Association of Serum Myostatin with Body Weight, Visceral Fat Volume, and High Sensitivity C-Reactive Protein But Not With Muscle Mass and Physical Fitness in Premenopausal Women

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ABSTRACT

Background The myokine myostatin regulates muscle mass and has been linked to insulin resistance and metabolic syndrome. However, data on its role in humans is still limited. We, therefore, investigated the associations of serum myostatin with muscle mass, physical fitness, and components of the metabolic syndrome in a cohort of premenopausal women.

Methods We undertook a cross-sectional analysis of 233 women from the monocenter study PPSDiab, conducted in Munich, Germany. Participants had recently completed a pregnancy with or without gestational diabetes. Our analysis included medical history, anthropometrics, oral glucose tolerance testing, laboratory chemistry, cardiopulmonary exercise testing, and magnetic resonance imaging (n = 142) of visceral fat volume, left quadriceps muscle mass, and muscle fat content. Serum myostatin was quantified by a competitive enzyme-linked immunosorbent assay.

Results We observed positive correlations of serum myostatin with body mass index ($\rho = 0.235$; $p = 0.0003$), body fat percentage ($\rho = 0.166$; $p = 0.011$), waist circumference ($\rho = 0.206$; $p = 0.002$), intraabdominal fat volume ($\rho = 0.182$; $p = 0.030$) and high-sensitivity C-reactive protein ($\rho = 0.175$; $p = 0.008$). These correlations were reproduced in linear regression analyses with adjustment for age and time after delivery. We saw no correlations with muscle mass, physical fitness, insulin sensitivity, triglycerides, HDL cholesterol, and blood pressure.

Conclusions Our observation of elevated serum myostatin in women with a higher body fat percentage, visceral obesity, and elevated c-reactive protein suggests that this myokine contributes to the altered muscle-adipose tissue crosstalk in metabolic syndrome. Elevated myostatin may advance this pathophysiologic process and could also impair the efficacy of exercise interventions. Further mechanistic studies, therefore, seem warranted.

Introduction

Myostatin, also known as growth-differentiation factor 8 (GDF8), is a member of the transforming growth factor-β superfamily. Its name-giving function is the negative regulation of muscle cell proliferation [1]. Myostatin is expressed in and secreted from skeletal muscle and, to a lower extent, from adipose tissue [1–3]. Absence or blockage of myostatin increases muscle mass and reduces adipose tissue volume [1, 4]. However, these functions of myostatin have mainly been investigated in mouse models and human subjects with specific genetic mutations or severe concomitant conditions, such as end-stage renal disease [1, 4–7]. Data from healthy human subjects is still inconclusive.

Elevated serum or plasma myostatin has been linked to insulin resistance and metabolic syndrome in some previous studies. However, these have mainly included subjects with severe obesity in groups of less than 30 individuals [8–10]. Brandt et al., on the other hand, examined plasma and muscle myostatin in insulin-resistant individuals with type 2 diabetes in comparison to healthy controls in groups of 76 and 92 individuals, respectively [11]. In their study, plasma myostatin did not differ between the 2 groups. Only muscle myostatin mRNA was elevated with type 2 diabetes.

To help clarify the contradicting results of previous studies, we examined serum myostatin in a cohort of premenopausal women after a recent pregnancy with or without gestational diabetes (GDM). A preceding GDM identifies women at high risk for subsequent permanent diabetes. The underlying pathophysiology often includes insulin resistance and metabolic syndrome [12]. Therefore, we were able to cover a wide range of metabolic states and insulin sensitivity within this study cohort. In the same cohort, we had also demonstrated previously that physical fitness was lower in women after GDM than in women after a normoglycemic pregnancy [13].

The specific aims of this cross-sectional analysis were to examine the association of serum myostatin with physical fitness and skeletal muscle mass on the one hand and with obesity, insulin resistance, and other components of metabolic syndrome on the other hand. Insights into these associations could help to understand the role of myostatin in metabolism, specifically in the altered crosstalk between musculature and adipose tissue in metabolic syndrome [10, 14–16].

Materials and Methods

Study design

All data for this cross-sectional analysis were collected at the baseline visit of the Prediction, Prevention, and Subclassification of Type 2 Diabetes (PPSDiab) study. Detailed information on this study has been published previously [17]. In short, 304 women 3–16 months after a completed pregnancy were recruited from the Diabetes Center and the obstetrics department of the Ludwig-Maximilians-University Hospital in Munich, Germany (LMU Klinikum München). Women after GDM and women after a normoglycemic pregnancy were included in a 2:1 ratio. The main exclusion criteria for this study were alcohol or substance abuse, pre-pregnancy diabetes, and chronic diseases requiring systemic medication (except for medication related to hypothyroidism [n = 52], mild hypertension [n = 4], gastroesophageal reflux [n = 2], and a history of pulmonary embolism resulting in Rivaroxaban prophylaxis [n = 1]). GDM during the preceding pregnancy was diagnosed by a 75 g oral glucose tolerance test (oGTT) using the cut-off values of the International Diabetes and Pregnancy Study Groups recommendations [18]. The ethics committee of the medical faculty of the LMU approved the PPSDiab Study (ID: 300-11), and written informed consent was obtained from all participants.

Study cohort

At the baseline visit of PPSDiab, all women received an oGTT, anthropometric measurements, and questionnaires. They were also invited to participate in a cardiopulmonary exercise test and, on a separate day, a whole-body magnetic resonance imaging (MRI) test. These tests were not mandatory, and not all women participated due to time constraints or family obligations.

We excluded 16 of the 304 PPSDiab study participants from this analysis: 11 due to missing values, 2 because of newly diagnosed type 1 diabetes, 2 because of hyperthyroidism, and 1 because of an upper respiratory infection at the time of the relevant study visit. Of the remaining 288 women, 233 participated in cardiopulmonary exercise testing and had a valid test result. These women constituted the study cohort for this analysis. MRI measurements were available from 142 of these participants who thus constituted the MRI subcohort of this analysis (▶ **Fig. 1**).

oGTT

A 5-point, 75 g oGTT with measurements of plasma glucose and serum insulin was performed after an overnight fast, as described previously [17]. The insulin sensitivity index (ISI) according to Matsuda and deFronzo was calculated as: [10 000/(SQRT(glucose0’ * insulin0’ * (glucose0’ * 15 + glucose30’ * 30 + glucose60’ * 30 + glucose90’ * 30 + glucose120’ * 15)/120 + (insulin0’ * 15 + insulin30’ * 30 + insulin60’ * 30 + insulin90’ * 30 + insulin120’ * 15)/120))] [19].

In a previous study, we had already validated ISI against hyperinsulinemic-euglycemic clamp data in the PPSDiab cohort [17].

Anthropometrics and physical examination

Bodyweight, body fat content (as a percentage of body weight), and muscle mass were quantified using a bioelectrical impedance analysis (BIA) scale (Tanita BC-418; Tanita Corporation, Tokyo, Japan). Height and waist circumference were measured to the nearest centimeter. The body mass index (BMI) was calculated as weight (kg)/height (m)^2. Blood pressure readings were obtained in a seated position with repeated measurements on both arms, separated by at least 15 min. The average from the “higher” arm was recorded. The mean blood pressure was calculated as (diastolic value × 2 + systolic value)/3.

Biochemical measurements

Plasma glucose (Glucose HK Gen.3, Roche Diagnostics, Mannheim, Germany), serum insulin (CLIA, DiaSorin LIASON systems, Saluggia, Italy), high sensitivity c-reactive protein (hsCRP; wide-range CRP, Siemens Healthcare Diagnostics, Erlangen, Germany), and blood lipids (HDL cholesterol, triglycerides; enzymatic caloric test, Roche Diagnostics, Mannheim, Germany) were quantified in a central laboratory [13].

Myostatin serum levels were measured in duplicate by a competitive ELISA (myostatin ELISA; Immundiagnostik AG, Bensheim,

Germany) from serum samples taken after an overnight fast. The samples had been snap-frozen on dry ice immediately after centrifugation and had been stored at − 80 °C until used for the ELISA. The assay measures total myostatin immunoreactivity. Its calibration curve has an optimal range from 0.3 to 83.3 ng/ml, and the inter- and intra- assay coefficients of variability of our measurements were in the reported target ranges (11 % and 10 %, respectively) [20].

Cardiopulmonary exercise testing

We conducted a cardiopulmonary exercise test until exhaustion on a bicycle ergometer (MasterScreen CPX; CareFusion, Höchberg, Germany) as described previously [13]. The workload was increased by 25 W every 3 min until exhaustion, where the maximum workload (W_{max}) was determined. Peak oxygen uptake (VO_{2peak}) was defined as the maximum oxygen uptake that was reached during workload. For a valid exercise test, a maximal respiratory exchange ratio (RER) of at least 1.05 had to be reached [13].

MRI

All MRI tests were conducted on a 3 Tesla system (Ingenia or Achieva; Philips Health Care, Hamburg, Germany). Intraabdominal visceral fat volume was quantified by a semi-manual segmentation method from a whole-body axial sequence using the 2-point Dixon technique (SliceOmatic image analysis software version 5.0 rev. 7, TomoVision, Magog, Canada). The left quadriceps muscle was chosen for muscle volume quantification [21], and the cross-sectional area at 40 % of the femur length was measured [22]. The distance between the femoral head and the lateral condyle defined the femur length. Muscle volume (MV) was estimated by multiplying the cross-sectional area with muscle length and a shape factor [23].

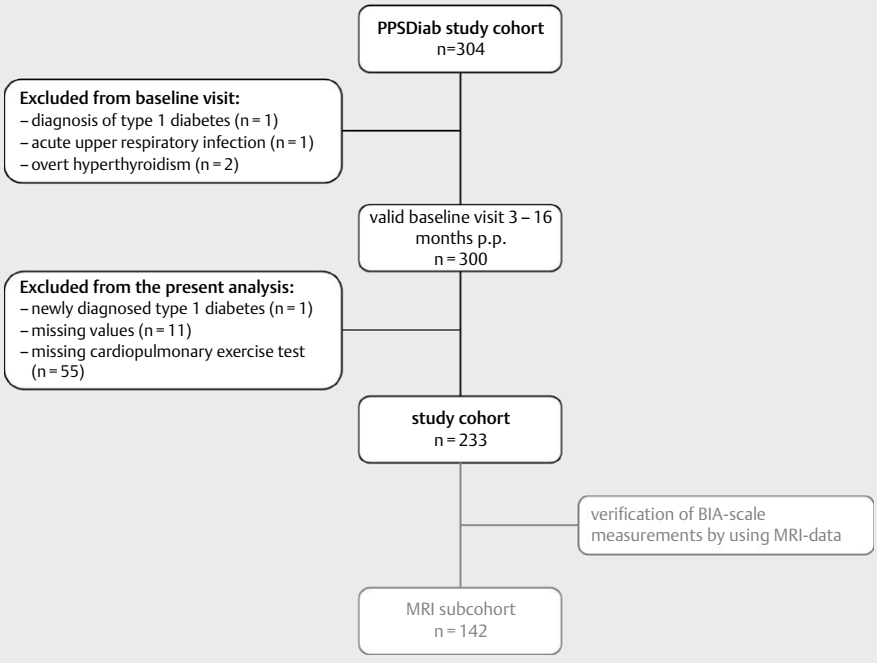
For intramyocellular lipid content, a single-voxel (^{1}H) magnetic resonance spectroscopy (MRS) of the left anterior tibial muscle was conducted by using a point resolved spectroscopy (PRESS) according to Torriani and colleagues [24]. The spectroscopy was analyzed by using the jMRUI software (version 4.0, jMRUI Consortium, Brno, Czech Republic).

Statistics

Normally distributed, metric variables are presented as mean ± standard deviation, other metric variables as median (first quartile-third quartile). Categorical variables are shown as frequency (percent). Nonparametric Spearman correlation analyses were computed. For linear regression models, serum myostatin was logarithmized as the dependent variable to achieve a near-normal distribution. Three or more groups were compared applying the Kruskal-Wallis-Test followed by the Dwass, Steel, Critchlow-Fligner post-hoc procedure for pairwise comparisons. Comparisons of the MRI subcohort with the group of women who did not participate in this test (Suppl. ▶ **Table 1**) were conducted using the Mann-Whitney-U Test for metric variables and the χ^2 or Fisher exact test for categorical variables. A p-value less than 0.05 was considered statistically significant. All analyses were done using SAS University Edition version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

The baseline characteristics of the study cohort are listed in ▶ **Table 1**. All 233 participants were premenopausal women after a recent pregnancy, which was complicated by GDM in 150 cases (64 %). The time since delivery was 9.3 ± 2.8 months. Women with previous GDM had comparable serum myostatin to women after a normoglycemic pregnancy (44.5 ± 11.0 ng/ml vs. 44.6 ± 13.6 ng/ml; p = 0.75). At



▶ **Fig. 1** Flow chart describing the study cohorts. MRI: magnetic resonance imaging.

▶ **Table 1** Baseline characteristics of the study cohort (n = 233).

Age [years]	35.9 ± 4.1	
Time since delivery [months]	9.3 ± 2.8	
Glucose tolerance status	NGT	178 (76 %)
	IFG	21 (9 %)
	IGT	19 (8 %)
	IFG + IGT	10 (5 %)
	T2D	5 (2 %)
GDM in preceding pregnancy	yes	150 (64 %)
	no	83 (36 %)
Breast feading status	full	4 (2 %)
	partial	86 (37 %)
	no	143 (61 %)
BMI [kg/m^2]	23.3 (21.2 - 26.9)	
Waist circumference [cm]	78 (73 - 86)	
Body muscle mass [kg]	44.2 ± 4.6	
Body fat content [%]	31.6 ± 7.8	
Systolic blood pressure [mmHg]	117.2 ± 11.4	
Diastolic blood pressure [mmHg]	73.4 ± 9.1	
Mean blood pressure [mmHg]	88.0 ± 9.4	
Triglycerides [mg/dl]	67 (63 - 72)	
HDL cholesterol [mg/dl]	62 (53 - 90)	
ISI (missing n = 1)	5.4 (3.6 - 7.5)	
hsCRP [mg/dl]	0.05 (0.01 - 0.14)	
W_{max} [W]	130 ± 27	
VO_{2peak} [ml/min]	1856 ± 367	
Myostatin [ng/ml]	44.5 ± 11.9	
additional variables in the MRI subcohort (n = 142)		
Intraabdominal fat volume [dm^3]	1.63 (1.06 - 2.79)	
Quadriceps muscle volume [dm^3]	1.31 (1.16 - 1.43)	
IMCL [%] (missing n = 27)	0.93 (0.61 - 1.44)	

NGT = normal glucose tolerance; IFG = impaired fasting glucose; IGT = impaired glucose tolerance; T2D = type 2 diabetes; GDM = gestational diabetes; BMI = body mass index; ISI = insulin sensitivity index; hsCRP = high sensitivity C-reactive protein; W_{max} = maximum workload; VO_{2peak} = peak oxygen uptake; IMCL = intramyocellular lipid content (Musculus tibialis anterior).

the time of the study visit, 178 women (76 %) were normoglycemic, 50 women (22 %) had prediabetes, and 5 women (2 %) had incident type 2 diabetes diagnosed by the study oGTT. Serum myostatin of women with prediabetes or diabetes did not differ from that of normoglycemic women (44.0 ± 10.8 ng/ml vs. 44.7 ± 12.3 ng/ml; p = 0.78). Furthermore, breastfeeding status (full and partial vs. no) was not associated with changes in serum myostatin (44.6 ± 11.4 ng/ml vs. 44.6 ± 12.3 ng/ml; p = 0.81). The women in the MRI subcohort had baseline characteristics comparable to the women who did not participate in this test (Suppl. ▶ **Table 1**).

In the whole study cohort, serum myostatin correlated positively with BMI, waist circumference, body fat percentage, intraabdominal fat volume, and hsCRP. No correlations were observed between serum myostatin and body muscle mass, musculus quadriceps volume, VO_{2peak}, W_{max}, ISI, triglycerides, HDL cholesterol, and mean blood pressure (▶ **Table 2**; ▶ **Fig. 2**). In linear regression analyses

▶ **Table 2** Correlations of selected parameters with serum myostatin level (n = 233).

	ρ	p-value
BMI	**0.235**	**<0.001**
Body fat content	0.166	**0.011**
Waist circumference	0.206	**0.002**
Intraabdominal fat volume (n = 142)	0.182	**0.030**
Body muscle mass	0.095	0.148
Quadriceps muscle volume (n = 142)	−0.132	0.118
IMCL (n = 115)	−0.031	0.745
W_{max}	−0.108	0.101
VO_{2peak}	0.021	0.752
ISI	−0.082	0.213
Triglycerides	0.0864	0.189
HDL cholesterol	−0.053	0.419
Mean blood pressure	0.068	0.300
hsCRP	0.175	**0.008**

BMI = body mass index; IMCL = intramyocellular lipid content (M. tibialis anterior); W_{max} = maximum workload; VO_{2peak} = peak oxygen uptake; ISI = Insulin sensitivity index; hsCRP = high sensitivity C-reactive protein; ρ = Spearman correlation coefficient; significant p-values are marked in bold.

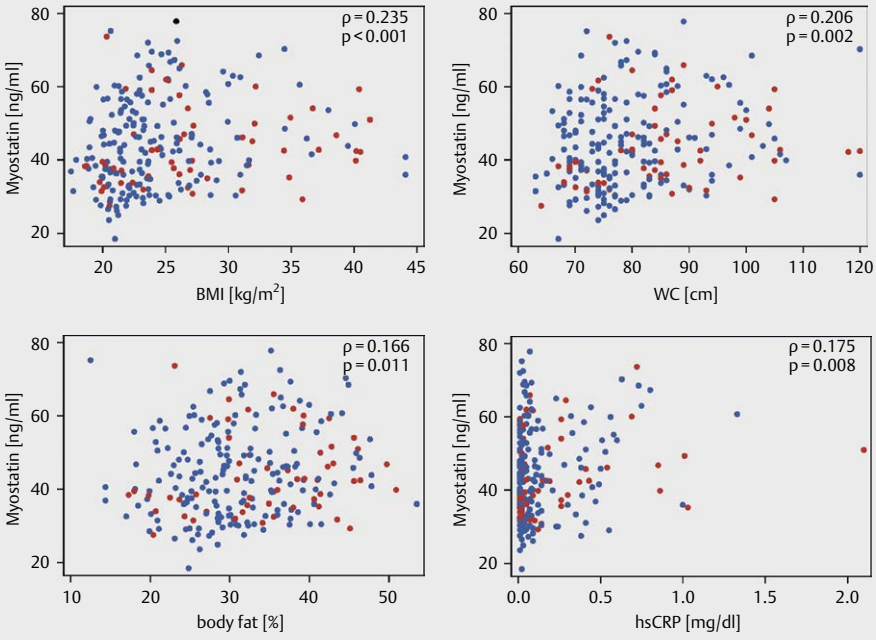
adjusted for age and time since delivery, the associations of serum myostatin with BMI, body fat content, waist circumference, intraabdominal fat volume, and hsCRP remained significant (▶ **Table 3**). ▶ **Fig. 3** illustrates the serum myostatin concentration for waist circumference and hsCRP in combination. For this analysis, we grouped the study cohort according to the medians of these 2 parameters. Within the resulting 4 groups, only the one with both parameters below their median differed significantly from the one with both parameters above their median.

Discussion

In a cohort of premenopausal women, serum myostatin was associated with higher BMI and body fat percentage, visceral obesity (waist circumference and intraabdominal fat volume), and elevated hsCRP. However, it was not associated with physical fitness, muscle mass, insulin resistance, dyslipidemia, and hypertension.

Myostatin, visceral obesity, and metabolic inflammation

Our results are in line with previous human studies that examined severely obese individuals [9, 16]. However, they also extend previous work by demonstrating that less extreme amounts of body fat are similarly associated with higher serum myostatin. Furthermore, our findings suggest a connection of circulating myostatin specifically with visceral obesity and metabolic inflammation, exemplified by hsCRP in our study. These 2 components appeared to have additive effects. Due to the cross-sectional, observational design of our analysis, we cannot elucidate the physiology underlying these associations. Higher myostatin secretion from the musculature may support adipose tissue inflammation, as suggested by Dong et al. based on mouse studies [25]. Alternatively, visceral

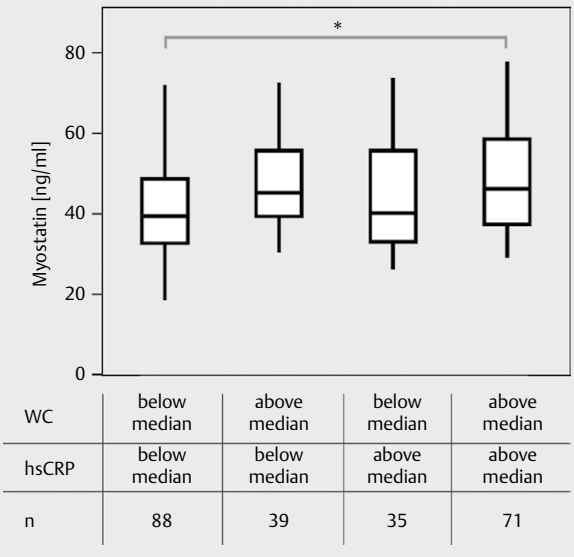


▶ **Fig. 2** Scatter plots of the significant correlations of clinical parameters and serum myostatin in the whole study cohort (see also ▶ **Table 2**). Blue dots indicate normoglycemia, red dots prediabetes (IFG and/or IGT), or type 2 diabetes.

▶ **Table 3** Linear regression analyses with logarithmized serum myostatin as the dependent variable; each analysis adjusted for age and time since delivery (n = 233).

	Standardized Beta-Coefficient	p-value
BMI	0.181	**0.006**
Body fat content	0.155	**0.018**
WC	0.200	**0.002**
hsCRP	0.199	**0.002**
Intraabdominal fat volume (n = 142)	0.169	**0.048**

BMI = body mass index; WC = waist circumference; hsCRP = high sensitivity C-reactive protein; significant p-values are marked in bold.



▶ **Fig. 3** Serum myostatin levels stratified by waist circumference and high-sensitivity C-reactive protein (hsCRP) below vs. above the respective median of the study cohort; Kruskal-Wallis-Test with p = 0.01; * indicates the only pairwise significance in the Dwass, Steel, Critchlow-Fligner post-hoc procedure.

adiposity could drive inflammation [26] and also a rise in serum myostatin, maybe via adipokines like follistatin [27]. This hypothesis is supported by data from Carvalho et al. who compared metabolically "healthy" versus "unhealthy" obese individuals. In their study, serum myostatin was only elevated in the metabolically "unhealthy" group [8].

Myostatin and insulin resistance

Despite serum myostatin correlating with some components of metabolic syndrome in our study, we did not observe a significant association with insulin resistance. Such an association has been demonstrated in previous analyses but only in severely obese individuals (BMI > 40 kg/m² or ~ 35 kg/m²) [9, 28], which were underrepresented in our study cohort (3 % and 7 %, respectively).

Myostatin, muscle mass, and physical fitness

The link between a loss of function mutation in the myostatin gene or short-term pharmacologic use of myostatin antagonists and increased muscle mass has been demonstrated in mice and humans

[4, 5, 29–31]. Furthermore, serum myostatin is increased in sarcopenia associated with end-stage renal disease [6, 7]. Our study included a whole-body estimate of muscle mass from bioimpedance and direct quantification of left quadriceps muscle volume. However, for both parameters, we did not observe a correlation with serum myostatin. Similarly, we detected no correlation between serum myostatin and physical fitness and muscle fat content. These results are consistent with previous work [11, 32]. However, Carvalho et al., who also saw no correlation of myostatin with physical fitness, observed a negative correlation with muscle mass [8].

Our findings may indicate that circulating myostatin is not relevant as a regulator of adult muscle mass in premenopausal women. Alternatively, the serum level of the myokine may not accurately reflect its physiologically relevant concentration since myostatin circulates mainly in inactive states and only gets activated at its target sites [33]. In that case, tissue measurements would be needed to make an accurate assessment.

Strengths and limitations

The main strength of this work is its study cohort, which includes individuals with a broad range of metabolic states but is otherwise very homogeneous. Additionally, the sample size exceeded that of most previous studies and serum myostatin was quantified by a well-established assay. The most important limitation of this study is the potential difference between the measured serum myostatin concentration and its physiologically relevant concentration at its target sites. This phenomenon may obscure important links but is difficult to resolve in human cohort studies. An additional limitation is the cross-sectional nature of this analysis, which precludes the inference of cause-effect relationships. Finally, we did not estimate insulin sensitivity with the gold-standard technique of a hyperinsulinemic clamp. However, we had previously validated the oGTT-derived ISI with clamp data in the same cohort [17].

Conclusion

We observed elevated serum myostatin in association with higher body weight and body fat percentage, visceral obesity, and elevated hsCRP. These results suggest a role of myostatin in the altered muscle adipose tissue crosstalk in metabolic syndrome and related conditions in premenopausal women. Moreover, elevated serum myostatin could impair the efficacy of exercise interventions. Therefore, further mechanistic investigations of this myokine in the context of human metabolism seem warranted.

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Author Contributions

Conceptualization, S.K.M, C.G., and A.L.; formal analysis, S.K.M. and A.L.; investigation S.K.M, C.G., L.W., S.H., A.L.P., C.T., and N.H.; data curation, C.G., A.L.; writing: original draft, S.K.M; writing: review and editing, all; visualization, S.K.M; supervision, J.S. and A.L.; project administration A.L.; funding acquisition A.L., J.S.; A.L. is the guarantor of this work. This work was not published previously.

Data Availability

Data from the PPSDiab study are available from the corresponding author upon reasonable request.

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflict of interest.

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