Polymorphism of the AHSG gene is associated with increased adipocyte β2-adrenoceptor function

Catharina Lavebratt,* Elisabeth Dungner,† and Johan Hoffstedt[1,†]

Departments of Molecular Medicine* and Medicine,† Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

Abstract **The alpha$_2$ Heremans-Schmid glycoprotein (AHSG) gene is implicated in the regulation of body fat and insulin sensitivity. The Met/Met genotype of the common single-nucleotide polymorphism (SNP), *rs4917*, in the AHSG gene has been shown to be associated with reduced plasma levels as well as lower body fat. Here, we studied the association of this variation with subcutaneous adipocyte lipolysis. Ninety-three obese and nonobese healthy men were genotyped for Thr230Met, and subcutaneous adipose tissue biopsies were analyzed for lipolysis characteristics. The Met/Met genotype was associated with a marked increase of 1.5 log units in the lipolytic sensitivity to the β2-adrenoceptor agonist terbutaline ($P = 0.0008$) as compared with the Thr/Thr and Thr/Met genotypes. This corresponds to an approximately 35-fold increase in β2-adrenoceptor function. The genotype effect was independent of body mass index and waist circumference. In contrast, lipolytic sensitivity to both the β1-adrenoceptor agonist dobutamine ($P = 0.25$) and the α2A-adrenoceptor agonist clonidine ($P = 0.54$) was unaffected by the Thr230Met variation. Moreover, no difference in either maximal stimulation or inhibition of lipolysis was found between genotypes. We conclude that a common variation (Thr230Met) in the AHSG gene is associated with a marked increase in β2-adrenoceptor sensitivity in subcutaneous fat cells, which may be of importance in body weight regulation.**—Lavebratt, C., E. Dungner, and J. Hoffstedt. **Polymorphism of the AHSG gene is associated with increased adipocyte β2-adrenoceptor function. *J. Lipid Res.* 2005.** 46: **2278–2281.**

Supplementary key words alpha$_2$ Heremans-Schmid glycoprotein • adipose • alpha-adrenoceptor • beta-adrenoceptor • fat cell

Obesity with associated metabolic complications constitutes a well-known risk factor for both cardiovascular morbidity and mortality. Although lifestyle factors including eating behavior and physical activity are crucial, family studies have provided evidence also for a strong genetic influence on obesity development. In searching for genes that may affect body fat content and metabolism, the alpha$_2$ Heremans-Schmid glycoprotein (AHSG), also known as fetuin-A, is an attractive candidate. AHSG is a glycoprotein synthesized in the liver and circulating in plasma at high concentrations (1). Mice null for the AHSG gene are insulin sensitive, resistant to weight gain, and have decreased body fat content, which in turn may be due to altered lipid metabolism or higher energy expenditure (2). A double-single nonsynonymous nucleotide substitution [single-nucleotide polymorphism (SNP)] at amino acid positions 230 (*rs4917*, Thr230Met) and 238 (*rs4918*, Thr238Ser) has been described (3) and was recently found to be associated with decreased plasma concentrations of AHSG (4). In agreement with these findings, we have shown that homozygosity for the *rs2593813*:G–*rs4917*:Met–*rs4918*:Ser haplotype of the AHSG gene conferred an increased risk for leanness in men (5), which in turn suggests a protective role of AHSG gene variation in body fat accumulation in man.

A fundamental aspect of adipose tissue metabolism is the process of triglyceride breakdown, lipolysis. In subcutaneous fat cells, catecholamines either stimulate lipolysis by binding with β-adrenoceptors or inhibit triglyceride breakdown by binding to α2-adrenoceptors (6). The balance between the two adrenoceptor subtypes determines whether the catecholamine-induced effect will be of a stimulatory or inhibitory nature in subsequent cAMP production and activation of hormone-sensitive lipase, which catalyses the rate-limiting step in the lipolytic cascade.

The present study was designed to evaluate whether the *rs4917* (Thr230Met) variation in the AHSG gene is associated with fat cell function by investigating subcutaneous fat cell lipolysis in 93 healthy obese and nonobese male subjects.

METHODS

Subjects

Ninety-three men who were healthy and free of medication were recruited in order to study the influence of genetic variance

Manuscript received 19 May 2005 and in revised form 15 July 2005.

Published, JLR Papers in Press, July 16, 2005.
DOI 10.1194/jlr.M500201-JLR200

[1] To whom correspondence should be addressed.
e-mail: johan.hoffstedt@medhs.ki.se

on adipocyte lipolysis regulation. Body mass index (BMI) ranged between 20 and 51 kg/m^2 and age between 21 and 72 years. All subjects were living in the Stockholm area and were at least second-generation Scandinavian. None of the subjects were completely sedentary or involved in athletic performances. All ate a standard Swedish diet. None had undergone a weight loss program or experienced a change in body weight >1 kg within the 6 months prior to the study, according to self-report. At ~07:30 AM, after an overnight fast, a venous blood sample was obtained for DNA extraction and for analyses of plasma levels of glucose, insulin, triglycerides, cholesterol, and HDL-cholesterol, which were performed by the hospital's accredited chemistry laboratory. Systolic and diastolic blood pressures were measured in the supine position after 15 min of rest. Thereafter, an adipose sample (1–2 g) was obtained by needle biopsy from the abdominal subcutaneous area under local anesthesia, as described (7). The study was explained in detail to each subject and his informed consent was obtained. The study was approved by the hospital's committee on ethics.

Fat cell studies

The adipose tissue was collagenase treated, and isolated fat cells were collected and subjected to lipolysis experiments exactly as described (8). In brief, fat cells were incubated in buffer (pH 7.4) containing albumin and glucose at 37°C in the absence (basal) or presence of increasing concentrations of either dobutamine (a selective β1-adrenoceptor agonist), terbutaline (a selective β2-adrenoceptor agonist), clonidine (a selective α2A-adrenoceptor agonist), or forskolin (an adenylyl cyclase activator that increases cAMP). After 2 h incubation, the medium was removed for measurement of glycerol, which is a quantitative marker for lipolysis. Glycerol release was related to the number of fat cells that were incubated. All agonists caused a concentration-dependent stimulation of glycerol release that reached a plateau at the highest agonist concentration. Consequently, it was always possible to determine the concentration of agonist producing a half-maximum effect on glycerol release, which, in turn, is an indirect measure of adrenoceptor sensitivity. These EC_{50} values (expressed as log mol/l) were determined by log-logit transformation of the ascending (β-agonist) or descending (α2-agonist) parts of the individual concentration-response curves. The base 10 logarithm of the EC_{50} values was used in the calculations, because this value is a pharmacological representation of receptor sensitivity (pD_2). The accuracy of the lipolysis method has been validated in detail (8).

Genotyping

Genotype determination of the *rs4917* C (Thr) > T (Met) polymorphism of the AHSG gene (http://www.ncbi.nlm.nih.gov/SNP) was accomplished using restriction fragment length polymorphism (RFLP) analysis, exactly as described (3). For 54 subjects, RFLP-generated genotypes were verified using pyrosequencing, as described (5).

Power calculation

We made a calculation to estimate the smallest mean difference in adrenoceptor lipolysis sensitivity between genotypes that can be detected in the present study material of 93 subjects to have a power of 80% to yield a statistically significant result (9). The calculation was done assuming an allele frequency of 0.7 (A) and 0.3 (B) for two alleles of a specific SNP, which would correspond to homozygous genotype AA (n = 46) and homo/heterozygous genotype AB/BB (n = 46). The criterion for significance was set at 0.05. The test was 2-tailed, which means that an effect in either direction was interpreted. On average, a study of this design would enable us to report a mean adrenoceptor lipolysis sensitivity (pD_2) difference of 0.8 between AA and AB/BB genotypes with a common within-group standard deviation of 1.4.

Statistical analysis

The Hardy-Weinberg equilibrium test was applied to the study material as a whole and to the obese and nonobese groups separately to ensure independent segregation of alleles (10). Parameter distributions were normalized when necessary by base 10 logarithm transformation. Testing for differences among clinical parameters between genotype groups was performed using univariate 1-way ANOVA, or ANCOVA, with BMI or waist circumference as covariates. The analyses were performed using StatView version 6.0 (Stata Corporation; College Station, TX). Values are expressed as mean ± standard deviation.

RESULTS

The allele distribution of the AHSG polymorphism Thr230Met was in Hardy-Weinberg equilibrium, and the frequency of the minor allele T (Met) was 40%.

In **Table 1**, the effect of Thr230Met on clinical parameters is shown. No effect of the genotype on any of the examined clinical parameters was found.

The results of the subcutaneous fat cell lipolysis measurements are shown in **Table 2**. There were no differences between the genotypes, either in basal lipolysis, in maximum stimulation of lipolysis induced by dobutamine, terbutaline, or forskolin or in maximum inhibition of lipolysis induced by clonidine. In contrast, with respect to lipolytic adrenoceptor sensitivity, a marked effect was found for terbutaline. Men homozygous for the AHSG *rs4917* Met allele had approximately 1.5 log units higher sensitivity for adipocyte β2-adrenoceptor stimulation than men heterozygous or homozygous for the Thr allele (P = 0.0008 comparing three genotypes, P = 0.0024 after Bonferroni adjustment for multiple comparison of adrenoceptor subtype sensitivity). In other words, β2-adrenoceptor sensitivity of fat cells from Met-allele-homozygous men was about 35-fold higher than that of fat cells from men with Thr/Met or Thr/Thr genotype (**Fig. 1**). The genotype effect on terbutaline sensitivity remained statistically

TABLE 1. Effect of Thr230Met on clinical parameters

AHSG *rs4917* Genotype	CC	CT	TT	P
N	32	47	14	
Age (years)	42 ± 13	38 ± 12	40 ± 11	0.47
BMI (kg/m^2)	32 ± 8	31 ± 7	28 ± 7	0.20
Waist (cm)	108 ± 21	105 ± 21	99 ± 22	0.40
P-glucose (mmol/l)	5.4 ± 0.5	5.4 ± 1.1	4.9 ± 0.4	0.12
Log P-insulin (mU/l)	1.0 ± 0.3	1.0 ± 0.3	0.9 ± 0.2	0.30
Log HOMA	0.4 ± 0.3	0.4 ± 0.4	0.2 ± 0.3	0.27
P-cholesterol (mmol/l)	5.2 ± 1.0	5.2 ± 1.0	5.2 ± 1.0	0.94
P-HDL-cholesterol (mmol/l)	1.1 ± 0.3	1.1 ± 0.3	1.3 ± 0.5	0.23
Log P-triglycerides	0.13 ± 0.4	0.14 ± 0.25	0.03 ± 0.22	0.35
Systolic blood pressure (mmHg)	129 ± 18	127 ± 17	122 ± 9	0.48
Diastolic blood pressure (mmHg)	80 ± 9	77 ± 11	75 ± 6	0.30

Values are mean ± standard deviation and were compared using ANOVA. AHSG, alpha$_2$ Heremans-Schmid glycoprotein gene; BMI, body mass index; HOMA, Homeostasis Model Assessment Index.

TABLE 2. Results of subcutaneous fat cell lipolysis measurements

AHSG *rs4917* Genotype	CC	CT	TT	*P*
N	32	47	14	
Lipolytic response (μmol glycerol/107 cells)				
Basal	8.5 ± 6.5	8.6 ± 5.6	6.0 ± 4.1	0.34
Maximum stimulation				
Dobutamine	26 ± 15	28 ± 12	26 ± 12	0.73
Terbutaline	27 ± 15	29 ± 12	27 ± 12	0.78
Forskolin	30 ± 16	30 ± 13	26 ± 11	0.55
Maximum inhibition				
Clonidine	3.2 ± 1.6	2.7 ± 1.4	2.2 ± 1.7	0.28
Lipolytic sensitivity (pD_2)				
Dobutamine	7.5 ± 0.7	7.7 ± 0.9	7.9 ± 1.0	0.25
Terbutaline	7.5 ± 1.4	7.7 ± 1.1	9.1 ± 1.4	0.0008
Clonidine	9.2 ± 1.1	9.4 ± 0.7	9.5 ± 0.8	0.54

Values are mean ± standard deviation and were compared using ANOVA.

significant when adjusted for BMI ($P = 0.002$) or waist circumference ($P = 0.0003$) using ANCOVA. The effect of the AHSG *rs4917* SNP on adipocyte adrenoceptor sensitivity was restricted to terbutaline. No difference in either β1 (dobutamine)- or α2A (clonidine)-adrenoceptor sensitivity was found between genotypes.

DISCUSSION

Human fat cells express an excess of α- and β-adrenoceptors and only a fraction of the receptor population has to be occupied to give a maximum response (6). The sensitivity to agonist stimulation is determined by receptor density, which, in turn, determines the concentration of agonist required to elicit a full response. Consequently, the maximum effect on lipolysis will be decreased only when the number of receptors is markedly reduced. Thus, variation in the maximum lipolytic capacity of the adipocyte is primarily due to postreceptor events, eventually resulting in HSL-dependent hydrolysis of the triglycerides. The striking finding of this study was the marked increase

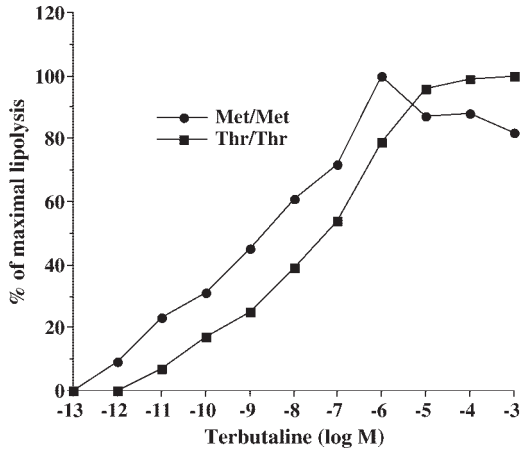

Fig. 1. Relationship between AHSG genotypes and terbutaline (β2-adrenoceptor agonist)-induced lipolysis. Concentration response curves from two representative subjects carrying the Thr/Thr and Met/Met genotypes, respectively, are shown.

of 1.5 log units for β2-adrenoceptor sensitivity in subcutaneous fat cells from men homozygous for the Met allele of the AHSG Thr230Met variation. Consequently, in Met/Met-carrying men, a half-maximum effect on lipolysis stimulation can be achieved by an approximately 35-fold lower concentration of β2-adrenoceptor agonist than in men with Thr/Met or Thr/Thr genotypes. In contrast, both β1- and α2A-adrenoceptor sensitivity was independent of Thr230Met. Moreover, basal as well as maximum stimulation and inhibition of lipolysis was not different between Thr230Met genotypes. Thus, given previous findings in mice and humans on the Thr230Met SNP (2, 4, 5) it may be hypothesized that a decreased expression of AHSG in men with genotype Met/Met influences human fat cell function at an early step of the lipolytic cascade, preferably by specifically inducing β2-adrenoceptor function. An increased capacity to mobilize triglycerides from subcutaneous adipose tissue for a given concentration of receptor agonist would follow, in turn resulting in resistance to body fat accumulation. Although there was no significant association of the Thr230Met SNP with BMI in the present study, the results are in agreement with our previous finding (7) that homozygosity for the *rs2593813*:G–*rs4917*:Met–*rs4918*:Ser haplotype spanning from intron 1 to the last exon 7 is more common among lean than overweight or obese men, suggesting a protective role of the Met allele in obesity development.

A consequence of increased body fat is the development of resistance to insulin-stimulated glucose uptake. Indeed, previous studies have implicated a pathophysiologic role of AHSG in regulating insulin sensitivity. Serum levels of AHSG have been found to be increased in women with gestational diabetes mellitus and to correlate with maternal insulin resistance parameters (11). AHSG has been shown to inhibit insulin-stimulated insulin receptor tyrosine kinase activity and autophosphorylation (12, 13), and mice null for the AHSG gene are insulin sensitive (2). Moreover, in adipose tissue from obese and nonobese women, a common (rare allele frequency 48%) SNP located in the 5′ region of the AHSG gene (*rs2077119*) was recently found to be associated primarily with insulin-mediated regulation of lipolysis (14). However, no data on adrenoceptor regulation of lipolysis were reported. We found no association of the AHSG Thr230Met variation and clinical parameters. However, the mean values for several features of the metabolic syndrome, including waist circumference, plasma glucose, triglycerides, and insulin, as well as Homeostasis Model Assessment (HOMA) Index and blood pressure levels, were lower in the Met/Met genotype group. Failure to find association of Thr230Met with these clinical parameters may be due to the small sample size. Nevertheless, the present finding of increased β2-adrenoceptor sensitivity in Thr230Met Met/Met men suggests a role also for adipose tissue adrenoceptor function in mediating the insulin-signaling modulating effects of AHSG. With respect to human lipolytic function, a wide variation in sensitivity to catecholamine-induced lipolysis has been shown, which has been attributable mainly to variation in β2-adrenoceptor sensitivity (8). Upper-body

obese women (15), as well as men with insulin resistance and the metabolic syndrome (16), are characterized by lipolytic catecholamine resistance due to reduced numbers of β2-adrenoceptors, as determined by radioligand binding studies. In the latter study, a strong correlation ($r = 0.67$) between β2-adrenoceptor number and insulin sensitivity was also found, implicating a pathophysiological role of reduced β2-adrenoceptor function in insulin resistance, possibly due to a compensatory increase in the sympathetic activity (17).

We previously reported a high degree of linkage disequilibrium ($|D'| \geq 0.97$) between the presently studied *rs4917* (Thr230Met), the *rs4918* (Thr238Ser), and the *rs2593813* (intron 1) SNPs of the AHSG gene (7). Whether one of these SNPs is true functional is unknown. However, the Thr230Met nucleotide substitution changes the existence of putative exonic splicing enhancer sites from the SF2/ASF and SRp40 sites (cognate score from 2.5 to below 0, and 2.7 to 1.3, respectively) to an SRp55 site (score from 1.16 to 3.76), as discussed (5).

In conclusion, a common SNP, *rs4917* (Thr230Met) of the AHSG gene is markedly associated with β2-adrenoceptor function in subcutaneous adipose tissue. Men homozygous for the Met allele display an approximately 35-fold higher sensitivity for lipolysis induced by the selective β2-adrenoceptor agonist terbutaline than do men carrying the Thr/Met or Thr/Thr genotype.

This study was supported by grants from the Swedish Research Council, Foundation of Thuring, and the Swedish Medical Society. The authors are grateful for the excellent technical assistance of Britt-Marie Leijonhufvud, Katarina Sjöberg, Kerstin Wåhlén, and Eva Sjölin.

REFERENCES

1. Dziegielewska, K. M., and W. M. Brown. 1995. Fetuin. Springer, Berlin, Heidelberg, New York.
2. Mathews, S. T., G. P. Singh, M. Ranalletta, V. J. Cintron, X. Qiang, A. S. Goustin, K-L. C. Jen, M. J. Charron, W. Jahnen-Dechent, and G. Grunberger. 2002. Improved insulin sensitivity and resistance to weight gain in mice null for the *Ahsg* gene. *Diabetes.* **51:** 2450–2458.
3. Osawa, M., K. Umetsu, T. Ohki, T. Nagasawa, T. Suzuki, and S. Takeichi. 1997. Molecular evidence for human alpha 2-HS glycoprotein (AHSG) polymorphism. *Hum. Genet.* **99:** 18–21.
4. Osawa, M., W. Tian, H. Horiuchi, M. Kaneko, and K. Umetsu. 2005. Association of 2-HS glycoprotein (AHSG, fetuin-A) polymorphism with AHSG and phosphate serum levels. *Hum. Genet.* **116:** 146–151.
5. Lavebratt, C., S. Wahlqvist, L. Nordfors, J. Hoffstedt, and P. Arner. 2005. AHSG gene variant is associated with leanness among Swedish men. *Hum. Genet.* **117:** 54–60.
6. Arner, P. 1992. Adrenergic receptor function in fat cells. *Am. J. Clin. Nutr.* **55 (Suppl.):** 228–236.
7. Kolaczynski, J. W., L. M. Morales, J. H. Moore, Jr., R. V. Considine, Z. Pietrzkowski, P. F. Noto, J. Colberg, and J. F. Caro. 1994. A new technique for biopsy of human abdominal fat under local anaesthesia with Lidocaine. *Int. J. Obes. Relat. Metab. Disord.* **18:** 161–166.
8. Lönnquist, F., H. Wahrenberg, L. Hellström, S. Reynisdottir, and P. Arner. 1992. Lipolytic catecholamine resistance due to decreased beta2-adrenoceptor expression in fat cells. *J. Clin. Invest.* **90:** 2175–2186.
9. Armitage, P., G. Berry, and J. Matthews. 2002. Statistical Methods in Clinical Research. Blackwell Science, Ltd., Oxford.
10. Guo, S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics.* **48:** 361–372.
11. Kalabay, L., K. Cseh, A. Pajor, E. Baranyi, G. M. Csákány, Z. Melczer, G. Speer, M. Kovács, G. Siller, I. Karádi, et al. 2002. Correlation of maternal serum fetuin/2-HS-glycoprotein concentration with maternal insulin resistance and anthropometric parameters of neonates in normal pregnancy and gestational diabetes. *Eur. J. Endocrinol.* **147:** 243–248.
12. Auberger, P., L. Falquerho, J. O. Contreres, G. Pages, G. Le Cam, B. Rossi, and A. Le Cam. 1989. Characterization of a natural inhibitor of the insulin receptor tyrosine kinase: cDNA cloning, purification and anti-mitogenic activity. *Cell.* **58:** 631–640.
13. Srinivas, P. R., A. S. Wagner, L. V. Reddy, D. D. Deutsch, M. A. Leon, A. S. Goustin, and G. Grunberger. 1993. Serum alpha 2-HS-glycoprotein is an inhibitor of the human insulin receptor at the tyrosine kinase level. *Mol. Endocrinol.* **7:** 1445–1455.
14. Dahlman, I., P. Eriksson, M. Kaaman, H. Jiao, C. M. Lindgren, J. Kere, and P. Arner. 2004. alpha2-Heremans-Schmid glycoprotein gene polymorphisms are associated with adipocyte insulin action. *Diabetologia.* **47:** 1974–1979.
15. Reynisdottir, S., H. Wahrenberg, K. Carlstrom, S. Rossner, and P. Arner. 1994. Catecholamine resistance in fat cells of women with upper-body obesity due to decreased expression of beta 2-adrenoceptors. *Diabetologia.* **37:** 428–435.
16. Reynisdottir, S., K. Ellerfeldt, H. Wahrenberg, H. Lithell, and P. Arner. 1994. Multiple lipolysis defects in the insulin resistance (metabolic) syndrome. *J. Clin. Invest.* **93:** 2590–2599.
17. Smith, U., and I. Lager. 1989. Insulin-antagonistic effects of counterregulatory hormones: clinical and mechanistic aspects. *Diabetes Metab. Rev.* **5:** 511–525.