α_2-Heremans-Schmid Glycoprotein/ Fetuin-A Is Associated With Insulin Resistance and Fat Accumulation in the Liver in Humans

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OBJECTIVE — The α_2-Heremans-Schmid glycoprotein (AHSG; fetuin-A in animals) impairs insulin signaling in vitro and in rodents. Whether AHSG is associated with insulin resistance in humans is under investigation. In an animal model of diet-induced obesity that is commonly associated with hepatic steatosis, an increase in *Ahsg* mRNA expression was observed in the liver. Therefore, we hypothesized that the AHSG plasma protein, which is exclusively secreted by the liver in humans, may not only be associated with insulin resistance but also with fat accumulation in the liver.

RESEARCH DESIGN AND METHODS — Data from 106 healthy Caucasians without type 2 diabetes were included in cross-sectional analyses. A subgroup of 47 individuals had data from a longitudinal study. Insulin sensitivity was measured by a euglycemic-hyperinsulinemic clamp, and liver fat was determined by ^{1}H magnetic resonance spectroscopy.

RESULTS — AHSG plasma levels, adjusted for age, sex, and percentage of body fat, were higher in subjects with impaired glucose tolerance compared with subjects with normal glucose tolerance ($P = 0.006$). AHSG plasma levels were negatively associated with insulin sensitivity ($r = -0.22$, $P = 0.03$) in cross-sectional analyses. Moreover, they were positively associated with liver fat ($r = 0.27$, $P = 0.01$). In longitudinal analyses, under weight loss, a decrease in liver fat was accompanied by a decrease in AHSG plasma concentrations. Furthermore, high AHSG levels at baseline predicted less increase in insulin sensitivity ($P = 0.02$).

CONCLUSIONS — We found that high AHSG plasma levels are associated with insulin resistance in humans. Moreover, AHSG plasma levels are elevated in subjects with fat accumulation in the liver. This is consistent with a potential role of AHSG as a link between fatty liver and insulin resistance.

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Insulin resistance plays a crucial role in the development of type 2 diabetes (1). Multiple mechanisms are thought to be involved in its pathogenesis. Among them, the human α_2-Heremans-Schmid glycoprotein (AHSG) was found to be important in animals and in in vitro studies. It is an abundant serum protein in mammals. Bovine and murine fetuin-A and pp63 in rats are homologues of AHSG (2,3). In humans, except for the tongue and the placenta, it is exclusively expressed in the liver (4). It is a natural inhibitor of the insulin-stimulated insulin receptor tyrosine kinase (3). Acute injection of human recombinant AHSG inhibited insulin-stimulated tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 in rat liver and skeletal muscle (3). In addition, AHSG knockout mice display improved insulin sensitivity and are resistant to weight gain on a high-fat diet (5).

While these data reflect that AHSG is an important candidate among the factors that induce insulin resistance, the role of this protein in the natural history of type 2 diabetes is still unclear (6). Recent reports from genetic studies suggest that single nucleotide polymorphisms in the *AHSG* gene are associated with adipocyte insulin action in humans (7) and with type 2 diabetes (8). Whether AHSG plasma levels are associated with insulin resistance in humans in vivo is still under investigation.

In a rat model of diet-induced obesity, which commonly displays fatty liver, an increase in *Ahsg* mRNA expression was observed in the liver (9). Because in humans AHSG is almost exclusively secreted from the liver and because the liver, particularly the fatty liver, plays a crucial role in the development of type 2 diabetes (10,11), it can be hypothesized that *AHSG* expression is upregulated in liver dysfunction. So far, information on AHSG secretion in humans is only available from reports on cases of severe liver damage, including cirrhosis, acute viral hepatitis, and cancer (12). Except for hepatitis, AHSG plasma levels are decreased in individuals with these diseases. However, it is not known whether fat accumulation in the liver is associated with high AHSG production.

RESEARCH DESIGN AND METHODS

— Data from 106 Caucasians were included in the present analyses. These individuals participate in an ongoing study (Tübingen Lifestyle Internet Program [TULIP]) to reduce adiposity and to prevent type 2 diabetes. Individuals are included in the study when they

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Abbreviations: AHSG, α_2-Heremans-Schmid glycoprotein.

Additional information on this article can be found in an online appendix at http://care.diabetesjournals.org.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Demographics and metabolic characteristics of all subjects*

	Cross sectional (n = 106)	Longitudinal (n = 47)		
		Baseline	Follow-up	P
Demographics				
Sex (men/women)	47/59	20/27	—	—
Normal glucose tolerance/ impaired glucose tolerance	70/36	30/17	—	—
Age (years)	45 ± 1	44.2 ± 1.7	45.0 ± 1.7	—
Body weight (kg)	85.6 ± 1.9	86.8 ± 2.9	83.6 ± 2.7	<0.0001
Body fat (%)	31.4 ± 0.8	31.6 ± 1.2	30.5 ± 1.2	0.048
Metabolic				
Fasting glucose (mmol/l)	5.12 ± 0.05	5.09 ± 0.06	5.02 ± 0.07	0.07
2-h glucose (mmol/l)	7.09 ± 0.16	7.04 ± 0.23	6.33 ± 0.22	0.02
Fasting insulin (pmol/l)	60 ± 4	53 ± 4	45 ± 4	0.004
2-h insulin (pmol/l)	533 ± 42	459 ± 53	326 ± 43	0.0007
Insulin sensitivity (μmol · kg^{-1} · min^{-1} · pmol/l^{-1})	0.063 ± 0.003	0.067 ± 0.005	0.073 ± 0.007	0.02
Liver fat (%)	5.57 ± 0.64*	5.25 ± 1.00	3.45 ± 0.66†	0.0002
AHSG (mg/ml)	269 ± 11	260 ± 17	192 ± 14†	0.006

Data are means ± SE. Available in *90 and in †30 subjects. P for paired differences.

fulfill at least one of the following criteria: a family history of type 2 diabetes, a BMI >27 kg/m^2, previous diagnosis of impaired glucose tolerance, or gestational diabetes. A BMI >27 kg/m^2 was chosen because in our population, a BMI above this cutoff is particularly frequently associated with impaired glucose tolerance (N.S., unpublished observation). All individuals underwent a 75-g oral glucose tolerance test. The participants did not take any medication known to affect glucose tolerance or insulin sensitivity. They were considered healthy according to a physical examination and routine laboratory tests. A group of 47 subjects had follow-up data after they underwent dietary counseling and increased physical activity. Due to claustrophobia or metal implantations, not all individuals underwent measurements of liver fat. AHSG levels at follow-up were measured only in subjects who had data on liver fat at follow-up. Participants had ~10 sessions with a dietitian. During each visit, they presented a 3-day food diary and discussed the results with the dietitians. The counseling aimed to reduce body weight and total intake of calories, particularly intake of calories from fat, and to increase intake of fibers. Furthermore, participants were asked to reduce intake of saturated fat. Subjects were instructed to increase physical activity and to perform at least 3 h of moderate sports per week. From interviews with the participants, we learned that they were motivated to stick to the program because of the intense contact with the dietitians and the physicians. Also, in general participants were allowed to retain their common dietary habits. However, these were individually modified by the dietitians. A similar approach was chosen for aerobic exercise. Informed written consent was obtained from all participants, and the local medical ethics committee had approved the protocol.

Body composition

Body fat was measured by the bioelectrical impedance method (RJL, Detroit, MI). In brief, electrodes are attached to various parts of the body and a small electric signal is circulated. With this method, the impedance or resistance to the signal as it travels through the water that is found in muscle and fat is measured. The more fat a person has, the more resistance to the current exists.

^{1}H magnetic resonance spectroscopy for quantitative analysis of liver fat content

Liver fat was determined by localized proton magnetic resonance spectroscopy as previously described (13).

Oral glucose tolerance test

All individuals underwent a 75-g oral glucose tolerance test, and venous plasma samples were obtained at 0, 30, 60, 90, and 120 min for determination of plasma glucose and insulin. Glucose tolerance was determined according to the 1997 World Health Organization diagnostic criteria (14).

Euglycemic-hyperinsulinemic clamp

Insulin sensitivity was determined as previously described (13). In brief, subjects received a primed insulin infusion at a rate of 40 mU/m^2 per min for 2 h. Plasma was drawn every 5 min for determination of plasma glucose, and a glucose infusion was appropriately adjusted to maintain the fasting glucose level. An insulin sensitivity index (in μmol · kg^{-1} · min^{-1} · pmol/l^{-1}) for systemic glucose uptake was calculated as the mean infusion rate of glucose (in μmol · kg^{-1} · min^{-1}) necessary to maintain euglycemia during the last 40 min of the euglycemic-hyperinsulinemic clamp divided by the steady-state plasma insulin concentration. The latter was the mean insulin concentration at minute 100, 110, and 120 of the clamp (mean [±SE] for all subjects: 525 ± 12 pmol/l).

Analytical procedures

Plasma glucose was determined using a bedside glucose analyzer (glucose-oxidase method; YSI, Yellow Springs Instruments, Yellow Springs, CO). Plasma insulin was determined by microparticle enzyme immunoassay (Abbott Laboratories, Tokyo, Japan). EDTA plasma samples were collected, frozen immediately and stored at −80°C. Fasting plasma levels of AHSG were determined by a sandwich ELISA (BioVendor Laborytory Medicine, Brno, Czech Republic). The antibodies are highly specific for the human AHSG protein, and the assay has a sensitivity of 3.5 μg/ml.

Statistical analyses

Data are given as means ± SE. Data that were not normally distributed (Shapiro-Wilk W test, e.g., AHSG plasma levels, insulin sensitivity) were logarithmically transformed. Differences between parameters at baseline and at follow-up were tested using the matched-pairs t test. ANOVA was used for simple and multivariate linear regression analysis. A P value <0.05 was considered statistically significant. The statistical software package JMP 4.0 (SAS Institute, Cary, NC) was used.

RESULTS

Cross-sectional data

Characteristics of the population are shown in Table 1. AHSG plasma levels

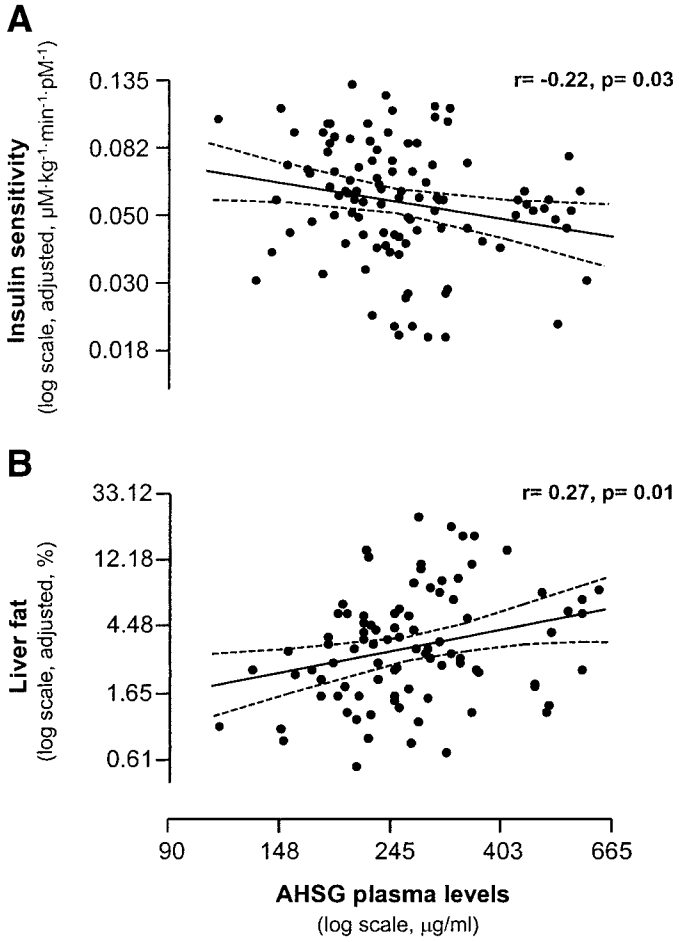

Figure 1—*Cross-sectional data. Relationships of AHSG plasma levels with insulin sensitivity* (A) *and with liver fat* (B) *after adjustment for age, sex, and percentage of body fat in multivariate linear regression models (regression line and 95% CI).*

were not statistically different between male (271 ± 18 μg/ml) and female (268 ± 13 μg/ml, $P = 0.78$) subjects. They were negatively associated with age ($r = -0.33$, $P = 0.006$) and not associated with percentage of body fat ($r = 0.12$, $P = 0.23$). AHSG plasma levels were positively associated with fasting insulinemia ($r = 0.24$, $P = 0.01$) and 2-h glycemia ($r = 0.23$, $P = 0.02$) and insulinemia ($r = 0.23$, $P = 0.01$) after adjustment for age and sex. The relationship between AHSG levels and fasting glycemia was statistically not significant ($r = 0.18$, $P = 0.08$).

AHSG plasma levels were higher in individuals with impaired glucose tolerance (307 ± 19 μg/ml) compared with individuals with normal glucose tolerance (250 ± 13 μg/ml, $P = 0.006$). Moreover, they were negatively associated with insulin sensitivity determined during the euglycemic-hyperinsulinemic clamp. This was independent of age, sex, and percentage of body fat (Fig. 1*A*). In addition, high AHSG plasma levels were associated with high liver fat (Fig. 1*B*), after adjustment for age, sex, and percentage of body fat.

Longitudinal data

In longitudinal analyses, under a lifestyle intervention the mean time of follow-up was 9.3 ± 0.4 months. There was a decrease in percentage of body fat, fasting and 2-h insulinemia, and 2-h glycemia. In contrast, insulin sensitivity increased from baseline to follow-up (Table 1). Data from food diaries revealed that there was a significant decrease in energy intake (−7%, $P = 0.03$) and, particularly, a decrease in intake of saturated fat (−11%, $P = 0.03$). Aerobic physical fitness, as measured by the lactate threshold on a treadmill, increased ($P = 0.002$). It is of particular note that there was a large decrease in liver fat (−34%) that was paralleled by a decrease in AHSG plasma levels (−27%). These large changes were only partially explained by changes in energy intake or intake of fat and to a larger extent by physical fitness (data not shown).

Subjects were divided in tertiles according to change in liver fat. AHSG plasma levels decreased in the lowest tertile (−58%, individuals with the largest mean decrease in liver fat) and in the middle tertile (−44%). In contrast, AHSG plasma levels increased in the upper tertile (+21%) (Fig. 2).

Prospective analyses

Based on the correlation of AHSG plasma levels with insulin sensitivity in the cross-sectional analysis, we further assessed the predictive effect of AHSG plasma levels at baseline on fold-change in insulin sensitivity (follow-up over baseline) using multivariate linear regression models. Fold-change in insulin sensitivity was

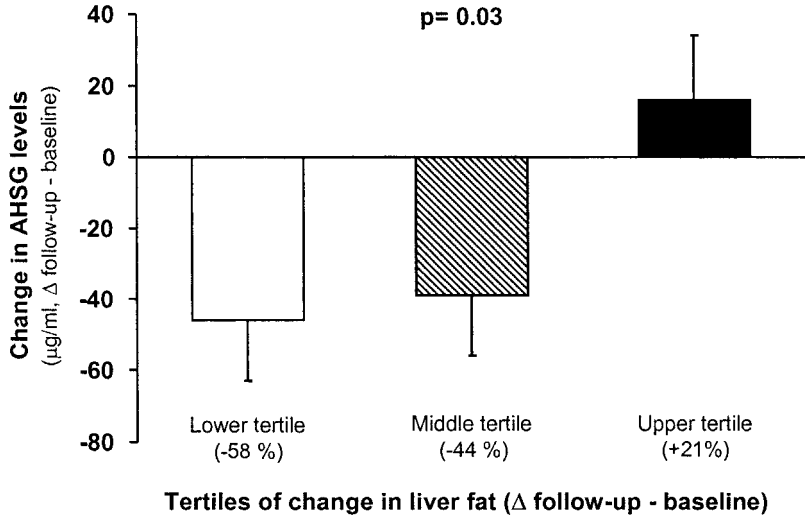

Figure 2—*Longitudinal data. Change in AHSG plasma levels, adjusted for sex, in relationship with tertiles according to change in liver fat.* P *for statistical significance (ANOVA).*

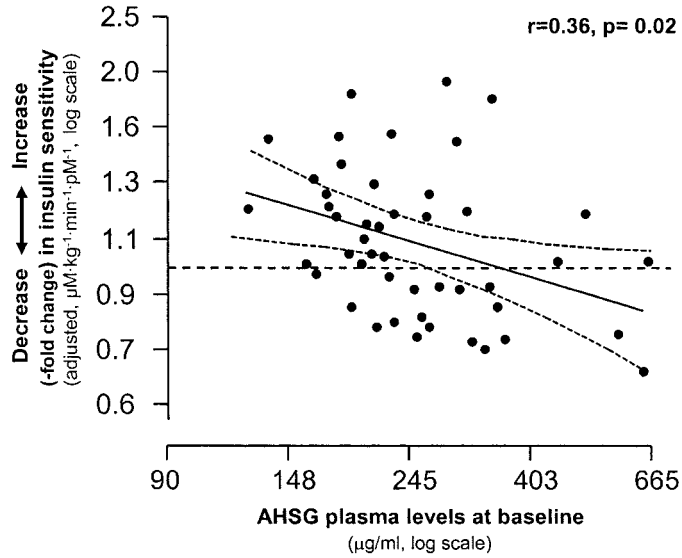

Figure 3—*Prospective data. AHSG plasma levels at baseline and fold-change in insulin sensitivity, adjusted for insulin sensitivity at baseline, sex, and percentage of body fat at baseline, and at follow-up in multivariate linear regression models (regression line and 95% CI).*

chosen as the dependent variable, and sex, insulin sensitivity at baseline, and percentage of body fat at baseline and at follow-up were used as fixed covariates. Fold-change in insulin sensitivity was independently predicted by insulin sensitivity at baseline ($P = 0.01$). Inclusion of AHSG plasma levels at baseline as another covariate increased the r^2 of the model from 0.15 to 0.26, and AHSG plasma levels at baseline turned out to be an independent determinant of fold-change in insulin sensitivity (Fig. 3).

CONCLUSIONS — In the present study, we found that high AHSG plasma levels are associated with insulin resistance in humans. Together with the data on effects of AHSG on insulin signaling in vitro and in animals (3,5,15), this finding suggests that AHSG may represent a new target for prevention of insulin resistance. In in vitro studies, particularly the phosphorylated form of AHSG was found to be the potent regulator of insulin receptor autophosphorylation (3). In future studies, it remains to be determined whether particularly the phosphorylated form may be closely associated with insulin resistance.

There is not much information on the role of AHSG as a regulator of insulin sensitivity in humans. In one study (16), maternal AHSG serum levels were found to be positively associated with indexes of maternal insulin resistance in pregnant women. This finding, together with the well-known effects on insulin signaling and the reports on genetic variants in the *AHSG* gene that are associated with adipocyte insulin action and with type 2 diabetes (7,8), led us to investigate the role of AHSG in the natural course of insulin resistance in humans.

Using measurements from the euglycemic-hyperinsulinemic clamp, we found that AHSG plasma levels were higher in insulin-resistant compared with insulin-sensitive individuals. To further explore this relationship, we investigated whether AHSG plasma levels at baseline predicted change in insulin sensitivity. In subjects who underwent a lifestyle intervention program with diet and increase in physical activity, we found that high AHSG plasma levels at baseline were associated with less increase in insulin sensitivity. This relationship was independent of other determinants of insulin action. We recognize that both the number of subjects included in the longitudinal study and the observed effects are small. Also, a number of other factors including adipocytokines may play an important role and putatively explain a large part of the variability in the change in insulin sensitivity. Therefore, it is difficult to infer a general statement from these data about the functional relevance of AHSG in human plasma. This may eventually be resolved with administration of recombinant AHSG to humans. Nevertheless, we feel that our findings are strengthened by the animal data (5,15) and by the fact that there was an association of the AHSG plasma levels with insulin sensitivity not only in the cross-sectional but also in the longitudinal study.

We also investigated factors that might be associated with high AHSG plasma levels. In humans, AHSG is almost exclusively secreted by the liver (4). Particularly, the fatty liver plays a crucial role in the development of type 2 diabetes (10,11). Therefore, we hypothesized that AHSG plasma levels, which are associated with insulin resistance, may be increased when there is accumulation of fat in the liver. An interesting point is that in a rat model of diet-induced obesity, an increase in *Ahsg* mRNA expression was observed in the liver (9). In a cross-sectional analysis, we found that AHSG plasma concentrations were elevated in subjects with high liver fat.

The longitudinal data allowed us to investigate whether change in liver fat was associated with change in AHSG plasma levels. We observed a relatively large mean decrease in liver fat and in AHSG plasma levels. In contrast, the decrease in percentage of body fat was small. A similar relationship was shown in a recent study (17). A possible explanation for these findings may be that liver fat is a lipid pool that is rapidly regulated. It may not only depend on overall adiposity but also on energy balance and on fat intake (17,18). In the present study, intake of fat and intake of saturated fat decreased, and these changes did not explain all of the variability in change in liver fat. Other factors, like increased physical fitness, may play an additional important role. We further hypothesized that this large change in liver fat may have contributed most to the improvement in metabolism. Liver fat is an important regulator of hepatic insulin sensitivity (10,11), and hepatic insulin sensitivity was found to be a strong predictor of glucose tolerance (19). Unfortunately, hepatic insulin sensitivity was not measured in the present study.

While analyzing the longitudinal data, we found that a decrease in liver fat was accompanied by a decrease in AHSG plasma levels. Thus, it is tempting to speculate that fat accumulation in the liver may result in an increased secretion of AHSG. It is of note that we do not have proof that in humans with fat accumulation in the liver, *AHSG* mRNA expression is upregulated. However, we found that *Ahsg* expression was significantly elevated in mice with fatty liver (online appendix [available at http://care.diabetesjournals.org]). Although, these findings need to be replicated in humans, they further sup-

port the hypothesis of a relationship between fat accumulation in the liver and high AHSG plasma levels.

There is only little information on the regulation of *Ahsg* expression in the liver. Recombinant human interleukin-6, interleukin-1β (20), and tumor necrosis factor-α and partial hepatectomy (21) were found to downregulate the synthesis of *Ahsg*. In contrast, *AHSG* mRNA expression was upregulated upon treatment of hepatocellular carcinoma cells with thyroid hormones (22) and after overexpression of signal transducer and activator of transcription-3 (23). The mechanisms by which liver fat induces AHSG production are unknown and require clarification.

In summary, we show that high AHSG plasma levels are associated with insulin resistance in humans. Moreover, we hypothesize that accumulation of fat in the liver may result in increased production of AHSG, which, in turn, may impair insulin signaling in muscle and liver. This hypothesis, and precise mechanisms explaining our findings, need to be tested in further studies.

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