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# Relationship between testosterone, sex hormone binding globulin, angiotensin related growth factor and insulin resistance in normal weight and obese men

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## KEYWORDS

Angiotensin related growth factor;  
Insulin resistance;  
Obesity;  
Sex hormone binding globulin;  
Testosterone

**Abstract** *Background:* Angiotensin related growth factor (AGF) is a liver derived factor that potentially antagonizes obesity and insulin resistance (IR).

*Aim:* The objective of this work is to determine AGF serum levels and evaluate its relationship with total testosterone (TT), calculated free testosterone (cFT), sex hormone binding globulin (SHBG), insulin and IR in normal weight and obese men.

*Subjects:* A total of 60 men were included: twenty normal weight subjects with body mass index (18.5–24.7 kg/m<sup>2</sup>) and 40 obese men with BMI (30–39.5 kg/m<sup>2</sup>).

*Methods:* Serum AGF was measured by enzyme linked immunosorbent assay. Serum TT, SHBG, and insulin were analyzed by chemiluminescent immunoassay.

*Results:* Angiotensin related growth factor was significantly lower in the obese group as compared with normal weight group. In all subjects, AGF correlated positively with TT and SHBG and negatively with 2 h oral glucose tolerance test (OGTT) glucose and fatty liver index (FLI). In normal weight group, AGF correlated positively with age, SHBG, fasting insulin, HOMA-IR, AST and

*Abbreviations:* AGF, angiotensin related growth factor; FLI, fatty liver index; MAPK, mitogen activated protein kinase; PPARs, peroxisome proliferator activated receptors; PGC-1 $\alpha$ , peroxisome proliferator activated receptor  $\gamma$  coactivator 1 $\alpha$ .

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ALT and negatively with BMI, TC and LDL-C. In the obese group, it correlated positively with BMI and negatively with TG. Multiple linear regression analysis revealed that SHBG and fasting glucose were positive predictors of AGF serum levels. In the total sample, SHBG correlated negatively with BMI, fasting glucose, fasting insulin, HOMA-IR, FLI and positively with AGF, QUICKI and HDL-C.

*Conclusion:* The present results revealed for the first time an association between SHBG and AGF serum levels. It could be suggested that they overlap to regulate metabolic homeostasis in normal weight men and that the disturbed inter-relationship could contribute to the pathogenesis of insulin resistance and obesity. Moreover, the observed relationship between SHBG and AGF in the present study could clarify the unresolved controversies regarding specific mechanistic relationships between SHBG abnormalities and abnormalities in glucose homeostasis.

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## 1. Introduction

Obesity – a condition of excess adipose tissue in the body – is emerging as an important cause of adverse health outcomes. It is a worldwide epidemic that continues to grow at an alarming rate. It has been postulated that in addition to increased food intake,<sup>1</sup> defective biochemical pathways involved in energy expenditure may contribute significantly to obesity.<sup>2</sup>

Angiopoietin-related growth factor (AGF), also known as angiopoietin-like protein 6 (Angptl 6), is secreted predominantly from the liver into the systemic circulation. Data provide very compelling evidence that AGF is a powerful modulator of energy metabolism and adiposity.<sup>3</sup> In animal experiments, AGF increased energy expenditure and improved insulin sensitivity and lipid profiles.<sup>4,5</sup> It has been proposed that AGF stimulates fat burning in peripheral tissues through the activation of p38 mitogen activated protein kinase (MAPK) pathway and downstream effects on respiration and gene expression linked to mitochondrial uncoupling and energy expenditure.<sup>4</sup> It was also reported that AGF suppresses gluconeogenesis via the activation of phosphoinositide 3-kinase/Akt signaling cascades resulting in reduced transcriptional activity of FOXO1, a key transcription factor of glucose-6-phosphatase expression.<sup>5</sup>

Testosterone (T), the predominant sex hormone in men, is an anabolic hormone with a wide range of beneficial effects on men's health. Both animal and human studies suggest that T has favorable effects on insulin sensitivity in the male. Castration of male rats results in marked insulin resistance, which is abolished by physiological T replacement.<sup>6</sup> In men, low T concentrations are associated with insulin resistance and adiposity.<sup>7,8</sup> In contrast, T administration to obese men with low normal T levels was associated with an improvement in insulin sensitivity.<sup>9</sup>

It is well known that androgen mediates its biological functions by binding with a high affinity to specific androgen receptors, which are found in normal human liver, the main site of AGF expression. These androgen receptors belong to the family of nuclear receptors that act as transcription factors regulating the expression of several genes.<sup>10</sup> Moreover, SHBG which binds tightly to T and regulates free sex hormone bioavailability to target tissues has emerged as a stronger correlate with insulin resistance.<sup>11,12</sup> The plasma membranes of various kinds of cells, including the liver, have been shown to be capable of binding specifically and with a high affinity to

SHBG, mediating sex hormone signaling at the cell membrane through SHBG receptors.<sup>13,14</sup>

## 2. Aim of the work

The aim of the present work is to determine AGF serum levels and evaluate its potential relationships with TT, cFT, SHBG, insulin and IR in normal weight and obese men.

## 3. Subjects

Sixty adult males, from working staff in Medical Research Institute and their relatives, age ranging between 38 and 60 years, were enrolled in this study. All participants gave their approval to participate in the study and a written consent was obtained from each subject. The Ethics Committee of the Medical Research Institute, Alexandria University, approved the study protocol and all experimental procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

All subjects were apparently healthy with no previous diagnosis or evidence, upon physical examination, of hypertension (BP < 130/85 mmHg), cardiovascular disease, diabetes mellitus, endocrine disorders, renal or liver impairment, and were not using any medications or supplements.

According to the WHO criteria for definition of obesity on the basis of body mass index (BMI), subjects with BMI (18.5–24.9) were included in the normal weight group ( $n = 20$ ) and those with BMI (30–39.9) were included in the obese group ( $n = 40$ ).

## 4. Methods

All the studied participants were subjected to:

### 4.1. Anthropometric measurements

Body mass index (BMI) was calculated as weight in kilograms divided by square of the height in meters ( $\text{kg}/\text{m}^2$ ). In addition, waist and hip circumferences were measured and the waist to hip ratio (WHR) was then calculated.

### 4.2. Laboratory investigations which included

4.2.1. Fasting and 2 h serum glucose concentrations after ingestion of 75 g glucose solution were determined.

Subjects with impaired glucose tolerance (fasting glucose >110 mg/dl and/or 2 h OGTT glucose > 140 mg/dl) were excluded from the present work.

- 4.2.2. Complete lipid profile (TC, HDL-C, LDL-C, and TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transpeptidase (GGT) activities were measured. Analysis was performed on the autoanalyzer Konelab 30i system using reconstituted freeze dried forms of multianalyte calibrators for the serum samples.
- 4.2.3. We evaluated the fatty liver condition with the validated fatty liver index (FLI) derived from TG levels, BMI, waist circumference, and GGT levels as follows:  $\exp[0.953 \times \ln(\text{TG}) + 0.139 \times \text{BMI} + 0.718 \times \ln(\text{GGT}) + 0.053 \times \text{waist-15.745}]/(1 + \exp[0.953 \times \ln(\text{TG}) + 0.139 \times \text{BMI} + 0.718 \times \ln(\text{GGT}) + 0.053 \times \text{waist-15.745}]) \times 100$ .<sup>15</sup>
- 4.2.4. Fasting insulin, total testosterone, and SHBG were analyzed by chemiluminescent immunoassays on an IMMULITE 2000 auto analyzer.
- 4.2.5. Insulin resistance was estimated using the homeostatic model assessment of insulin resistance (HOMA-IR).<sup>16</sup> The equation used was:  $\text{HOMA-IR} = (\text{fasting insulin } [\mu\text{IU/ml}] \times \text{fasting glucose } [\text{mmol/L}])/22.5$ .
- 4.2.6. Insulin sensitivity was estimated using the quantitative insulin sensitivity check index (QUICKI)<sup>17</sup> using the following equation:  $\text{QUICKI} = 1/[\log(\text{fasting insulin } (\mu\text{IU/mL}) + \log(\text{fasting glucose } (\text{mg/dL}))]$ .
- 4.2.7. Total testosterone and SHBG levels were used to calculate free testosterone levels.<sup>18</sup> Free testosterone levels (nmol/L) =  $([-a + \sqrt{b}]/c)/10^{-9}$  with  $a = \text{SHBG (nmol/L)} - \text{TT(nmol/L)} + 23.43$ ,  $b = a^2 + (4 \times 23.43 \times \text{TT [nmol/L]})$  and  $c = 2 \times 23.43 \times 10^9$  for a standard average albumin concentration of 4.3 g/dl.

- 4.2.8. Serum AGF concentration was measured by Enzyme-Linked Immunosorbant assay using a commercially available kit AG, AdioGen Inc. Incheon, Korea according to manufacturer's instructions.

### 4.3. Statistical analysis

Statistical analysis was performed using SPSS software. Normality was assessed by the Kolmogorov–Smirnov test. Data were described as (mean  $\pm$  SD) or median (range). Differences between groups were analyzed with unpaired Student's *t*-test, or Mann–Whitney *U* test for normally and non-normally distributed parameters, respectively. Pearson's and Spearman Rank correlation coefficients were calculated to evaluate the inter-variable associations. To adjust the effects of covariates and identify independent relationships, multiple linear regression analysis was performed. Covariables were added to the models either because they were identified as significantly associated with AGF or SHBG in this study, or because they had been previously described as determinants for AGF or SHBG. The predictors which caused multicollinearity problems were omitted. Variables that were not normally distributed such as age, SHBG, and FLI, were log-transformed to approximate normal distributions. A value of  $P < 0.05$  was considered as statistically significant for all analyses.

## 5. Results

The clinical and metabolic variables of normal weight ( $n = 20$ ) and obese groups ( $n = 40$ ) are shown in Table 1. Obese men had significantly higher anthropometric indices (BMI, waist circumference, and WHR), fasting and 2 h OGTT glucose,

**Table 1** Clinical and metabolic variables of normal weight and obese groups.

Variables	Normal ( $n = 20$ )	Obese ( $n = 40$ )	<i>P</i> value
Age (years)	41(39–60)	43.5(38–57)	0.147
BMI (kg/m <sup>2</sup> )	21.5(18.5–24.7)	32.6(30–39.5)	<0.001**
Waist circumference (cm)	86.15 $\pm$ 7.32	110.95 $\pm$ 6.08	<0.001**
Waist-hip-ratio	0.89(0.79–1.2)	0.92(0.77–1.34)	<0.001**
AGF (ng/ml)	631.13 $\pm$ 112.9	464.55 $\pm$ 126.68	<0.001**
TT (nmol/L)	23.81 $\pm$ 7.32	15.17 $\pm$ 3.87	<0.001**
cFT (nmol/L)	0.38 $\pm$ 0.13	0.35 $\pm$ 0.09	0.38
SHBG (nmol/L)	50.7(22.2–136)	26.2(11.8–42.2)	<0.001**
Fasting glucose (mg/dl)	90.85 $\pm$ 6.92	96.82 $\pm$ 9.65	0.017*
2 h OGTT glucose (mg/dl)	84.17 $\pm$ 10.52	101.62 $\pm$ 15.9	<0.001**
Fasting insulin ( $\mu$ IU/ml)	5.05 $\pm$ 1.89	10.97 $\pm$ 5.6	<0.001**
HOMA-IR	1.14 $\pm$ 0.47	2.62 $\pm$ 1.35	<0.001**
QUICKI	0.38 $\pm$ 0.02	0.33 $\pm$ 0.02	<0.001**
Total cholesterol (mg/dl)	169.65 $\pm$ 28.34	206.35 $\pm$ 33.51	<0.001**
HDL-cholesterol (mg/dl)	45.2 $\pm$ 9.35	39.98 $\pm$ 7.21	0.02*
LDL-cholesterol (mg/dl)	106.6 $\pm$ 24.69	143 $\pm$ 33.54	<0.001**
Triglyceride (mg/dl)	91.05 $\pm$ 52.97	125.68 $\pm$ 44.8	<0.01*
AST (u/l)	23.75 $\pm$ 9.1	24.08 $\pm$ 7.81	0.88
ALT (u/l)	18.95 $\pm$ 7.89	31.12 $\pm$ 11.76	<0.001**
GGT (u/l)	21.95 $\pm$ 7.52	32.7 $\pm$ 13	<0.001**
Fatty liver index	16.83(1.66 $\pm$ 41.46)	83.82(53.63 $\pm$ 97.44)	<0.001**

Data are shown as mean  $\pm$  SD or median (range).

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

fasting insulin, HOMA-IR, lipid profile (TC, LDL-C and TG) and liver parameters (ALT, GGT and FLI) than those of normal weight men. Serum levels of AGF, TT, SHBG, QUICKI and HDL-C were significantly lower in the obese group as compared with normal weight group. No significant differences among the normal weight and obese group as regard age, cFT and AST were detected. The correlations between serum AGF levels and other clinical and metabolic variables are shown in Table 2. When all subjects were considered for analysis ( $n = 60$ ), AGF serum levels correlated positively with TT and SHBG and negatively with BMI, waist circumference, WHR, 2 h OGTT glucose, TC, LDL-C, TG, ALT and FLI. In normal weight group ( $n = 20$ ), AGF serum levels correlated positively with age, SHBG, FI, HOMA-IR, AST and ALT, and negatively with BMI, TC, LDL-C. In the obese group ( $n = 40$ ), AGF correlated positively with BMI and negatively with TG. To determine factors affecting serum AGF levels, multiple linear regression analysis was performed. The model included AGF (as a dependent variable) and age, SHBG, FG, FI, TC and TG (as independent variables). As shown in Table 3, SHBG and fasting glucose were positive, while TC and TG were negative predictors of AGF serum levels. ( $R^2 = 31.5$ ,  $F = 5.51$ ,  $P < 0.0005$ ).

The correlations between serum TT, cFT and SHBG and other clinical and metabolic variables, in all studied subjects ( $n = 60$ ), are shown in Table 4. Total testosterone correlated positively with SHBG and both correlated positively with AGF and QUICKI, and negatively with BMI, WC, FG, fasting insulin, HOMA-IR, TC, LDL-C, and FLI. Sex hormone binding globulin also correlated significantly and positively with HDL-C and negatively with ALT and GGT. As regards cFT, no correlations were detected except a significant positive correlation with TT. Multiple linear regression analysis was

performed to evaluate the degree to which age, fasting glucose, fasting insulin, AGF, FI and TG predicted SHBG. As shown in Table 5, age was positive whereas fasting insulin and FLI were negative predictors of SHBG serum levels ( $R^2 = 54.4$ ,  $F = 12.74$ ,  $P < 0.0005$ ). In addition, FLI correlated positively and significantly with FI and HOMA-IR in both the normal weight group, FI ( $r^s = 0.532$ ,  $P = 0.01$ ), HOMA-IR ( $r^s = 0.594$ ,  $P = 0.006$ ) and in obese group, FI ( $r^s = 0.580$ ,  $P < 0.001$ ), HOMA-IR ( $r^s = 0.564$ ,  $P < 0.001$ ).

## 6. Discussion

Deposition of excess fatty acids into fat cells in the form of triglycerides is the biochemical basis of obesity, thus any imbalance in food intake and energy utilization may result in obesity. This homeostasis is complex and is regulated by a multitude of poorly understood metabolic and endocrine factors.<sup>19</sup>

Angiopoietin-related growth factor is a liver derived circulating factor that counteracts obesity and related insulin resistance through increased energy expenditure.<sup>4</sup> Both visceral and subcutaneous fat depots were significantly increased in AGF-deficient mice, and sections of white adipose tissue from these mice showed increased adipocyte size relative to those from wild-type mice. Furthermore, a large amount of lipid accumulation in liver and skeletal muscle was observed in AGF-deficient mice. Whereas no increase was observed in daily food intake in AGF-deficient mice compared with wild-type mice, it was suggested that inactivation of AGF in vivo leads to decreased energy expenditure and obesity.<sup>4</sup>

Human studies showed that serum AGF levels were paradoxically higher in subjects with metabolic syndrome and diabetes than in healthy groups.<sup>20,21</sup> It has been suggested

**Table 2** Correlations between AGF serum levels and various parameters in total, normal weight and obese groups.

Variables	AGF		
	Total ( $n = 60$ )	Normal ( $n = 20$ )	Obese ( $n = 40$ )
Age (years)	-0.036 <sup>a</sup> /0.78	0.646 <sup>a</sup> /0.03*	-0.014 <sup>a</sup> /0.93
BMI (kg/m <sup>2</sup> )	-0.347 <sup>a</sup> /0.007**	-0.569 <sup>a</sup> /0.009**	0.34 <sup>a</sup> /0.03*
Waist circumference (cm)	-0.495/0.001**	-0.249/0.29	0.086/0.59
Waist-hip-ratio	-0.370 <sup>a</sup> /0.004**	0.053 <sup>a</sup> /0.82	-0.251 <sup>a</sup> /0.12
Total testosterone (nmol/L)	0.413/0.001**	0.397/0.08	-0.104/0.52
cFT (nmol/L)	0.28/0.83	-0.117/0.62	-0.014/0.93
SHBG (nmol/L)	0.357 <sup>a</sup> /0.005**	0.447 <sup>a</sup> /0.04*	-0.13 <sup>a</sup> /0.43
Fasting glucose (mg/dl)	-0.028/0.83	0.086/0.72	0.205/0.2
2 h OGTT glucose (mg/dl)	-0.335/0.009**	0.018/0.94	-0.113/0.48
Fasting insulin (μIU/ml)	-0.185/0.15	0.485/0.03*	0.091/0.57
HOMA-IR	-0.174/0.18	0.472/0.036*	0.012/0.46
QUICKI	0.245/0.059	-0.388/0.09	-0.081/0.62
Total cholesterol (mg/dl)	-0.434/0.001**	-0.473/0.03*	-0.143/0.37
HDL-cholesterol (mg/dl)	0.026/0.84	-0.212/0.37	-0.155/0.34
LDL-cholesterol (mg/dl)	-0.340/0.008**	-0.468/0.03*	0.02/0.9
Triglyceride (mg/dl)	-0.405/0.001**	0.066/0.78	-0.469/0.002**
AST (u/L)	0.094/0.47	0.467/0.038*	-0.047/0.77
ALT (u/L)	-0.314/0.01*	0.472/0.036*	-0.229/0.15
GGT	-0.199/0.13	0.078/0.74	0.2/0.91
FLI	-0.443 <sup>a</sup> /0.001**	-0.211 <sup>a</sup> /0.37	0.077 <sup>a</sup> /0.64

Coefficients ( $r$ ) and  $P$  values are calculated using Pearson's correlation.

<sup>a</sup> Spearman's correlation analysis.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

**Table 3** Multiple linear regression analysis with serum AGF levels as dependent variable. ( $R^2 = 31.5$ ,  $P < 0.0005$ ).

Independent variables	Unstandardized coefficients		Standardized coefficients Beta	<i>t</i>	<i>P</i> value
	<i>B</i>	Std. error			
Age	−254.539	391.409	−.085	−.650	.518
SHBG	240.352	97.148	.355	2.474	.017*
Fasting glucose	4.246	1.915	.270	2.217	.031*
Fasting insulin	3.195	3.487	.121	.916	.364
Total cholesterol	−1.197	.496	−.298	−2.411	.019*
Triglyceride	−.982	.361	−.339	−2.717	.009**

\*  $P < 0.05$ .\*\*  $P < 0.01$ .**Table 4** Correlations between TT, cFT and SHBG serum levels and various parameters ( $N = 60$ ).

Variables	TT	cFT	SHBG
Total testosterone (nmol/L)	1.000		
cFT (nmol/L)	0.587/0.001**	1.000	
SHBG (nmol/L)	0.646 <sup>a</sup> /0.001**	−0.031 <sup>a</sup> /0.81	1.000
Age (years)	−0.03 <sup>a</sup> /0.81	−0.208 <sup>a</sup> /0.11	0.100 <sup>a</sup> /0.44
BMI (kg/m <sup>2</sup> )	−0.595 <sup>a</sup> /0.001**	−0.211 <sup>a</sup> /0.1	−0.504 <sup>a</sup> /0.001**
Waist circumference (cm)	−0.579 /0.001**	−0.111/0.4	−0.487 <sup>a</sup> /0.001**
Waist-hip-ratio	−0.262 <sup>a</sup> /0.04*	−0.153 <sup>a</sup> /0.24	−0.23 <sup>a</sup> /0.07
AGF(ng/ml)	0.413/0.001**	0.28/0.83	0.357 <sup>a</sup> /0.005**
Fasting glucose (mg/dl)	−0.262/0.04*	−0.056/0.67	−0.315 <sup>a</sup> /0.01*
2 h OGTT glucose (mg/dl)	−0.371/0.004**	−0.148/0.26	−0.233/0.07
Fasting insulin (μIU/ml)	−0.368/0.004**	−0.131/0.32	−0.465 <sup>a</sup> /0.001**
HOMA-IR	−0.384/0.002**	−0.135/0.3	−0.498 <sup>a</sup> /0.001**
QUICKI	0.44/0.001**	0.172/0.18	0.485 <sup>a</sup> /0.001**
Total cholesterol (mg/dl)	−0.475/0.001**	−0.136/0.29	−0.361 <sup>a</sup> /0.005**
HDL-cholesterol (mg/dl)	0.052/0.69	−0.14/0.28	0.265 <sup>a</sup> /0.04*
LDL-cholesterol (mg/dl)	−0.472/0.001**	−0.145/0.27	−0.368 <sup>a</sup> /0.004**
Triglyceride (mg/dl)	−0.055/0.67	0.145/0.27	−0.236 <sup>a</sup> /0.07
AST (u/L)	0.182/0.16	−0.115/0.38	−0.109 <sup>a</sup> /0.4
ALT (u/L)	−0.234/0.07	−0.102/0.43	−0.381 <sup>a</sup> /0.003**
GGT	−0.244/0.06	.002/0.98	−0.460 <sup>a</sup> /0.001**
FLI	−0.482 <sup>a</sup> /0.001**	−0.049 <sup>a</sup> /0.71	−0.575 <sup>a</sup> /0.001**

Coefficients (*r*) and *P* values are calculated using Pearson' correlation.<sup>a</sup> Spearman's correlation analysis.\*  $P < 0.05$ .\*\*  $P < 0.01$ .**Table 5** Multiple regression analysis with SHBG as dependent variable. ( $R^2 = 54.4$ ,  $P < 0.0005$ ).

Independent variables	Unstandardized coefficients		Standardized coefficients Beta	<i>t</i>	<i>P</i> value
	<i>B</i>	Std. error			
Age	1.583	.426	.358	3.714	.000**
Fasting glucose	−.004	.002	−.163	−1.625	.110
Fasting insulin	−.010	.004	−.266	−2.560	.013**
AGF	.000	.000	.179	1.666	.102
Fatty liver index	−.232	.062	−.500	−3.771	.000**
Triglyceride	.001	.000	.160	1.407	.165

\*\*  $P < 0.01$ .

that the up regulation of AGF might be a compensatory mechanism that partly limits hyperglycemia in humans.

In the present study, all participants had normal glucose tolerance. Our results revealed that AGF serum levels and

insulin sensitivity index (QUICKI) were significantly lower while fasting insulin and HOMA-IR index were significantly higher in obese as compared to normal weight group. Furthermore, anthropometric indices (BMI, WC, and WHR) were



significantly higher in the obese group and correlated negatively with AGF serum levels in all studied subjects. These results confirm the physiological role of circulating AGF suggested in antagonizing obesity.<sup>4</sup>

Several studies indicate that skeletal muscle regulates energy expenditure, mediated by PPARs (PPAR $\alpha$ , PPAR $\delta$ , and PPAR $\gamma$ ) and their coactivators (PGC-1 $\alpha$  and PGC-1 $\beta$ ) which govern the expression of major enzymes of oxidative phosphorylation.<sup>22,23</sup> Significant decreases in the expression of the genes encoding PPAR $\delta$  and PGC-1 $\alpha$  in skeletal muscle in AGF null mice, and increases in the expression of PPAR $\alpha$ , PPAR $\delta$  and PGC-1 $\alpha$  in skeletal muscle of AGF transgenic mice were indicated.<sup>4</sup> It was stated that over expression of AGF in vivo activates molecules involved in stimulating energy expenditure, and thereby leads to decreased adiposity. Moreover, Oike et al.<sup>4</sup> also reported that AGF protein enhances phosphorylation of p38 MAPK, which in turn, improves the stability and activation of PGC-1 $\alpha$  protein and increase in energy expenditure.<sup>24</sup>

It should be noted that, in people who are not significantly physically active, 60–75% of total energy expenditure is consumed as the resting metabolic rate (RMR), which can significantly affect weight gain or weight loss.<sup>25</sup> In a study by Mirzaei et al.<sup>26</sup> they detected significantly higher levels of RMR/kg in subjects with higher circulating AGF concentration and conversely lower levels of RMR/kg in those with lower circulating AGF concentration in which 72.3% of them were obese (BMI  $\geq$  30).

In addition to its effect on energy expenditure, convincing evidence has also presented that AGF potently antagonizes insulin resistance. One mechanism whereby AGF affects insulin sensitivity is inhibition of abnormal lipid stores in insulin target tissues. It was found that even on a high fat diet, AGF transgenic mice are protected against hepatic and muscle steatosis resulting in the maintenance of insulin sensitivity.<sup>4</sup> It was reported that AGF activates p38 MAPK<sup>4</sup> which plays a critical role in lipid metabolism in the liver as it has an inhibitory role in hepatic lipogenesis.<sup>27</sup> In humans, hepatic lipogenesis is strongly associated with fatty liver.<sup>28</sup> This could explain the significantly higher FLI detected in the obese group which correlated negatively with AGF serum levels and positively with fasting insulin and HOMA-IR in all studied subjects.

Angiopoietin-related growth factor could also affect insulin sensitivity by inhibiting gluconeogenesis. In an in vitro study by Kitazawa et al.<sup>5</sup>, AGF was reported to suppress glucose production in rat hepatocytes in a concentration dependent manner through reduced expression of glucose-6-phosphatase. This partially accounts for insulin sensitizing effect of AGF. Thus a lower AGF serum level may suggest over expression of glucose-6-phosphatase in liver which is sufficient to perturb whole glucose and lipid homeostasis.<sup>29</sup> Our results revealed that, fasting and 2 h OGTT glucose, TC, LDL-C and TG were all significantly higher while HDL-C was significantly lower in obese as compared to normal weight group. Moreover, significant negative correlations were detected between AGF serum levels and 2 h OGTT glucose, TC, LDL-C and TG in the total sample. These results are in accordance with those of Mirzaei et al.<sup>26</sup>, who demonstrated significant improvement of lipid profile in those with higher levels of AGF.

Contradictory to our results, Kadmatsu et al.<sup>3</sup>, found that AGF serum levels are elevated in diet induced obese mice and in obese humans; they considered that although the

normal production of AGF from liver may be up regulated to counteract weight gain and promote insulin sensitivity, the effect of AGF might also be attenuated in the obese state and does not reverse obesity at all. Moreover, in diabetic patients<sup>21</sup> AGF serum levels correlated positively with BMI and the authors suggested that AGF antagonizes IR before the onset of disease but is no longer an effective antagonist after the disease is manifest. It was suggested that AGF resistance may be attributed to decreased AGF sensitivity and impaired receptor or post receptor signaling in its target tissues.<sup>20,21</sup>

Interestingly, our results revealed that, AGF serum levels significantly and positively correlated with fasting insulin and HOMA-IR in normal weight men. This up regulation of AGF may be a physiological response to counteract insulin resistance and compensate for disturbed metabolic profile, prevent weight gain and dyslipidemia as noticed by the significant negative correlations detected between AGF serum levels and BMI, TC and LDL-C in normal weight men. This is in coincidence with the significant positive correlation detected in normal weight group between AGF serum levels and ALT, an index of worsening of metabolic factors<sup>30</sup> and an early marker of insulin resistance.<sup>31</sup> In the obese group, no such correlations were detected; only TG serum levels correlated negatively with AGF serum levels, while BMI correlated positively with AGF suggesting disturbed physiological homeostasis, or a sort of AGF resistance as previously suggested.<sup>20,21</sup>

In accordance with previous studies<sup>7,8,32</sup>, the present study detected significantly lower levels of TT and SHBG in the obese group as compared with normal weight group, and both correlated positively with QUICKI and negatively with BMI, WC, FG, and fasting insulin, HOMA-IR, TC and LDL-C. Calculated FT, which serves as a stronger marker than TT for androgen deficiency in males, showed no significant difference between both groups and it correlated only with TT. Total testosterone and SHBG both correlated positively and significantly with AGF serum levels. However no relationship could be detected between cFT and AGF serum levels, suggesting that SHBG may be the primary determinant of the apparent relationship between TT and AGF. Multiple liner regression analysis revealed that SHBG positively predicted AGF serum levels suggesting that SHBG signaling may regulate AGF expression in the liver and circulation.

The origin of low levels of TT in obese men is multifactorial<sup>33</sup>, and attributed, in part, to decreased SHBG levels. The low SHBG serum levels in obese individuals have been largely attributed to hyperinsulinemia.<sup>34</sup> Other investigators have suggested that insulin suppression of SHBG is nonspecific and probably reflects global reduction in hepatic protein secretion under non-physiologic experimental conditions,<sup>35</sup> this could also explain the lower AGF serum levels detected in the obese group. It was also suggested that excess monosaccharide consumption and levels of fasting glucose rather than elevated insulin levels are the actual determinants of liver SHBG production.<sup>36,37</sup> In addition, Selva et al.<sup>36</sup>, have previously described the role of de novo lipogenesis in the down regulation of hepatic SHBG expression, and a strong association between fatty liver disease and low circulating SHBG levels has been reported by Peter et al.<sup>37</sup> This is in coincidence with our results in which SHBG correlated negatively with FG, fasting insulin and FLI. Moreover, those studies could explain the significant positive correlation detected in the present study between AGF

and SHBG serum levels, as it was previously mentioned that AGF activates p38 MAPK<sup>4</sup> which has an inhibitory role on hepatic lipogenesis.<sup>27</sup> In addition, multiple linear regression analysis revealed that fasting insulin and FLI negatively predicted SHBG serum levels.

It was demonstrated that alterations in circulating levels of SHBG appear to be associated with changes in glucose homeostasis among individuals without diabetes,<sup>38</sup> and that low SHBG levels may precede the development of impaired glucose metabolism.<sup>39</sup> Moreover, lower SHBG levels predict metabolic syndrome<sup>40</sup> and T<sub>2</sub>DM,<sup>41</sup> independent of serum androgen levels, and the exact mechanism by which SHBG influences metabolic components remains largely unclear. The observed relationship between SHBG and AGF in the present study could clarify the unresolved controversies regarding specific mechanistic relationships between SHBG abnormalities and abnormalities in glucose homeostasis.<sup>40,41</sup> The small sample size is one of the limitations of the current study. Also the cross-sectional design of the study makes interpretation of the results limited. It does not allow clarification of any cause-effect mechanism.

## 7. Conclusion

The present results revealed for the first time an association between SHBG and AGF. Considering the findings of the present study together with previous observations, it could be suggested that SHBG and AGF may overlap to regulate metabolic homeostasis and that the disturbed relationship between them could contribute to the pathogenesis of insulin resistance and obesity. Prospective studies with large sample identifying more in depth the relationship between SHBG and AGF would be an important area for future research. The benefits of AGF administration in obese subjects are to be defined by large and long clinical trials.

## Conflict of interest

None declared.

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## References

1. Swinburn BA, Sacks G, Lo SK, Westterterp KR, Rush EC, Rosenbaum M, et al. Estimating the changes in energy flux that characterize the rise in obesity prevalence. *Am J Clin Nutr* 2009;**89**:1723–8.
2. Lowell BB, Bachman ES. Beta-Adrenergic receptors, diet-induced thermogenesis, and obesity. *J Biol Chem* 2003;**278**:29385–8.
3. Kadamatsu T, Tabata M, Oike Y. Angiopoietin-like proteins: emerging targets for treatment of obesity and related metabolic diseases. *FEBS J* 2011;**278**:559–64.
4. Oike Y, Akao M, Yasunaga K, Yamauchi T, Morisada T, Ito Y, et al. Angiopoietin-related growth factor antagonizes obesity and insulin resistance. *Nat Med* 2005;**11**:400–8.
5. Kitazawa M, Ohizumi Y, Oike Y, Hishinuma T, Hashimoto S. Angiopoietin-related growth factor suppresses gluconeogenesis through the Akt/forkhead fox class O1-dependent pathway in hepatocytes. *J Pharmacol Exp Ther* 2007;**323**:787–93.
6. Holmäng A, Björntorp P. The effects of testosterone on insulin sensitivity in male rats. *Acta Physiol Scand* 1992;**146**:505–10.
7. Osuna JA, Gómez-Pérez R, Arata-Bellarbarba G, Villaruel V. Relationship between BMI, total testosterone, sex hormone-binding-globulin, leptin, insulin and insulin resistance in obese men. *Arch Androl* 2006;**52**:355–61.
8. Abate N, Haffner SM, Garg A, Peshock RM, Grundy SM. Sex steroid hormones, upper body obesity, and insulin resistance. *J Clin Endocrinol Metab* 2002;**87**:4522–7.
9. Mårin P, Holmäng S, Jönsson L, Sjöström L, Kvist H, Holm G, et al. The effects of testosterone treatment on body composition and metabolism in middle-aged obese men. *Int J Obes Relat Metab Disord* 1992;**16**:991–7.
10. Boonyaratanakornkit V, Edwards DP. Receptor mechanisms mediating non-genomic actions of sex steroids. *Semin Reprod Med* 2007;**25**:139–53.
11. Rajala UM, Keinänen-Kiukaanniemi SM, Hirsso PK, Jokelainen MA, Laakso MA, Hiltunen LA, et al. Associations of total testosterone and sex hormone-binding globulin levels with insulin sensitivity in middle-aged Finnish men. *Diabetes Care* 2007;**30**:e13.
12. Li C, Ford ES, Li B, Giles WH, Liu S. Association of testosterone and sex hormone-binding globulin with metabolic syndrome and insulin resistance in men. *Diabetes Care* 2010;**33**:1618–24.
13. Rosner W, Hryb DJ, Khan MS, Nakhla AM, Romas NA. Sex hormone-binding globulin mediates steroid hormone signal transduction at the plasma membrane. *J Steroid Biochem Mol Biol* 1999;**69**:481–5.
14. Krupenko SA, Krupenko NI, Danzo BJ. Interaction of sex hormone-binding globulin with plasma membranes from the rat epididymis and other tissues. *J Steroid Biochem Mol Biol* 1994;**51**:115–24.
15. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The fatty liver index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006;**6**:33.
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher RC, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;**28**:412–9.
17. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000;**85**:2402–10.
18. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* 1999;**84**:3666–72.
19. Saad F, Aversa A, Isidori AM, Gooren LJ. Testosterone as potential effective therapy in treatment of obesity in men with testosterone deficiency: a review. *Curr Diabetes Rev* 2012;**8**:131–43.
20. Namkung J, Koh SB, Kong ID, Choi JW, Yeh. Serum levels of angiopoietin-related growth factor are increased in metabolic syndrome. *Metabolism* 2011;**60**:564–8.
21. Ebert T, Bachmann A, Lossner U. Serum levels of angiopoietin related growth factor in diabetes mellitus and chronic hemodialysis. *Metabolism* 2009;**58**:547–51.
22. Evans RM, Barish GD, Wang YX. PPARs and the complex journey to obesity. *Nat Med* 2004;**10**:355–61.
23. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 1999;**98**:115–24.
24. Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr Rev* 2003;**24**:78–90.



25. Connolly J, Romano T, Patruno M. Selections from current literature: effects of dieting and exercise on resting metabolic rate and implications for weight management. *Fam Pract* 1999;**16**: 196–201.
26. Mirzaei K, Hossein-Nezhad A, Chamari M, Shahbazi S. Evidence of a role of ANGPTL6 in resting metabolic rate and its potential application in treatment of obesity. *Minerva Endocrinol* 2011;**36**: 13–21.
27. Xiong Y, Collins QF, An J, Lupo Jr E, Liu HY, Liu D, et al. p38 mitogen-activated protein kinase plays an inhibitory role in hepatic lipogenesis. *J Biol Chem* 2007;**282**:4975–82.
28. Browning JD, Horton JD. Molecular mediators of hepatic steatosis and liver injury. *J Clin Invest* 2004;**114**:147–52.
29. Trinh KY, O'Doherty RM, Anderson P, Lange AJ, Newgard CB. Perturbation of fuel homeostasis caused by overexpression of the glucose-6-phosphatase catalytic subunit in liver of normal rats. *J Biol Chem* 1998;**273**:31615–20.
30. Oh SY, Cho YK, Kang MS, Yoo TW, Park JH, Kim HJ, et al. The association between increased alanine aminotransferase activity and metabolic factors in nonalcoholic fatty liver disease. *Metabolism* 2006;**55**:1604–9.
31. Bonnet F, Ducluzeau PH, Gastaldelli A, Laville M, Anderwald T, Konrad T, et al. Liver enzymes are associated with hepatic insulin resistance, insulin secretion, and glucagon concentration in healthy men and women. *Diabetes* 2011;**60**:1660–7.
32. Tchernof A, Labrie F, Bélanger A, Prud'homme D, Bouchard C, Tremblay A, et al. Relationships between endogenous steroid hormone, sex hormone-binding globulin and lipoprotein levels in men: contribution of visceral obesity, insulin levels and other metabolic variables. *Atherosclerosis* 1997;**133**:235–44.
33. Hammoud AO, Gibson M, Peterson CW, Hamilton BD, Carrell DT. Obesity and male reproductive potential minireview. *J Androl* 2006;**27**:619–26.
34. Pasquali R, Casimirri F, De Iasio R, Mesini P, Boschi S, Chierici R, et al. Insulin regulates testosterone and sex hormone-binding globulin concentrations in adult normal weight and obese men. *J Clin Endocrinol Metab* 1995;**80**:654–8.
35. Crave JC, Lejeune H, Brébant C, Baret C, Pugeat M. Differential effects of insulin and insulin-like growth factor I on the production of plasma steroid-binding globulins by human hepatoblastoma-derived (Hep G2) cells. *J Clin Endocrinol Metab* 1995;**80**: 1283–9.
36. Selva DM, Hogeveen KN, Innis SM, Hammond GL. Monosaccharide-induced lipogenesis regulates the human hepatic sex hormone-binding globulin gene. *J Clin Invest* 2007;**117**:3979–87.
37. Peter A, Kantartzis K, Machann J, Schick F, Staiger H, Machicao F, et al. Relationships of circulating sex hormone-binding globulin with metabolic traits in humans. *Diabetes* 2010;**59**: 3167–73.
38. Brand JS, Wareham NJ, Dowsett M, Folkerd E, van der Schouw RN, Luben RN, et al. Association of endogenous testosterone and SHBG with glycated haemoglobin in middle-aged and older men. *Clin Endocrinol (Oxf)* 2011;**74**:572–8.
39. Haffner SM, Shaten J, Stern MP, Smith GD, Kuller L. Low levels of sex hormone-binding globulin and testosterone predict the development of non-insulin-dependent diabetes mellitus in men. MRFIT research group. Multiple risk factor intervention trial. *Am J Epidemiol* 1996;**143**:889–97.
40. Shalender B, Guneet KJ, Michael P, Ralph Sr D'Agostino, Andrea DC, Ramachandran SV, et al. Sex hormone-binding globulin, but not testosterone, is associated prospectively and independently with incident metabolic syndrome in men. The Framingham heart study. *Diabetes Care* 2011;**34**:2464–70.
41. Le TN, Nestler JE, Strauss 3rd JF, Wickham 3rd EP. Sex hormone-binding globulin and type 2 diabetes mellitus. *Trends Endocrinol Metab* 2012;**23**:32–40.