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The Pro12Ala Polymorphism of the PPAR Gamma 2 Gene Influences Sex Hormone–Binding Globulin Level and its Relationship to the Development of the Metabolic Syndrome in Young Finnish Men

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Association of low sex hormone–binding globulin (SHBG) level with the risk of the metabolic syndrome (MetS) in men has previously been reported. A proline to alanine substitution in codon 12 of the peroxisome proliferator activated receptor gamma 2 (*PPARγ2*) gene has been shown to be related to high insulin sensitivity. The relationship of SHBG levels with the Pro12Ala polymorphism of *PPARγ2* in men has not been previously studied. Therefore, we investigated the effect of the Pro12Ala polymorphism of *PPARγ2* on SHBG levels in 202 young Finnish men. The range of SHBG was from 3.30 to 73 nmol/L (geometric mean = 17.90; 95%CI = 16.62–19.25 nmol/L). Baseline SHBG levels tended to be lower in men who developed the MetS (*n* = 11) compared to men who did not develop the MetS (*n* = 169) (22.85 vs 17.26 nmol/L) on a high-caloric diet during their 6 mo military service. SHBG levels tended to be higher among the subjects with the Ala12Ala genotype compared to subjects with the Pro12Pro or Pro12Ala genotypes of the *PPARγ* gene (27.7 vs 21.7 and 22.7 nmol/L). Among the carriers of the Pro12Pro genotype, those who developed the MetS (*n* = 8) had significantly lower levels of SHBG compared to men who did not develop the MetS (*n* = 93) (13.23 vs 24.22 nmol/L, *p* = 0.027). Among the subjects who developed the MetS those with the Pro12Pro genotype (*n* = 3) had significantly lower levels of SHBG compared to subjects with X12Ala (*n* = 8) (13.23 vs 28 nmol/L, *p* = 0.025). We conclude that the 12Ala allele of *PPARγ2* may influence SHBG levels in young Finnish men.

Key Words: Metabolic syndrome; PPAR2 gene; SHBG; young Finnish men.

Introduction

Sex hormone–binding globulin (SHBG), mainly secreted from hepatocytes in humans, is an extracellular protein that transports sex steroid hormones in the body *(1)*. An association of low plasma levels of SHBG with the development of the metabolic syndrome (MetS) and type 2 diabetes in men has previously been reported *(2,3)*. High levels of SHBG have been associated with high insulin sensitivity and reduced risk of the MetS in aging men *(4)*.

Our previous studies in a population of young Finnish men who were on a high-caloric diet in military service for 6 mo, showed a trend toward insulin resistance with decreased serum adiponectin levels, and the development of the MetS according to the International Diabetes Federation (IDF) definition *(5)*. In that particular population, we showed an interaction of genetic and environmental factors in the regulation of serum adiponectin concentration *(6,7)*.

Peroxisome proliferator-activated receptor γ2 (*PPARγ2*), which belongs to the family of nuclear receptors, is expressed in adipose tissue and is a major regulator of adipogenesis *(8, 9)*. A proline to alanine substitution in codon 12 (exon B) of the *PPARγ2* gene has been related to high insulin sensitivity *(10)*. Treatment with thiazolidinediones, which are *PPARγ* agonists, elevates SHBG levels in women with polycystic ovary syndrome (PCOS) and insulin resistance *(11–14)*.

To our knowledge no previous study has examined the association of SHBG level with the Pro12Ala polymorphism of *PPARγ2* in relation to the development of the MetS among men. Because the SHBG level is associated with insulin sensitivity and because PPARγ2 regulates insulin action, we hypothesized that the PPARγ2 polymorphism could modify the association of insulin resistance–related characteristics with SHBG level in young Finnish men.

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Table 1
Correlation of SHBG Level
with the Components of the Metabolic Syndrome

	Correlation	*p*
Smoking	–0.00534[1]	0.940
Adiponectin (μg/mL)	0.05053[1]	0.475
Body mass index (kg/m^2)	–0.33595[1]	<0.001
Waist (cm)	–0.30413[1]	<0.001
Diastolic blood pressure (mmHg)	–0.11489[1]	0.104
Systolic blood pressure (mmHg)	–0.13259[1]	0.060
HDL cholesterol (mmol/L)	0.17687[1]	0.012
LDL cholesterol (mmol/L)	0.01988[1]	0.779
Cholesterol (total) (mmol/L)	0.01541[1]	0.828
Triglyceride (mmol/L)	–0.24572[2]	0.001
Fasting glucose (mmol/L)	–0.09704[2]	0.170
Fasting insulin (mU/L)	–0.39634[2]	<0.001
QUICKI1	0.37561[1]	<0.001

[1]Pearson correlations.
[2]Spearman correlations.
HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

Table 2
Multiple Linear Regression Analysis
with SHBG (log) as a Dependent Variable

Independent variable	Parameter estimate	Standard error	*t*	*p*
Smoking	–1.46612	1.79578	–0.82	0.4153
Adiponectin (μg/mL)	–0.13018	0.24921	–0.52	0.6020
Body mass index (kg/m^2)	–0.38298	0.26309	–1.46	0.1471
Systolic blood pressure (mmHg)	–0.02234	0.06939	–0.32	0.7479
HDL cholesterol (mmol/L)	0.45686	3.32502	0.14	0.8909
Log of triglycerides (mmol/L)	–2.74954	2.27462	–1.21	0.2282
Log of fasting insulin (mU/L)	–7.84299	2.97718	–2.63	0.0091

HDL: High-density lipoprotein.

Table 3
Prevalence of the Components of the Metabolic Syndrome

Risk factor at the baseline	Defining level*	Prevalence in this study
Waist circumference	≥94 cm	28.2%
Triglycerides	≥1.7 mmol/L	4.4%
HDL cholesterol	<1.03 mmol/L	36.4%
Fasting plasma glucose	≥5.6 mmol/L	12.6%
Blood pressure	≥130/85 mm Hg	44.7%
Metabolic syndrome		10.7%

*International Diabetes Federation (IDF) definition *(5)*

Results

The range of SHBG was from 3.30 to 73 nmol/L (geometric mean = 17.90 and 95%CI = 16.62–19.25 nmol/L). SHBG was inversely related to waist-to-hip ratio (WHR), body mass index (BMI), plasma insulin, and triglyceride (TG) levels, and positively to serum high-density-lipoprotein (HDL) cholesterol and quantitative insulin sensitivity check index (QUICKI) (Table 1). In multiple linear regression analysis, only insulin level was significantly associated with SHBG, independently of other variables included in the model (Table 2).

The diagnosis of the MetS was made when waist circumference was ≥ 94 cm and any two of the components of MetS shown in Table 3 were present. Eleven subjects in this population developed the MetS after 6 mo follow-up on a high-caloric diet. As we have previously reported *(6)*, total, low-density-lipoprotein (LDL) and HDL cholesterol, triglycerides, and insulin significantly increased and serum adiponectin concentrations significantly decreased in the entire population during 6 mo military service. SHBG level did not change significantly (baseline: 21.64; follow-up 21.84 nmol/L) during this period. SHBG levels were significantly lower among the subjects with the MetS at baseline compared to subjects without the MetS (13.5 vs 22.5 nmol/L, $p < 0.001$).

Men with SHBG in the lowest quartile (≤11.4) had significantly higher levels of TG and insulin, they were more obese, and had higher waist and hip circumference and lower serum adiponectin levels compared to subjects with high SHBG levels (Table 4).

Baseline plasma insulin, weight, and WHR were significantly higher in men who developed the MetS during the 6 mo military service, whereas SHBG level tended to be lower among them compared to subjects who remained healthy during the 6 mo of service (Table 5).

Altogether 171 subjects were genotyped for the Pro12Ala polymorphism of *PPARγ2* in this study (31 subjects who did not complete the follow up tests were omitted). The genotype distribution was as follows: 118 (69%) Pro/Pro, 48 (28.1%) Pro/Ala, and 5 (2.9%) Ala/Ala. The genotype distri-bution was in Hardy–Weinberg equilibrium.

SHBG tended to be higher among carriers with the Ala12Ala genotype compared to carriers of the Pro12Pro and Pro12Ala genotypes of *PPARγ2*, but the difference was not statistically significant (27.7 vs 21.7 vs 22.7 nmol/L). Men with the Pro12Pro genotype of *PPARγ2* who developed the MetS had significantly lower levels of SHBG compared to men who did not develop the MetS (Fig. 1). SHBG level correlated (Spearman correlation coefficient) significantly with insulin level ($r = –0.519$, $p < 0.001$) and QUICKI ($r = 0.503$, $p < 0.001$) among carriers of the Pro12Pro genotype. The corresponding correlations among carriers of the 12Ala allele were –0.325 ($p = 0.017$) and 0.324 ($p = 0.018$).

Subjects who developed the MetS and who had the Pro12 Pro genotype ($n = 8$) had significantly lower level of SHBG

Table 4
Variables Related to the Metabolic Syndrome According to the Level of SHBG (Cut-off Point is the Lowest Quartile 11.4 nmol/L)

	SHBG level at the baseline				
	≤11.4 nmol/L) $n = 51$		>11.4 nmol/L $n = 151$		
	Mean	95% CI for mean	Mean	95% CI for mean	*p*
Adiponectin (μg/mL)	10.34	9.40–11.27	11.60	3.66	0.032
Body mass index (kg/m^2)	27.16	25.97–28.35	24.54	223.83–25.24	<0.001
Waist (cm)	91.54	88.30–94.76	85.94	83.95–87.92	0.005
Diastolic blood pressure (mmHg)	75.43	73.15–77.71	73.74	72.18–75.30	0.265
Systolic blood pressure (mmHg)	131.1	126.1–135.23	128.8	126.8–130.7	0.266
HDL cholesterol (mmol/L)	1.10	1.02–1.18	1.16	1.11–1.20	0.213
LDL cholesterol (mmol/L)	2.20	2.03–2.37	2.19	2.10–2.30	0.877
Cholesterol (total) (mmol/L)	3.74	3.55–3.93	3.70	3.60–3.81	0.7135
Triglyceride (mmol/L)	0.85	0.76–0.95	0.70	0.67–0.75	0.012[1]
Fasting glucose (mmol/L), median	5.00	4.80–5.20	4.80	4.70–4.90	0.086[2]
Fasting insulin (mU/L)	10.19	9.25–11.22	7.85	7.43–8.30	<0.001[1]
QUICKI1	0.34	0.33–0.34	0.35	0.35–0.36	<0.001

[1]Based on log-transformed value.
[2]Mann–Whitney *U* test.

Table 5
Levels of the SHBG and Cardiovascular Risk Factors
According to the Development of the Metabolic Syndrome During 6 mo Follow-up

	Metabolic syndrome				
	Healthy at baseline and 6 mo later		Healthy at baseline but developed the metabolic syndrome during 6 mo		
	Mean	95% CI for mean	Mean	95% CI for mean	*p*
SHBG (nmol/L)	19.09	17.62–20.68	15.05	11.26–20.12	0.224[+]
Adiponectin (μg/mL)	11.48	10.92–12.04	11.98	10.27–13.68	0.662
Body mass index (kg/m^2)	23.95	23.39–24.49	30.48	29.09–31.87	<0.001
Waist (cm)	83.97	82.45–85.48	101.7	98.01–105.43	<0.001
Diastolic blood pressure (mmHg)	73.40	72.00–74.81	76.00	70.95–81.05	0.369
Systolic blood pressure (mmHg)	127.5	125.76–129.32	132.9	121.32–144.50	0.159
HDL cholesterol (mmol/L)	1.19	1.14–1.22	1.08	0.94–1.21	0.229
LDL cholesterol (mmol/L)	2.16	2.06–2.24	2.31	1.75–2.86	0.424
Cholesterol (total) (mmol/L)	3.68	3.57–3.78	3.75	3.15–4.35	0.743
Triglyceride (mmol/L)	0.69	0.65–0.72	0.75	0.60–0.94	0.478[+]
Fasting glucose (mmol/L), median	4.8	4.7–4.9	5.10	4.8–5.4	0.391[#]
Fasting insulin (mU/L)	7.66	7.31–8.04	10.36	8.53–12.57	0.011[+]
QUICKI1	0.36	0.352–0.358	0.34	0.324–0.349	0.019

[+]Based on log-transformed value.
[#]Mann–Whitney *U* test.
HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

compared to carriers of the 12Ala allele ($n = 3$) (13.23 vs 28 nmol/L, $p = 0.025$).

Discussion

Our study demonstrated that men who developed the MetS on a high-caloric high-fat diet during their 6 mo military service, especially if they possessed the Pro12Pro genotype of *PPARγ2*, had lower SHBG levels compared to those who had the 12Ala allele. Furthermore, among subjects who were carriers of the Pro12Pro genotype and who developed the MetS, they had significantly lower SHBG level compared to subjects who did not develop the MetS during the

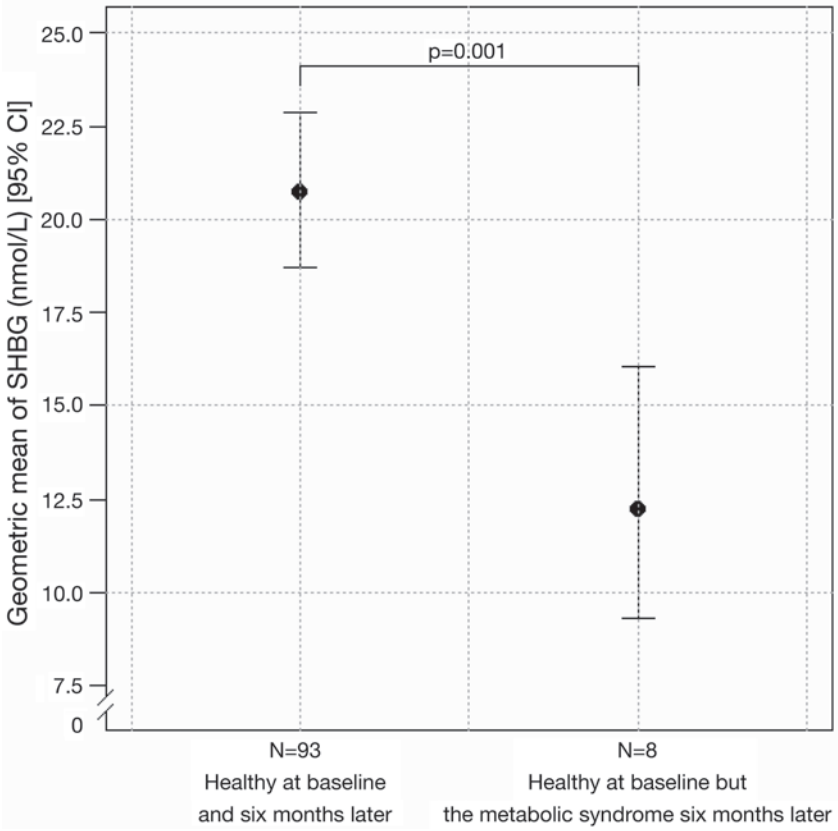

Fig.1. SHBG levels in carriers of the Pro12Pro genotype of *PPARγ2* according to the development of the metabolic syndrome during 6 mo follow-up.

follow-up. The caloric content of ordinary meals served in Finnish military service was 3200–3600 kcal per day and additional energy was obtained from the snacks such as doughnuts and confectionary *(16)*. According to the questionnaire, servicemen reported that they consumed more snacks during the service compared to their normal life *(16)*. This high-caloric diet was the main reason for an increase in plasma lipids during the 6 mo follow-up in military service.

Our study also showed that SHBG levels were higher in subjects with the Ala12Ala genotype of *PPARγ2* in the entire study population, although this difference was not statistically significant. Therefore, low SHBG level in subjects with the MetS could be partly explained by the Pro12Ala polymorphism.

Previous studies have shown that treatment with thiazolidinediones, which are PPARγ receptor agonists, elevate SHBG levels in women with PCOS *(11–14)*, but none of these studies reported the effect of the *PPARγ2* polymorphism on SHBG levels. According to these results and our findings it is likely that *PPARγ* agonists and the Pro12Ala polymorphisms of *PPARγ2* may regulate SHBG levels in humans.

The mechanism(s) via which the PPARγ polymorphism may influence the production and secretion of SHBG are not clear. One possible mechanism could be the effect of PPARγ activation or polymorphisms on plasma insulin levels. The present study showed a significant independent correlation of plasma insulin and QUICKI with SHBG. Insulin could influence and significantly modulate SHBG levels. Insulin has been also shown to be an inhibitor of SHBG production in hepatoma cells in vitro *(18)*. Because acute ectopic expression of *PPARγ* has been shown to induce massive accumulation of triglycerides in the pancreatic beta cells and to impair glucose-stimulated insulin secretion *(19)*, and other studies have also reported that *PPARγ* agonists decrease plasma insulin level and increase SHBG *(11–14)*, one could speculate that the Pro12Ala polymorphism in the present study decreased insulin secretion and therefore increased SHBG level. Another possibility is the linkage disequilibrium between the Pro12Ala polymorphism of *PPARγ2* and other genes potentially involved in the production of SHBG.

Our study showed that men with SHBG in the lowest quartile (≤11.4 nmol/L) had low serum adiponectin levels and high levels of other cardiovascular risk factors, such as TG, insulin, BMI, and waist- and hip-circumference. These results demonstrate that subjects with the MetS have low levels of circulating SHBG. The relationship between SHBG levels and the risk of the MetS has been previously studied *(3)*, but to our knowledge the relationship between serum adiponectin and SHBG in men is a novel finding. Reduced levels of serum adiponectin in obese women with PCOS

and a negative correlation between delta4-androstenedione and serum adiponectin levels have already been demonstrated *(20)*. Testosterone treatment has been shown to cause a specific reduction of high-molecular-weight adiponectin in mice *(21)*. We did not measure testosterone levels in our subjects, but it is possible that men with low SHBG levels could also have low serum adiponectin concentration due to their high concentrations of free testosterone, which is associated with the development of the MetS and type 2 diabetes.

In conclusion, a significant decrease was found in SHBG level among carriers of the Pro12Pro genotype of *PPARγ2* who developed the MetS. In contrast, the 12Ala allele was associated with elevated SHBG levels. These results imply that *PPARγ2* can modulate the risk of the MetS via effects on SHBG levels. Further studies with a longer follow-up and a larger number of subjects are needed to better determine the relationship of *PPARγ* with SHBG.

Materials and Methods

Subjects and Study Design

A population of 202 young Finnish servicemen, aged 17–28 yr, were recruited in this study. The subjects were healthy and were not on any medication. The study sample was drawn in 1995–1997 from two garrisons (Ostrobothnian Brigade and First Signal Company) located in the northern part of Finland. The effect of the military lifestyle on insulin resistance–associated risk factors and adiponectin levels as well as the influence of the genetic background and its interaction with the environment in the development of MetS were investigated among the subjects. As previously described *(15)*, the Ethics Committees of Oulu Deaconess Institute and the Finnish Defence Forces approved the study protocol. All recruited servicemen were informed about the study and gave their written consent.

Methods

Anthropometric and Biochemical Measurements

As previously described *(16)*, systolic and diastolic blood pressures were measured by a trained medical assistant. If systolic blood pressure was > 140 mmHg or diastolic blood pressure > 80 mmHg, the measurement was repeated twice after a 5–10 min rest in a sitting position, and the calculated mean of the two measurements was used in statistical analysis. The subjects were weighed in light clothing. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). Waist circumference was defined as the smallest girth midway between the lowest rib margin and the iliac crest. Hip circumference was measured at the level of the greater trochanter. Waist-to-hip ratio (WHR) was calculated as waist circumference divided by hip circumference. Fasting serum total cholesterol, LDL and HDL cholesterol and TG, fasting plasma insulin, and fasting glucose were measured with the methods that have been described previously *(16)*. Quantitative insulin sensitivity check index (QUICKI) was determined from fasting insulin and glucose values according to the equation: QUICKI = 1/ [log (I0) + log (G0)], in which I0 is fasting insulin and G0 is fasting glucose *(17)*.

Definition of the Metabolic Syndrome

The MetS was defined according to the International Diabetes Federation (IDF) definition as follows: waist circumference ≥ 94 cm plus any two of the following four factors: TG level ≥ 1.7 mmol/L, HDL cholesterol <1.03 mmol/L, systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥ 85 mmHg, fasting plasma glucose ≥ 5.6 mmol/L *(5)*.

Measurement of Serum Adiponectin and SHBG Levels

Fasting serum adiponectin concentration was determined by the Human Adiponectin ELISA Kit (B-Bridge International, Inc., CA, US). This ELISA is a sandwich-type enzyme-linked immunoassay consisting of primary (mouse anti-adiponectin monoclonal) antibody-coated plate, secondary (rabbit anti-human adiponectin polyclonal) antibody, detection (HRP-conjugated goat anti-rabbit IgG) antibody, substrate for HRP, and a (recombinant human) adiponectin standard. One human serum sample with a known adiponectin concentration was used as reference. The coefficient of variation of this assay was 5.8%.

Fasting serum SHBG levels were determined with SHBG ELISA (IBL Immuno-Biological Laboratories GmbH, Hamburg and Germany). This assay is a solid-phase enzyme-linked immunosorbent assay based on the sandwich principle. The coefficient of variation for this assay is 11.67.

DNA Analysis

Genomic DNA was obtained from whole blood leukocytes. The Pro12Ala polymorphism of *PPARγ2* was determined by the TaqMan polymerase chain reaction (PCR) method. The following primers and probes were used: forward primer 5'-GACAAAATATCAGTGTGAATTACA GC-3' and reverse primer 5'-CCCAATAGCCGTATCTG GAAGG-3' (product size, 167 bp).

Statistical Analysis

All statistical analyses were performed using the SPSS programs (v.11.5) for Windows. The normality of the distribution of variables was evaluated using the Kolmogorov–Smirnov test. SHBG level, fasting plasma insulin, and TG were log-transformed to correct the skewed distribution. The correlations between SHBG and other variables were tested with Pearson and Spearman correlations when appropriate. Independent sample *t* test or Mann–Whitney *U* test was applied to compare the means of different variables of subjects without the MetS at baseline and 6 mo later, and those without the MetS at the baseline who developed the MetS during the 6 mo of service. Multivariate linear regression analysis was used to investigate the association of different variables with SHBG level. p values <0.05 were considered statistically significant.

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