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CLINICAL STUDY

Correlation of maternal serum fetuin/α_2-HS-glycoprotein concentration with maternal insulin resistance and anthropometric parameters of neonates in normal pregnancy and gestational diabetes

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Abstract

Objective: Human fetuin/α_2-HS-glycoprotein (AHSG) is a 49 kDa serum and tissue protein which is a natural inhibitor of insulin receptor signaling. We investigated serum AHSG levels during pregnancy and whether the protein is involved in insulin resistance observed in healthy pregnant women and patients with gestational diabetes.

Design: One hundred and four healthy pregnant women and 23 of their neonates, 30 patients with gestational diabetes and their neonates and 30 healthy age-matched non-pregnant females as a control group were investigated in a case-control cross-sectional study.

Methods: Serum AHSG was determined by radial immunodiffusion.

Results: We observed an increase of serum AHSG concentration in the second and third trimesters. Gestational diabetes patients had significantly higher AHSG levels than healthy pregnant women and non-pregnant controls. There was a highly significant positive correlation between serum AHSG concentration and indirect parameters of insulin resistance, i.e. tumor necrosis factor-α (TNF-α), leptin, C-peptide and C-peptide/blood glucose ratio. There was also a negative correlation between maternal AHSG, TNF-α, leptin levels and head circumference, body length and body weight of newborns.

Conclusion: AHSG, TNF-α and leptin may contribute to insulin resistance during normal pregnancy and gestational diabetes. AHSG along with these cytokines may also negatively regulate neonatal skeletal development.

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Introduction

Human fetuin/α_2-HS-glycoprotein (AHSG) is a 49 kDa serum and tissue protein which plays a role in host defense and bone metabolism (1, 2). The protein is one of the major components of the non-collagenous bone matrix, especially in the fetal age. The main source of its serum isoform is the liver, where its phosphorylated variant decreases the signal transduction of the insulin receptor; hence, it may contribute to cellular insulin resistance (3–5). The tumor necrosis factor (TNF) system (6) and leptin (7) were also found to contribute to insulin resistance in obesity and type 2 diabetes, and may influence the insulin secretory capacity of the β-cells, too. Both cytokines may also be involved in intrauterine bone development (8, 9).

Progressive increase in insulin resistance has repeatedly been demonstrated during the course of normal pregnancy (10). Decreased insulin sensitivity is even more pronounced in patients with gestational diabetes (11). The status of hepatic insulin sensitivity in pregnancy and gestational diabetes is less clear. Studies on pregnant rats described an increased hepatic insulin resistance (12). Because of its established role in signal transduction of the insulin receptor we studied the potential contribution of AHSG in insulin resistance accompanying normal pregnancy and gestational diabetes mellitus (GDM).

Table 1 Clinical and laboratory parameters of healthy pregnant women, patients with GDM and non-pregnant controls (means±S.D.).

	GDM patients	Healthy pregnant women	Non-pregnant women
n	30	104	30
Age (years)	28.0±2.8	27.8±2.8	28.3±3.5
Gestational age (weeks)	27.67±6.1	24.3±13.6	—
Body mass index (kg/m^2)	33.4±6.4*	25.8±2.7	22.9±2.5
Fasting blood glucose (mmol/l)	4.70±1.50	4.48±0.37	4.48±0.27
Fasting C-peptide (ng/ml)	6.00±3.01	1.85±0.97	1.11±0.82
C-peptide/blood glucose ratio	0.40±0.15	1.27±0.51	0.27±0.13
HbA_{1c} (%)	5.11±0.71	5.30±0.80	4.96±0.45
Serum fructosamine (μmol/l)	203.3±15.4	191.0±18.5	—
Daily insulin dose (U)	33.30±20.83	—	—
Daily insulin dose/body weight (U/kg)	0.41±0.21	—	—

*$P < 0.01$ as compared with values of healthy pregnant women, calculated by the Mann–Whitney test.

It can be expected that some of the neonatal complications of diabetic fetopathy (e.g. alterations in body composition and anthropometric parameters) may be explained by the pathophysiological influence of the TNF system and leptin. Therefore, in a case-control cross-sectional study we measured maternal serum AHSG, TNF-α, soluble TNF receptor-1 and -2 (sTNFR-1 and -2) and leptin concentrations in healthy pregnant women being in different trimesters of pregnancy and in patients with GDM. The relationships between indirect parameters of maternal insulin resistance (fasting C-peptide level, C-peptide/fasting blood glucose ratio) and the concentration of the above mentioned proteins were studied. Different anthropometric parameters of the neonates (body length, body weight and head circumference) were compared with the maternal cytokine and AHSG levels.

Subjects and methods

One hundred and four healthy pregnant women (35 in the first, 31 in the second, and 38 in the third trimester), 30 GDM patients with high fasting C-peptide levels in the 20–40th gestational weeks and 30 healthy age-matched non-pregnant females as a control group were investigated (Table 1). All participants gave their written informed consent. The diagnostic criteria of GDM were according to the WHO classification (13) by a 75 g oral glucose tolerance test. To maintain normoglycemia all of these patients required intensified insulin treatment (three or more short-acting shots, bedtime intermediate insulin).

Anthropometric parameters (body weight, length and head circumference) of 30 neonates (13 boys, 17 girls) of mothers with GDM, 23 newborns (11 boys, 12 girls) delivered by healthy pregnant women between the 38th and 40th week in the third trimester have been analyzed in correlation with the maternal clinical and laboratory parameters. All deliveries of the mothers with GDM also succeeded between the 38th and 40th gestational weeks. Table 2 shows the anthropometric parameters of neonates.

Serum AHSG concentration was determined by radial immunodiffusion using a monospecific goat anti-human AHSG antibody (IgG fraction, 13.7 mg/ml, Cat. No. 81931; Incstar, Stillwater, MN, USA) in a final concentration of 84 μl/11.5 ml gel (mean coefficient of variance (IACV) 3.6%, mean inter-assay coefficient of variance (IECV) 6.2%). TNF-α (Sigma, St Louis, MO, USA) (IACV: 4.8%, IECV: 6.7%), sTNFR-1 (Bender MedSystem, Vienna, Austria) (IACV: 1.9%, IECV: 8.6%), sTNFR-2 (Bender MedSystem) (IACV: 1.4%, IECV: 2.0%) and leptin (DRG International, Mountainside, NJ, USA) (IACV: 4.6%, IECV: 6.6%) concentrations were determined by ELISA, and serum fasting C-peptide concentration by RIA (Biodata, Rome, Italy) (IACV: 5.6%, IECV: 7.3%, normal fasting range 0.66–2.50 ng/ml). Glycosylated hemoglobin (HbA_{1c}) was measured by HPLC (BioRad, Hercules, CA, USA) (normal value in non-diabetics 4.3–5.8%), serum fructosamine by a Boehringer (Mannheim, Germany) automatic analyzer kit (normal value in non-pregnant non-diabetics 185–280 μmol/l). Statistical analysis was performed by the Mann–Whitney linear correlation (Spearman), and multivariate analysis using the SPSS v10 statistical program.

Table 2 Anthropometric parameters, absolute values and percentiles of the newborns (means±S.D.).

	Of patients with GDM ($n = 30$)	Of healthy pregnant women ($n = 23$)
Boys/girls	13/17	11/12
Body weight (g)	3151±672*	3575±365
Percentile	55.87±30.29	64.75±16.10
Body length (cm)	51.90±3.40*	55.80±2.80
Percentile	86.4±31.88	110.90±16.24
Head circumference (cm)	33.80±1.70*	34.75±1.15
Percentile	65.50±22.84	71.30±32.14

*$P < 0.01$ as compared with neonates of healthy pregnant women calculated by the Mann–Whitney test.

Table 3 Serum TNF-α, sTNFR-1 and sTNFR-2, leptin, fasting C-peptide concentrations and the C-peptide/blood glucose ratio in patients with GDM, different trimesters of the healthy pregnant group and non-pregnant control females (means±S.D.).

		Healthy pregnant women (trimester)			
	GDM	1st	2nd	3rd	**Non-pregnant controls**
TNF-α (pg/ml)	6.3±0.6**	4.1±0.4$^{\#,+}$	4.4±0.4$^{\#,+}$	5.5±0.7*	4.1±0.4
sTNFR-1 (ng/ml)	3.2±0.5*	2.1±0.5$^{\#,+}$	2.3±0.5$^{\#,+}$	2.8±0.9**	2.01±0.1
sTNFR-2 (ng/ml)	10.0±6.9**	4.7±2.1$^{\#,+}$	5.3±3.7$^{\#,++}$	5.7±2.6**	3.3±0.2
Leptin (ng/ml)	40.4±24.5**	11.4±7.2$^{\#,+}$	11.1±5.2$^{\#,+}$	33.5±22.0**	12.0±9.1
Fasting C-peptide	6.0±3.0**	1.2±0.8$^{\#,+}$	1.3±0.4$^{\#,+}$	3.1±1.7*	1.1±0.8
Fasting C-peptide/blood glucose ratio	1.4±0.5**	0.3±0.1$^{\#,+}$	0.3±0.1$^{\#,+}$	0.7±0.2*	0.2±0.1

*$P < 0.05$, **$P < 0.01$, as compared with the non-pregnant controls, $^{\#}P < 0.01$, as compared with patients with GDM, $^{+}P < 0.05$, $^{++}P < 0.01$, as compared with pregnant women in the third trimester, calculated by the Mann–Whitney test.

Results

Serum AHSG concentrations in non-pregnant and pregnant women and in patients with GDM

Compared with non-pregnant women there was an increase of serum AHSG concentration during the first, second, and third trimesters of pregnancy (Fig. 1). In patients with GDM, significantly elevated AHSG levels were observed compared with non-pregnant controls and of healthy pregnant women at any trimester (Fig. 1).

Significantly elevated ($P < 0.01$) serum TNF-α, sTNFR-1, sTNFR-2, leptin and C-peptide levels were found in GDM patients as compared with healthy non-pregnant controls and healthy pregnant females at any trimester (Table 3). In healthy pregnant females these values were also significantly higher ($P < 0.01$) in the third trimester compared with the first and second and with the non-pregnant controls (Table 3).

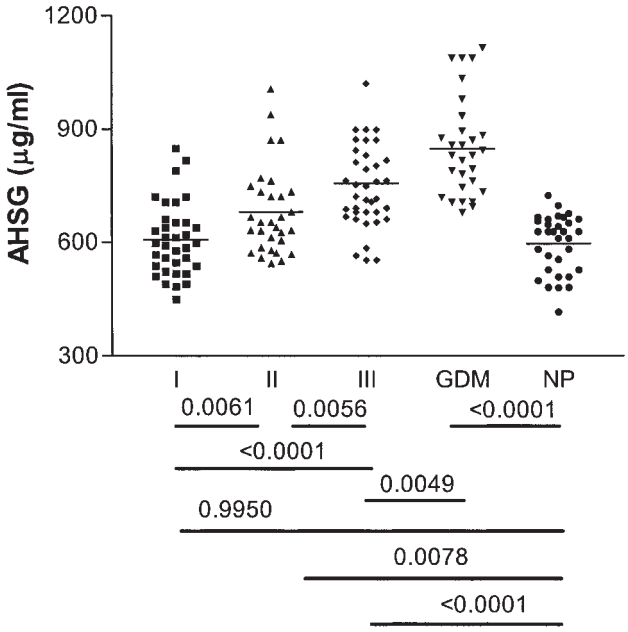

Figure 1 Serum AHSG in different trimesters (I, II, III) of normal pregnancy, the third trimester of GDM and non-pregnant women (NP). Horizontal lines within scattergrams represent means. Statistical differences between individual groups, represented by horizontal lines between groups, were calculated by the Mann–Whitney test.

Correlation between maternal serum AHSG concentration and the indirect parameters of insulin resistance

There was a significant positive correlation between serum AHSG concentration and the indirect parameters of maternal insulin resistance (fasting C-peptide concentration and C-peptide/blood glucose ratio) in GDM patients and in healthy pregnant women (Table 4). Maternal serum AHSG concentration was also found to be in a linear positive correlation with maternal serum TNF-α and leptin values in the GDM group and in the healthy pregnant groups. These correlations were not observed in non-pregnant controls (Table 4).

Multivariate analysis showed that TNF-α had a significant effect on AHSG concentrations both in GDM ($r = 0.781$, $P < 0.0001$) and in normal pregnancy ($r = 0.737$, $P = 0.004$). In this analysis, AHSG alone did not have a significant effect on indirect parameters of insulin resistance (C-peptide and C-peptide/blood sugar ratio); however, in combination with TNF-α and leptin it had a significant effect in GDM (adjusted $r^2 = 0.575$, $P = 0.001$) and in normal pregnancy (adjusted $r^2 = 0.823$, $P < 0.001$).

Correlation between maternal serum AHSG concentration and the anthropological parameters of newborns

The anthropological parameters of neonates (both sexes) delivered by the GDM mothers were slightly decreased as compared with those delivered by healthy pregnant women (Table 2).

Table 4 Correlation between AHSG and laboratory parameters of patients with GDM, healthy pregnant women, and non-pregnant controls calculated by Spearman's rank correlation. First row: correlation coefficient, second row: 95% CI. Significance values are in brackets in the third row.

	GDM ($n = 30$)	**Healthy pregnant women** ($n = 38$)	**Non-pregnant controls** ($n = 30$)
Body mass index	0.5702	0.2180	−0.0021
	0.2537–0.7764	−0.1099–0.5030	−0.3884–0.3513
	(0.0010)	(0.1766)	(0.9104)
TNF-α	0.6614	0.5853	−0.0541
	0.3858–0.8286	0.3263–0.7625	−0.3224–0.4158
	(<0.0001)	(<0.0001)	(0.7766)
sTNFR-1	0.0308	0.3956	−0.0091
	−0.3432–0.3963	0.0836–0.6353	−0.3779–0.3621
	(0.8718)	(0.0115)	(0.9618)
sTNFR-2	0.1003	0.2123	0.0318
	−0.2802–0.4535	−0.1157–0.4986	−0.3422–0.3972
	(0.5981)	(0.1884)	(0.8673)
Leptin	0.5775	0.3528	−0.1212
	0.2639–0.7807	0.0368–0.6047	−0.4702–0.2605
	(0.0008)	(0.0255)	(0.5233)
C-peptide	0.6699	0.4020	0.0519
	0.3988–0.8334	0.0939–0.6398	−0.3244–0.4139
	(<0.0001)	(0.0101)	(0.7855)
C-peptide/blood glucose ratio	0.5116	0.3241	0.2151
	0.0939–0.6398	0.0044–0.5867	−0.1683–0.5420
	(0.0039)	(0.0413)	(0.2537)

Body length and head circumference of the newborns were found to be in a significant negative correlation with the maternal AHSG concentration in GDM (Table 5). In healthy pregnant women a significant negative linear correlation was calculated with all three anthropological parameters of the newborns (Table 5). Among body length (bl), head circumference (hc), body weight (bw) of the newborns and maternal serum TNF-α ($r_{hc} = -0.4857$, 95% confidence interval (CI) -0.7254 -0.1411) and leptin ($r_{bl} = -0.4376$, 95% CI -0.6951 -0.0807, $P = 0.0156$; $r_{bw} = -0.3706$, 95% CI -0.6513 -0.0007, $P = 0.0438$) levels significant negative correlations have been calculated in the GDM group. In healthy pregnant women a significant negative linear correlation was observed only between maternal leptin concentration and the head circumference of the neonates ($r = -0.6001$, 95% CI -0.8283 -0.2010, $P = 0.0026$).

Multivariate analysis showed that maternal serum AHSG concentration alone did not have a significant influence either on head circumference or on body length of neonates in GDM and normal pregnancy. However, in combination with TNF-α, leptin and C-peptide AHSG had a significant effect on these parameters in GDM (adjusted $r^2 = 0.563$, $P = 0.002$), but not in normal pregnancy (adjusted $r^2 = -0.069$, $P = 0.583$).

Table 5 Correlation between AHSG concentration and anthropological parameters of the neonates of patients with GDM and healthy pregnant women calculated by Spearman's rank correlation. First row: correlation coefficient, second row: 95% CI. Significance values are in brackets in the third row.

	GDM ($n = 30$)	**Healthy pregnant women** ($n = 23$)
Head circumference	−0.6256	−0.6417
	−0.8085–−0.3325	−0.8374–−0.3003
	(0.0002)	(0.0010)
Body length	−0.4068	−0.5016
	−0.6752–−0.0433	−0.7628–−0.0098
	(0.0257)	(0.0147)
Body weight	−0.3098	−0.4998
	−0.6099–0.0680	−0.7773–−0.0594
	(0.0957)	(0.0248)

Discussion

The increase of maternal serum AHSG concentration during pregnancy has been observed but not analyzed and interpreted in detail (14). AHSG is considered as a negative acute phase reactant, the concentration of which decreases during infections, trauma and liver cirrhosis (15–17). It is tempting to speculate on the biological significance of this protein in clinical settings where its concentration increases, i.e. in pregnancy.

The aim of the present study was to investigate the role of AHSG in maternal insulin resistance in GDM and in normal pregnant women. The phosphorylated variant of AHSG was described among the first proteins which interfere with the intracellular signaling of the insulin receptor (1–5). No relationship between serum AHSG levels and any parameters of insulin resistance has been described so far. Only the phosphorylated form of AHSG is able to inhibit insulin

signaling *in vitro*, the dephosphorylated form is inactive (3, 5). AHSG isolated from human serum still retains some inhibitory activity on insulin receptor signaling, yet this activity is much less than that of recombinant AHSG produced in the baculovirus expression vector system (3). The presence of phosphorylated AHSG in human serum (approximately 20% of the total) has been recently demonstrated in healthy persons (18).

During the course of normal pregnancy increasing insulin resistance occurs, which is even more pronounced in GDM. Previously we found that the progressive elevation of the fasting C-peptide and C-peptide/blood glucose ratio are easily measurable parameters of progressive insulin resistance in normal pregnancy and in GDM (due to ethical reasons clamp or other i.v. models for exact estimation of insulin resistance were not performed in our subjects) (19). Therefore we considered these states as a model to study the contribution of different parameters (e.g. TNF system, leptin and also AHSG) to the transitory insulin resistance.

We found significant positive correlations among the TNF system, leptin, AHSG, and the indirect parameters of insulin resistance in both healthy pregnant women and GDM patients. These data suggest the contribution of the elevated AHSG levels to insulin resistance. The liver is the main site of AHSG synthesis; however, in pregnancy the fetoplacental unit can be another source of the protein. This is supported by the elevation of the protein concentration during the course of pregnancy, especially marked in the third trimester. In adults, AHSG can be expressed not only in the liver but in other organs, thus in the endometrium. In addition, autoantibodies against endometrial AHSG and transferrin have been demonstrated in patients with endometriosis (20). An increased hepatic synthesis during pregnancy, however, cannot be ruled out. Our observations may suggest that this increased synthesis of AHSG in the liver contributes to the decreased hepatic insulin sensitivity during normal pregnancy and GDM. TNF-α may also have a regulatory role on the synthesis of the protein.

The negative correlation between maternal serum leptin, TNF-α and head circumference of the newborns may raise the possibility that these cytokines can serve as negative regulators of neonatal bone development, as was suggested earlier in animal models (8, 9). The negative regulatory role of AHSG on bone development has also been postulated, e.g. AHSG prevented bone resorption and hydroxylapatite formation *in vitro* (21, 22). The biological significance of AHSG in the regulation of human neonatal bone development has not been clarified; however, several data from animal studies including mice knocked out for AHSG gene raise the possibility that the protein may prevent unwanted calcification (22). The negative correlation between the AHSG concentrations measured in maternal serum during normal pregnancy and GDM and the head circumference of the neonates further supports the hypothesis of the negative regulatory role of the protein in human neonatal bone development. Further studies are necessary on the exact role of AHSG in this process.

In conclusion, AHSG, TNF-α and leptin in combination may contribute to insulin resistance during normal pregnancy and GDM. These cytokines and AHSG may also negatively regulate neonatal skeletal development.

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