

# Plasma Adiponectin, Insulin Sensitivity, and Subclinical Inflammation in Women With Prior Gestational Diabetes Mellitus

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**OBJECTIVE** — Women with prior gestational diabetes mellitus (pGDM) are at increased risk of developing type 2 diabetes and associated vasculopathy. Because increased fat mass and inflammatory processes are angiopathic risk factors, the relationship between insulin sensitivity, parameters of subclinical inflammation, and plasma concentrations of adipocytokines was investigated in pGDM both at 3 months and 12 months after delivery.

**RESEARCH DESIGN AND METHODS** — Insulin sensitivity (through a frequently sampled intravenous glucose tolerance test) and plasma concentrations of ultrasensitive C-reactive protein (CRP), adiponectin, plasminogen activator inhibitor (PAI)-1, tumor necrosis factor- $\alpha$ , leptin, and interleukin-6 were measured in 89 pGDM (BMI  $26.9 \pm 0.5$  kg/m<sup>2</sup>, age  $32 \pm 0.5$  years) and in 19 women with normal glucose tolerance during pregnancy (NGT) ( $23.7 \pm 0.9$  kg/m<sup>2</sup>,  $31 \pm 1.3$  years).

**RESULTS** — pGDM showed lower ( $P < 0.0001$ ) plasma adiponectin ( $6.7 \pm 0.2$   $\mu$ g/ml) than NGT ( $9.8 \pm 0.6$   $\mu$ g/ml) and a decreased ( $P < 0.003$ ) insulin sensitivity index ( $S_i$ ) and disposition index ( $P < 0.03$ ), but increased plasma leptin ( $P < 0.003$ ), PAI-1 ( $P < 0.002$ ), and CRP ( $P < 0.03$ ). After adjustment for body fat mass, plasma adiponectin remained lower in pGDM ( $P < 0.004$ ) and correlated positively with  $S_i$  ( $P < 0.003$ ) and HDL cholesterol ( $P < 0.0001$ ) but negatively with plasma glucose (2-h oral glucose tolerance test [OGTT]) ( $P < 0.0001$ ), leptin ( $P < 0.01$ ), CRP ( $P < 0.007$ ), and PAI-1 ( $P < 0.0001$ ). On regression analysis, only HDL cholesterol, postload (2-h OGTT) plasma glucose, and  $S_i$  remained significant predictors of plasma adiponectin, explaining 42% of its variability. Of note, adiponectin further decreased ( $P < 0.05$ ) only in insulin-resistant pGDM despite unchanged body fat content and distribution after a 1-year follow-up.

**CONCLUSIONS** — Lower plasma adiponectin concentrations characterize women with previous GDM independently of the prevailing insulin sensitivity or the degree of obesity and are associated with subclinical inflammation and atherogenic parameters.

*Diabetes Care* 27:1721–1727, 2004

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Received for publication 8 January 2004 and accepted in revised form 23 March 2004.

**Abbreviations:** BFM, body fat mass; CRP, C-reactive protein; GDM, gestational diabetes mellitus; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; NGT, women with normal glucose tolerance during pregnancy; OGIS, insulin sensitivity estimated from OGTT data; OGTT, oral glucose tolerance test; PAI, plasminogen activator inhibitor; pGDM, women with prior GDM; pGDM-R, insulin-resistant pGDM; pGDM-S, insulin-sensitive pGDM; TNF, tumor necrosis factor.

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

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Plasma concentrations of adiponectin, an adipocyte-specific collagen-like molecule, are reduced in patients with obesity, coronary artery disease, and type 2 diabetes (1). Longitudinal studies indicate that a decrease in plasma adiponectin parallels the progression of the metabolic syndrome (2,3). Potentially, this observation suggests a predictive role of adiponectin also in the development of type 2 diabetes and atherosclerosis, where it could relate to insulin sensitivity and atherogenic parameters such as the lipid profile, cytokines, and subclinical inflammation. This concept is supported by low plasma adiponectin concentrations in states of enhanced insulin resistance in rhesus monkeys (4) and humans (5), when low plasma adiponectin precedes the decrease in whole-body insulin sensitivity (6,7). Moreover, adiponectin decreases both the attachment of monocytic THP-1 cells to human aortic endothelial cells and suppresses the secretion of tumor necrosis factor (TNF)- $\alpha$  from human monocytic macrophages (8). In mice, adiponectin deficiency mediates injury-induced neointimal formation and thereby argues for an adipovascular axis involving adiponectin and plasminogen activator inhibitor (PAI)-1 (9).

Gestational diabetes mellitus (GDM) carries considerable health risks for both the fetus and the mother (10). Although most women with GDM return to normal glucose tolerance after delivery, it is well established that glucose intolerance detected during pregnancy is predictive of later maternal type 2 diabetes (10,11). Particularly, women with impaired glucose metabolism or diabetes carry a greater cardiovascular risk than normoglycemic individuals (12). Indeed, women with prior GDM (pGDM) feature endothelial dysfunction that is associated with insulin resistance (13–15) and obesity, which are the most prominent risk factors for GDM and type 2 diabetes in pGDM and linked to both inflammatory processes and angiopathy (16).

Thus, both body fat and adipocytokines relate to the metabolic syndrome

Table 1—Clinical characteristics, metabolic parameters, and fasting plasma concentrations of adipocytokines and CRP of pGDM, pGDM-R, and pGDM-S compared with NGT 3 months after delivery

	pGDM	pGDM-R	pGDM-S	NGT
n	89	30	59	19
Age (years)	32.2 ± 0.5	32.0 ± 1.0	32.4 ± 0.6	31.9 ± 1.3
BMI (kg/m <sup>2</sup> )	26.9 ± 0.5*	29.1 ± 1.1	24.8 ± 0.5	23.7 ± 0.9†
BFM (kg)	26.0 ± 1.1*	31.5 ± 2.1	21.6 ± 1.1	18.7 ± 1.4†
Waist circumference (cm)	90.6 ± 1.3*	94.6 ± 2.3‡	88.2 ± 1.7	76.3 ± 1.7†§
Triglycerides (mg/dl)	104.9 ± 7.0*	110.9 ± 11.0	99.7 ± 9.7	71.8 ± 7.3†
HDL cholesterol (mg/dl)	55.3 ± 1.5*	54.6 ± 2.8	56.0 ± 1.7	63.6 ± 3.2†§
HbA <sub>1c</sub> (%)	5.60 ± 0.06*	5.65 ± 0.10	5.54 ± 0.07	5.17 ± 0.04†§
Fasting glucose (mg/dl)	90.7 ± 1.3*	93.2 ± 2.1‡	88.3 ± 1.5	82.8 ± 1.2†§
2-h glucose (mg/dl) <sub>OGTT</sub>	121.1 ± 3.7*	130.3 ± 7.0‡	115.1 ± 4.5	90.6 ± 3.5†§
OGIS (ml · min <sup>-1</sup> · m <sup>-2</sup> ) <sub>OGTT</sub>	439.5 ± 9.9*	391.7 ± 15.8‡	469.0 ± 11.6	504.2 ± 13.7†
S <sub>i</sub> [10 <sup>-4</sup> min <sup>-1</sup> (μU/ml) <sup>-1</sup> ] <sub>FSIGT</sub>	3.95 ± 0.29*	1.63 ± 0.10‡	5.22 ± 0.33	6.0 ± 0.6†
Disposition index (10 <sup>-2</sup> · min <sup>-1</sup> ) <sub>FSIGT</sub>	0.119 ± 0.01*	0.064 ± 0.01‡	0.148 ± 0.01	0.170 ± 0.02†
Adiponectin (μg/ml)	6.70 ± 0.23*	6.52 ± 0.40	6.93 ± 0.29§	9.80 ± 0.60†
Insulin (pmol/l)	61.2 ± 4.2	83.4 ± 9.0‡	41.5 ± 1.8	50.4 ± 3.6†
Leptin (ng/ml)	14.9 ± 0.8*	18.5 ± 1.3‡	13.3 ± 0.9§	9.2 ± 1.4†
PAI-1 (ng/ml)	28.7 ± 2.1*	34.3 ± 3.6‡	24.5 ± 2.5§	15.6 ± 1.7†
TNF-α (pg/ml)	5.2 ± 0.7	4.5 ± 1.2	5.6 ± 1.0	3.3 ± 0.4
IL-6 (pg/ml)	2.0 ± 0.3*	2.2 ± 0.6	1.8 ± 0.3	1.4 ± 0.6
CRP (mg/dl)	0.45 ± 0.06*	0.54 ± 0.11	0.38 ± 0.09	0.18 ± 0.04†

\*P < 0.05 pGDM vs. NGT; †P < 0.05 pGDM-R vs. NGT; ‡P < 0.05 pGDM-R vs. pGDM-S; §P < 0.05 pGDM-S vs. NGT.

and to associated endothelial dysfunction. Against that background, it is the aim of this study to determine at 3 and 12 months after delivery the association of adipocytokines with body fat mass (BFM) and distribution, as well as with insulin sensitivity and surrogate parameters of endothelial dysfunction in pGDM and age-matched women with normal glucose tolerance during pregnancy (NGT).

## RESEARCH DESIGN AND METHODS

A baseline cross-sectional analysis was performed at 3 months after delivery in 89 pGDM participating in a prospective longitudinal follow-up study (Vienna post-gestational diabetes project). All women were invited to undergo a reexamination 12 months after delivery. In a subgroup of these pGDM ( $n = 44$ ; 50%), who did not differ in baseline parameters from the other women (ANOVA, data not shown), plasma adiponectin concentrations could be reexamined after 1 year and related to changes in body fat content and distribution as well as to changes in glucose tolerance. Within the observation period, no woman developed diabetes.

pGDM were recruited from our division's outpatient service, where they had been seen during pregnancy. GDM had

been diagnosed according to the criteria of the 4th Workshop Conference of Gestational Diabetes (10). During pregnancy, 60 women (66%) were treated with diet plus insulin, because blood glucose exceeded 95 mg/dl at fasting and/or 130 mg/dl at 60 min postprandially. Nineteen age-matched women without any risk factors for diabetes and with normal glucose tolerance during and after pregnancy served as the control group (NGT). All subjects gave written informed consent for participation in the study, which was approved by the local ethics committee.

The relationship between adipocytokines, insulin sensitivity, and metabolic as well as inflammatory parameters was analyzed both in the total pGDM population and for the insulin-sensitive [pGDM-S:  $S_i > 2.8 \cdot 10^{-4} \text{ min}^{-1}/(\mu\text{U/ml})$ ] and the insulin-resistant [pGDM-R:  $S_i < 2.8 \cdot 10^{-4} \text{ min}^{-1}/(\mu\text{U/ml})$ ] subgroups. The cutoff value [ $2.8 \cdot 10^{-4} \text{ min}^{-1}/(\mu\text{U/ml})$ ] was obtained by analysis of  $S_i$  in NGT of the present study ( $n = 19$ ) plus control women ( $n = 22$ ) matched for age, weight, and parity from other studies (13,17). pGDM were then divided into subgroups with (pGDM-R,  $n = 30$ ) and without (pGDM-S,  $n = 59$ ) impaired insulin sensitivity.

## Metabolic characteristics at baseline

pGDM had higher fasting ( $P < 0.005$ ) and postload (2-h oral glucose tolerance test [OGTT]) glucose concentrations ( $P < 0.0001$ ), HbA<sub>1c</sub> ( $P < 0.0002$ ), and plasma triglycerides ( $P < 0.03$ ) but lower plasma HDL cholesterol concentrations ( $P < 0.03$ ) than NGT. Blood pressure did not differ between groups. The total pGDM group featured higher fat mass ( $P < 0.001$ ) and waist circumference ( $P < 0.0001$ ) than NGT, but pGDM-S did not differ from NGT in regard to BMI or body fat content (Table 1).

## Methods

All women ingested an isocaloric diet containing 200 g carbohydrate per day and refrained from exercise for at least 3 days before the studies. Metabolic tests were performed on different days during the first phase (days 5–8) of the menstrual cycle after a 10- to 12-h overnight fast.

## Frequently sampled glucose tolerance test

Glucose (time 0–0.5 min: 300 mg/kg body wt) and then normal insulin (time 20–25 min: 0.03 units/kg Humulin R; Eli Lilly, Indianapolis, IN) were intravenously infused, and venous blood sam-

**Table 2—Mean values of metabolic parameters and fasting plasma concentrations of adipocytokines after adjustment for waist circumference or BFM of pGDM, pGDM-R, and pGDM-S compared with NGT 3 months after delivery**

	pGDM	pGDM-R	pGDM-S	NGT
<i>n</i>	89	30	59	19
HbA <sub>1c</sub> (%)				
Adjusted for WC	5.54	5.48	5.53	5.24
Adjusted for BFM	5.57*	5.51	5.54	5.21
Fasting glucose (mg/dl)				
Adjusted for WC	89.00	88.51	87.85	82.33
Adjusted for BFM	89.93*	87.97	89.27	81.36
2-h glucose (mg/dl) <sub>OGTT</sub>				
Adjusted for WC	117.98*	121.01	114.40	90.85
Adjusted for BFM	119.82†	122.43	117.82‡	89.87#
S <sub>i</sub> [10 <sup>-4</sup> · min <sup>-1</sup> (μU/ml) <sup>-1</sup> ] <sub>FSIGT</sub>				
Adjusted for WC	4.18	1.79	5.29	4.16#
Adjusted for BFM	4.03*	1.75¶	4.77	5.34§
Adiponectin (μg/ml)				
Adjusted for WC	6.92*	6.85	6.95‡	9.25#
Adjusted for BFM	6.71*	6.21	6.76‡	9.60#
PAI-1 (ng/ml)				
Adjusted for WC	26.73	30.94	23.66	17.40
Adjusted for BFM	27.52*	31.03	39.41	18.14
Leptin (ng/ml)				
Adjusted for WC	14.08	15.97	13.42	15.42
Adjusted for BFM	14.26	15.24	14.43	13.52

FSIGT, frequently sampled intravenous glucose tolerance test; WC, waist circumference. \**P* < 0.05; †*P* < 0.005 pGDM vs. NGT; ‡*P* < 0.05 pGDM-S vs. NGT; §*P* < 0.0005 pGDM-R vs. NGT; ||*P* < 0.05; ¶*P* < 0.005 pGDM-R vs. pGDM-S; #*P* < 0.05 pGDM-R vs. NGT.

ples were taken in timed intervals (18). Analysis of plasma glucose and insulin concentrations provided indexes for insulin sensitivity (S<sub>i</sub>), which describe glucose disposal and the insulin effect on glucose disappearance (18). The disposition index was calculated as S<sub>i</sub> × ΔAIR<sub>G</sub> (acute insulin response to glucose) (incremental short-term insulin response after glucose bolus: 3–10 min) and gives a measure of the combined effects of insulin secretion and sensitivity on glucose disposal (19).

### 75-g OGTT

Glucose solution was ingested within 2 min, and venous blood samples were collected for measurements of plasma glucose, insulin, and C-peptide at timed intervals (20). Modeling analysis yielded estimates of basal insulin secretion on the basis of plasma C-peptide concentrations (20) and of insulin action by describing glucose dynamics with a mathematical model, which allows the quantification of glucose clearance per unit change of insulin (18). Insulin sensitivity estimated from OGTT data are termed OGIS (21).

### BFM

Body fat content was assessed from bioimpedance measurements (Akern-RJL Systems, Florence, Italy). Prediction errors of body composition equations estimating percent fat-free mass are based on empirically derived measurement errors associated with the reference method (hydrodensitometry). A standard error of estimation for percent fat-free mass of <2.8 kg for women has been calculated (22).

### Adipocytokines/hormones

All samples were stored at –70°C until analysis. Adiponectin was measured in duplicate in fasting plasma samples using the enzyme-linked immunosorbent assay (ELISA) system developed for the measurement of human plasma adiponectin concentrations (Department of Internal Medicine and Molecular Science, Osaka University, Suita, Osaka, Japan) (6). Human recombinant adiponectin was used as a standard. Insulin (Serono Diagnostics, Freiburg, Germany), C-peptide (CIS Bio International, Cedex, France), and total leptin (Human Leptin RIA kit; Linco

Research, St. Charles, MO) were determined in duplicate by commercially available radioimmunoassay kits with an interassay coefficient of variation of <5% for insulin and C-peptide and <8% for proinsulin. The intra-assay coefficient of variation for the determination of plasma leptin radioimmunoassay was 4.1%, whereas the respective interassay coefficient of variation was 5.5%.

Plasma concentrations of active PAI-1 antigen were measured by an ELISA system (Actibind PAI-1 ELISA; Technoclone, Vienna, Austria) according to the manufacturer's instructions. TNF-α was measured by a quantitative sandwich enzyme immunoassay technique (Quantikine HS Immunoassay kit), interleukin (IL)-6 by ELISA systems (both by R&D Systems, Wiesbaden, Germany), and C-reactive protein (CRP) by means of particle-enhanced immunonephelometry (N High Sensitivity CRP Reagent, BN Systems; Dade Behring, Deerfield, IL).

### Statistical analysis

Data are presented as means ± SE. Associations between continuous variables are described by correlation coefficients (Pearson and Spearman). Partial correlation analysis was performed to adjust for the effects of S<sub>i</sub>, BFM, and waist circumference on the relationship between plasma adiponectin concentrations, metabolic and inflammatory parameters, and other adipocytokines. ANOVA and ANCOVA were used for group comparisons, for adjustment of covariates, and for interaction between covariates and grouping variables. Multiple comparisons were by the Tukey-Kramer test, and stepwise regression analysis was carried out to identify independent regulators of plasma adiponectin concentrations. The baseline data and the data after 1 year were compared by paired *t* tests. Statistical significance was defined as a *P* value <0.05. The software package SAS version 8.2 (SAS Institute, Cary, NC) was used for all computations.

## RESULTS

### Baseline data

#### pGDM compared with NGT

**Adipocytokines.** Plasma adiponectin concentrations were lower (*P* < 0.0001) in pGDM than in NGT during and after pregnancy. pGDM showed higher plasma concentrations of leptin (*P* < 0.003),

**Table 3—Correlations of adiponectin plasma concentrations with parameters of glucose and lipid metabolism, adipocytokines, and inflammatory proteins (Spearman correlation coefficient) in all women as well as partial correlation analysis after adjustment of the effect of BFM, waist-to-hip ratio, or  $S_i$** 

	Adiponectin		Adjusted for BFM		Adjusted for waist-to-hip ratio		Adjusted for $S_i$	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
$S_i$	0.40	<0.0001	0.26	<0.009	0.20	0.04	—	—
Disposition index <sub>FSGIT</sub>	0.33	0.0008	0.23	<0.02	0.22	0.03	—	—
Basal insulin secretion <sub>OGTT</sub>	−0.31	0.001	−0.19	<0.05	−0.14	0.16	−0.15	0.14
Fasting glucose	−0.42	<0.0001	−0.26	<0.009	−0.20	0.04	−0.24	<0.02
Postload glucose <sub>2-h OGTT</sub>	−0.44	<0.0001	−0.36	<0.0003	−0.26	0.008	−0.30	<0.003
Triglycerides	−0.30	0.002	−0.22	<0.02	−0.13	0.19	−0.23	<0.03
HDL cholesterol	0.48	<0.0001	0.44	<0.0001	0.38	0.0001	0.41	<0.0001
PAI-1	−0.51	<0.0001	−0.44	<0.0001	−0.31	0.001	−0.38	<0.0001

pGDM plus healthy control subjects,  $n = 108$ .

PAI-1 ( $P < 0.001$ ), IL-6 ( $P = 0.05$ ), and CRP ( $P < 0.03$ ) but no difference in plasma TNF- $\alpha$  concentrations.

**Frequently sampled intravenous glucose tolerance test.** The pGDM group was characterized by decreased insulin sensitivity index ( $S_i$ ) ( $P < 0.0008$ ) and disposition index ( $S_i \times AIR_G$ ) ( $P < 0.03$ ) (Table 1).

**pGDM-R compared with pGDM-S and NGT.** Adiponectin did not differ between the pGDM-S and pGDM-R subgroups but was lower than in NGT ( $P < 0.001$ ). Lean pGDM-S further differed from NGT in plasma concentration of PAI-1 ( $P < 0.03$ ), leptin ( $P < 0.03$ ), and HDL cholesterol ( $P < 0.05$ ) and in waist circumference ( $P < 0.02$ ). Despite normal insulin sensitivity, pGDM featured lower disposition indexes ( $P < 0.0002$ ) than NGT.

**ANCOVA.** After adjustment for BFM, adiponectin remained lower ( $P < 0.0004$ ) in pGDM than in NGT, which applied to both pGDM-R and pGDM-S. Plasma concentrations of PAI-1 as well as parameters of glucose metabolism remained higher in pGDM than in NGT. pGDM-R still had the lowest  $S_i$ , whereas HbA<sub>1c</sub> and plasma glucose concentrations did not differ between the pGDM subgroups (Table 2).

After adjustment for waist circumference (Table 2) or waist-to-hip ratio (data not shown), adiponectin still remained lower ( $P < 0.02$ ) and 2-h glucose was higher in pGDM than in NGT.

**Correlations.** Adiponectin related positively to parameters of glucose metabolism including OGIS ( $P < 0.0003$ ,  $r = 0.35$ ) and HDL cholesterol, but negatively to the degree of obesity (BMI:  $P < 0.004$ ,

$r = -0.31$ ; BFM:  $P < 0.009$ ,  $r = -0.28$ ; waist circumference:  $P < 0.0001$ ,  $r = -0.39$ ) as well as to plasma concentrations of PAI-1 and other adipocytokines (leptin:  $P < 0.01$ ,  $r = -0.25$ ; IL-6:  $P < 0.03$ ,  $r = -0.22$ ) and CRP ( $P < 0.007$ ,  $r = -0.27$ ). Of note, adiponectin (measured postpartum) was also strongly associated with fasting ( $P < 0.02$ ,  $r = -0.24$ ) and stimulated ( $P < 0.001$ ,  $r = -0.38$ ) plasma glucose concentrations during pregnancy (diagnostic OGTT) (Table 3).

**Partial correlation analysis.** After adjustment for the effect of BFM, waist circumference, or insulin sensitivity, plasma adiponectin did not relate to leptin, IL-6, or CRP (data not shown), indicating that in the overall population such relation is at least partly mediated by BFM and distribution or  $S_i$ . No relation between plasma adiponectin and BFM was seen after adjustment for  $S_i$ .

**Stepwise linear regression.** Upon regression analysis with adiponectin as a dependent parameter, only HDL cholesterol, 2-h plasma glucose concentration,

and  $S_i$  remained as predictors, explaining 42% of plasma adiponectin (Table 4).

**Follow-up data at 1 year.** At 12 months after delivery, mean plasma adiponectin concentrations, glucose tolerance (2-h OGTT), insulin sensitivity (OGIS<sub>OGTT</sub>), BFM, and distribution (waist circumference) did not change in pGDM, whereas the lipid profile deteriorated and HbA<sub>1c</sub> improved (Table 5).

In pGDM-R ( $n = 16$ ) but not in pGDM-S ( $n = 28$ ), adiponectin fell by 10% ( $P < 0.01$ ) despite unchanged body weight, body fat composition and distribution, or glucose and lipid metabolism. Nevertheless, adiponectin still correlated with glucose tolerance (2-h OGTT:  $r = -0.5$ ,  $P < 0.004$ ), insulin sensitivity (OGIS<sub>OGTT</sub>:  $r = 0.4$ ,  $P < 0.02$ ), and lipids (triglycerides:  $r = -0.4$ ,  $P < 0.01$ ; HDL cholesterol:  $r = 0.6$ ,  $P < 0.0001$ ) as well as BFM ( $r = -0.4$ ,  $P < 0.008$ ) and waist circumference ( $r = -0.4$ ,  $P < 0.009$ ).

**CONCLUSIONS**— Plasma adiponectin concentrations are lower in

**Table 4—Stepwise linear regression of independent variables associated with adiponectin concentrations**

Independent variables	Parameter estimate	SE	<i>P</i>
Intercept	4.31	1.62	0.009
HDL cholesterol	0.073	0.019	0.0004
Postload glucose <sub>2-h OGTT</sub>	−0.018	0.007	0.02
$S_i$	0.204	0.099	0.04

Independent variables: fasting and postload glucose<sub>2-h OGTT</sub>, triglycerides, HDL cholesterol, PAI-1,  $S_i$ , disposition index<sub>FSGIT</sub>, basal insulin secretion<sub>OGTT</sub>, and waist circumference; 0.15 significance level for entry of variables into the model. Dependent variable = fasting plasma adiponectin concentration. FSGIT, frequently sampled intravenous glucose tolerance test.

**Table 5—12-month follow-up after delivery of pGDM and the respective pGDM-R and pGDM-S subgroups**

	pGDM	pGDM-R	pGDM-S
<i>n</i>	44	16	28
Body fat content (kg)	26.1 ± 1.8	32.9 ± 3.0	21.8 ± 1.8
Waist circumference (cm)	88.5 ± 1.9	95.0 ± 2.8	84.9 ± 2.4
Triglycerides (mg/dl)	123.1 ± 11.1*	111.2 ± 14.8	127.8 ± 16.5
HDL cholesterol (mg/dl)	49.7 ± 1.9†	47.7 ± 2.5	51.1 ± 2.8
HbA <sub>1c</sub> (%)	5.5 ± 0.1†	5.6 ± 0.3	5.3 ± 0.1
Fasting glucose (mg/dl)	95.7 ± 3.5	99.3 ± 8.2	112.9 ± 6.0
OGIS (ml · min <sup>-1</sup> · m <sup>-2</sup> ) <sub>OGTT</sub>	426.4 ± 28.2	398.8 ± 34.8	454.3 ± 17.8
Plasma adiponectin (μg/ml)	6.64 ± 0.4	6.08 ± 0.6*	6.83 ± 0.56

\**P* < 0.01; †*P* < 0.005 vs. baseline data.

women with prior GDM than in age-matched women who were glucose tolerant during the preceding pregnancy. Such reduced adiponectin concentrations are associated with insulin resistance independently of metabolic covariates and measures of body fat content and distribution. In addition, a negative association between circulating adiponectin levels and proinflammatory proteins, including CRP and PAI-1, was observed.

In the present study, adiponectin relates inversely to other adipocytokines such as leptin and PAI-1, but only marginally to plasma TNF- $\alpha$  concentrations (pGDM-R). In pGDM, plasma adiponectin and leptin concentrations differ from that in NGT, irrespective of the prevailing insulin sensitivity. Leptin acts as a signal for sufficient energy supplies, is persistently increased in women with GDM after delivery, and associates with hyperglycemia and insulin resistance as well as weight gain after delivery (23). Our data show interdependence of adiponectin and leptin with BFM and insulin sensitivity. Contrary to leptin, the interaction between adiponectin and insulin sensitivity is independent of BFM. Hence, plasma adiponectin concentrations appear more markedly associated with insulin sensitivity and glucose metabolism than with adiposity, extending previous results (4,5).

Of note, pGDM-R showed a significant decrease of plasma adiponectin concentrations 1 year after delivery, whereas pGDM-S maintained their plasma concentrations. Such a decrease occurred even though pGDM-R did not show any change in glucose tolerance, lipids, body weight, and body fat content or distribution. This is in line with the contention

that low plasma concentrations are not necessarily associated with weight gain in humans (24). On the other hand, because insulin resistance can associate with progression to impaired glucose metabolism and cardiovascular disease, this could indicate a high risk of these women developing type 2 diabetes and vascular complications at follow-up. Thus, decreased adiponectin preceded a decrease in whole-body insulin sensitivity (6) and has been related to incident diabetes, whereas high plasma adiponectin concentrations were protective against type 2 diabetes (7,25). In overweight Pima Indians, plasma adiponectin was found to be associated with skeletal muscle insulin receptor tyrosine phosphorylation (6). In rodents, administration of adiponectin increases insulin-induced tyrosine phosphorylation of the insulin receptor of skeletal muscle and improves glucose tolerance, relates to insulin action by increasing lipid oxidation in muscle (26), and enhances hepatic insulin action (27).

In our study, even lean and insulin-sensitive pGDM (pGDM-S) had markedly lower plasma adiponectin concentrations than NGT when matched for age and body fat content, underlining the importance of decreased adiponectin as an early marker for a population at risk for type 2 diabetes. However, pGDM-S still had higher mean waist circumferences, potentially indicating a higher degree of abdominal obesity. The observation that rosiglitazone increases adiponectin release from cultured human omental but not from subcutaneous adipocytes (28) supports the contention that decreased secretion of adiponectin from the omental rather than subcutaneous fat compartment accounts for its inverse relationship

with BMI. Nevertheless, the difference in adiponectin plasma concentrations between groups as well as their relationship with glucose tolerance (2-h glucose) and PAI-1 was independent of waist circumference or waist-to-hip ratio.

Our study shows that both pGDM subgroups have higher plasma PAI-1 concentrations than NGT, being highest in the insulin-resistant group even after correction for BFM. Accordingly, plasma PAI-1 concentrations are negatively correlated with plasma adiponectin concentrations, independent of anthropometric parameters as also found in first-degree relatives of patients with type 2 diabetes (2) or in overweight hypertensive patients (29), but also independent of insulin sensitivity, a new finding of this study. In this context, it is of note that hypofibrinolysis is a feature of type 2 diabetic patients and that increased PAI-1 and acute-phase proteins predict deterioration of glucose tolerance (30). Of interest, a PAI-1 knockout mouse model showed genetically diabetic and obese mice to display a reduction in body weight and improved glucose metabolism (31). Furthermore, protection from diet-induced obesity and insulin resistance in mice lacking PAI-1 was associated with the maintenance of peroxisome proliferator-activated receptor- $\gamma$  and adiponectin and with an increase of the metabolic rate (32). These findings seem to point at an independent role of PAI-1 in the pathophysiology of the metabolic syndrome, but also at an (indirect) interplay between PAI-1 and adiponectin, potentially mediated through modulation of the microenvironment and remodeling of the extracellular matrix component surrounding adipocytes (32).

A strong independent association with plasma HDL cholesterol and a weaker association with plasma triglyceride concentrations was found in pGDM, in agreement with other studies (2,3,7). This together with adiponectin's inverse association with PAI-1 and CRP could support the notion of a beneficial effect of adiponectin on the development of atherogenic lesions by exerting anti-inflammatory and antiatherogenic action (8).

Plasma adiponectin was also related to CRP, an inflammatory indirect surrogate marker of endothelial dysfunction (33) that relates to insulin sensitivity (34,35). In pGDM, this inverse association primarily depended on the degree of

obesity. This is in line with a study in premenopausal women with polycystic ovary syndrome, showing that obesity and not insulin resistance is the major determinant of serum inflammatory cardiovascular risk markers (36).

In conclusion, 1) decreased circulating adiponectin concentrations characterize pGDM independently of BFM and distribution or insulin sensitivity 3 months after delivery and 2) adiponectin further decreases in the insulin-resistant subgroup after 1 year despite unchanged anthropometric parameters and glucose tolerance status. In addition, decreased adiponectin plasma concentrations are associated with subtle changes in glucose metabolism and proinflammatory and hypofibrinolytic conditions in women with previous GDM.

**Acknowledgments**—These studies were supported by the Austrian Science Fund (P14515-MED to A.K.-W.). G.P. participated in this study within a cooperative project between Institute of Systems Science and Biomedical Engineering, Metabolic Unit, Italian National Research Council (formerly LADSEB) and the Department of Internal Medicine III, University of Vienna.

We are indebted to P. Nowotny and the laboratory staff of the Division of Endocrinology and Metabolism and to B. Putschögl for support in data management and statistical analysis.

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