	HOMA-IR
Group 1 (Normoglycemic)	Myostatin	r	0.003	0.174	-0.042
N: 27		*P*	0.988	0.406	0.842
Group 2 (Pre-DM)	Myostatin	r	0.161	0.424*	0.329
N: 30		*P*	0.395	0.02	0.076
Group 3 (DM)	Myostatin	r	0.267	0.395*	0.381*
N: 29		*P*	0.161	0.03	0.04

HOMA-IR: homeostatic model assessment-insulin resistance.
*$P < 0.05$ is considered statistically significant

Table 3. Correlation between preptin and values of myostatin, insulin, and HOMA-IR in the three study groups

Groups	Variables		Myostatin	Insulin	HOMA-IR
Group 1 (Normoglycemic)	Preptin	r	0.003	0.313	-0.117
N: 27		*P*	0.988	0.128	0.577
Group 2 (Pre-DM)	Preptin	r	0.161	0.456*	0.231
N: 30		*P*	0.395	0.01	0.219
Group 3 (DM)	Preptin	r	0.267	0.403*	0.370*
N: 29		*P*	0.161	0.03	0.04

HOMA-IR: homeostatic model assessment-insulin resistance
*$P < 0.05$ is considered statistically significant

Stepwise multiple regression analysis of myostatin, preptin, insulin, and HOMA-IR are presented in table 4. According to regression analysis, serum myostatin and preptin levels were independent from each other and from HOMA-IR. In diabetic patients, however, preptin was affected by insulin (β=5.68, *P*=0.03). Insulin, in addition to being greatly affected by preptin (β=0.029, *P*=0.03), only slightly was affected myostatin (β=0.034, *P*=0.03). Moreover, the only contributory factor to myostatin emerged to be insulin (β=42.12, *P*=0.034).

Table 4. Stepwise multiple linear regression analysis of myostatin, preptin, insulin, and HOMA-IR in three study groups

Variable		R square	β	95% Confidence interval	*P*
Myostatin (Pre-DM)	**Insulin**	0.180	37.17	6.42 – 67.92	0.02
Myostatin (DM)	**Insulin**	0.156	42.12	3.41 – 80.83	0.034
Preptin (Pre-DM)	**Insulin**	0.208	5.86	1.43 – 10.29	0.011
Preptin (DM)	**Insulin**	0.163	5.68	0.59 – 10.77	0.03
	Preptin	0.163	0.029	0.003 – 0.054	0.03
Insulin (DM)	**Preptin**	0.188	0.034	0.003 – 0.062	0.03
	Myostatin				
HOMA-IR (DM)	**Myostatin**	0.145	0	0	0.041

HOMA-IR: homeostatic model assessment-insulin resistance
*$P < 0.05$ is considered statistically significant

Discussion

The findings of the present study revealed that both myostatin and preptin levels were higher in T2DM patients; however, only pre-diabetics and T2DM patients demonstrated positive correlations between these two peptide hormones and serum insulin levels. Moreover, both serum myostatin and preptin levels were positively correlated with HOMA-IR only in patients with overt diabetes. No association was found between elevated levels of myostatin and preptin in serum. Furthermore, the increase in HOMA-IR was more related to myostatin than preptin.

In agreement with our findings, it has been shown that patients with diabetes (type 2 and gestational) have elevated levels of serum preptin (6,14). Contrary to the results obtained by Yang *et al*., (6), we observed no correlations between serum preptin and diastolic blood pressure, serum triglycerides, and serum HDL-C values. Additionally, they observed no relationship between serum preptin and insulin levels. In the current investigation, the findings of stepwise regression analysis revealed that serum preptin and insulin levels were closely related; however, no association was noticed between serum preptin levels and indices of lipid profile. Supporting these findings, previous experimental studies have shown a close relationship between preptin and insulin secretions, concluding that preptin stimulates glucose-mediated insulin release (15). Likewise, the positive association between preptin and insulin has been demonstrated in gestational diabetes patients (14).

So far, discordant data regarding myostatin levels have been obtained in T2DM; while Garcia-Fontana *et al*., (16), reported decreased levels of myostatin in these groups of patients and Brandt *et al*., (8), demonstrated no change in serum levels of myostatin between healthy

individuals and T2DM, our findings showed that pre-diabetic persons had higher levels of serum myostatin, with the highest levels reached in type 2 diabetics. Furthermore, Wang *et al*., (12), reported higher levels of serum myostatin in patients with T2DM.

The contribution of myostatin to insulin resistance has been evidenced as the findings of one investigation conducted on myostatin-deficient mice revealed enhanced insulin sensitivities as proved by hyperinsulinemic-hyperglycemic clamp test (17). Furthermore, pharmacological inhibition of myostatin in experimental models of chronic kidney disease leads to pronounced improvements in insulin sensitivities (18). It worth mentioning that in the study conducted by Brandt *et al*., (8), muscular gene expression of myostatin had been up-regulated in T2DM patients, despite having normal serum myostatin levels. Regression analysis further revealed that myostatin significantly contributes to the variations seen in HOMA-IR in our study, indicating a connection between myostatin and insulin resistance in patients with T2DM.

The strong association between preptin levels and HOMA-IR in obese subjects has previously been shown (19). Similarly, the association between myostatin and HOMA-IR in T2DMpatients has also been documented (12). Therefore we speculated that a possible connection between myostatin and preptin levels might exist. It was revealed in the present study that these two peptides increase independently in T2DM patients; however, both indices demonstrated a positive correlation with insulin levels and HOMA-IR values.

This study has some limitations; firstly, the relatively small sample size hinders the statistical power of the study to draw reliable analyses. Secondly, other pancreatic hormones co-secreted with insulin-like amylin and ghrelin, as well as other myokines such as irisin and muslin, with proven contributions to insulin resistance, has not been assessed in order to elucidate any possible relations. Finally, the inflammatory mediators including TNF-α and IL-6 have not been measured, as it is known that these cytokines greatly affect serum myostatin levels (17), which could be regarded as possible confounders.

In conclusion, pre-diabetics have higher levels of serum preptin and myostatin; and in T2DMpatients the serum levels of these two peptide hormones are further increased. Nonetheless, no association exists between serum preptin and myostatin levels, even in patients with overt T2DM. While preptin and insulin levels are closely related, myostatin has a slight relation with insulin and not HOMA-IR in patients of T2DM.

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