Osteocalcin Is Related to Enhanced Insulin Secretion in Gestational Diabetes Mellitus

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OBJECTIVE — There is growing evidence that osteocalcin, an osteoblast-derived protein locally acting on bone formation, can increase insulin secretion as well as insulin sensitivity and thus prevent the development of obesity and diabetes in experimental animals. In humans, osteocalcin has been reported to be decreased in patients with type 2 diabetes. Because gestational diabetes mellitus (GDM) can serve as a model of pre–type 2 diabetes, the aim of this study was to investigate osteocalcin in GDM.

RESEARCH DESIGN AND METHODS — Osteocalcin measurement and an oral glucose tolerance test were performed in 78 pregnant women (26 women had GDM and 52 women had normal glucose tolerance [NGT] during pregnancy; women were matched for age and BMI) and in 34 women postpartum.

RESULTS — During pregnancy osteocalcin was significantly higher in the women with GDM than in the women with NGT (15.6 ± 6.4 vs. 12.6 ± 4.0 ng/ml; $P < 0.015$), whereas no difference was observed between the two groups at 12 weeks postpartum (36.2 ± 10.2 vs. 36.2 ± 13.0 ng/ml), when osteocalcin was found to be increased compared with the level in the pregnant state in all women ($+145 \pm 102\%$ in GDM vs. $+187 \pm 119\%$ in NGT; $P < 0.0001$). Moreover, osteocalcin showed a significant correlation with basal and total insulin secretion in the whole study group ($R = 0.3$, $P < 0.01$).

CONCLUSIONS — In GDM osteocalcin was higher and thus less restrained than in women with NGT during pregnancy and furthermore correlated with insulin secretion parameters. Therefore, it could be hypothesized that osteocalcin can enhance insulin secretion in insulin-resistant states; alternatively an effect of hyperinsulinemia on osteocalcin secretion cannot be excluded.

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E vidence is growing regarding the reciprocal interaction between bone and energy metabolism (1). The findings that adipose tissue via leptin can regulate bone metabolism (2) and that insulin, as an anabolic hormone, influences bone metabolism via IGF-1 (3,4) raised the idea that bone in turn could also affect energy metabolism. Osteocalcin, an osteoblast-derived protein acting locally on bone formation, is suspected to be involved in the regulation of glucose and fat metabolism. Because animal models showed that mice lacking osteocalcin display insulin resistance and obesity, bone became a focus of interest concerning endocrine action as did adipose tissue when adipocytokines were detected. It has been demonstrated that osteocalcin can stimulate insulin secretion, acting directly on proliferation and secretion of the pancreatic β-cells. Osteocalcin might act via an endocrine pathway, and, indeed, osteocalcin has been reported to exert several hormone-specific features. Furthermore, it has been shown that osteocalcin can increase insulin sensitivity, probably by inducing the expression of adiponectin in adipocytes. Besides, mice lacking the *Esp* gene that encodes for a receptor-like protein called OST-PTP, which is thought to influence the bioactivity of osteocalcin, show increased energy expenditure and decreased levels of triglycerides and are protected from diet-induced obesity and diabetes (5).

In humans, osteocalcin has been reported to be decreased in patients with type 2 diabetes (6), negatively correlated with fasting plasma glucose, A1C, insulin resistance (assessed by homeostasis model assessment of insulin resistance), high-sensitivity C-reactive protein (hs-CRP), and BMI and increased with improved glycemic control (7,8). Moderate weight loss and regular exercise have also been found to increase osteocalcin concentrations, partly by direct effects of exercise on bone remodeling and partly by a reduction in visceral fat (9). Furthermore, weight loss after bariatric surgery has been reported to be associated with a significant increase in osteocalcin (10). These findings again emphasize the connection between osteocalcin and substrate metabolism.

Because gestational diabetes mellitus (GDM), defined as glucose intolerance first detected during pregnancy (11), serves as a model to study the early changes in the development of insulin resistance and type 2 diabetes, interest in the action and regulation of osteocalcin in this entity, which affects about 10% of pregnant women in the middle European population, has increased. Besides pronounced insulin resistance compared with that in women with normal glucose tolerance (NGT) during pregnancy (12), pancreatic β-cell dysfunction has been described as the main metabolic characteristic in women with GDM (13). Despite increased insulin release, women with GDM fail to cope with the increased insulin demand during the physiological insulin resistance of late pregnancy, re-

sulting in hyperglycemia. The majority of women with GDM regain NGT after delivery but have a life-long increased risk of developing type 2 diabetes, especially within the first 10 years after delivery (14). Therefore, the aim of this study was to investigate, for the first time, osteocalcin and its associations with glucose metabolism in GDM both during pregnancy and after delivery.

RESEARCH DESIGN AND

METHODS — The study was performed as a case-control study at the outpatient clinic of the Department of Internal Medicine III, Division of Endocrinology and Metabolism of the Medical University of Vienna. Healthy pregnant women, who were referred from the department of obstetrics and gynecology to the outpatient clinic for routine glucose tolerance testing between the 24th and 28th gestational weeks, were invited to take part in this investigation. None of these women had a high risk for GDM (e.g., a history of GDM or obstetric complications in a previous pregnancy, any history of impaired glucose tolerance, or signs of fetal macrosomia), and, therefore, no prior glucose tolerance testing was performed during the respective pregnancy. Women who gave informed consent underwent a 2-h oral glucose tolerance test (OGTT) and fasting plasma sampling for the measurement of osteocalcin, A1C, hs-CRP, and lipid profile. We used the American Diabetes Association criteria for the definition of GDM: fasting plasma glucose values ≥95 mg/dl, 1-h postload 75-g glucose value ≥180 mg/dl, or 2-h postload glucose value ≥155 mg/dl. GDM was diagnosed when one value was pathological. All women with GDM received dietary counseling and were requested to measure their blood glucose levels four times daily. If the aim to achieve blood glucose levels <90 mg/dl at fasting and 140 mg/dl 1 h after meal could not be achieved by nutrition therapy, additional insulin therapy was started.

Data from 26 women with GDM and 52 women with NGT during pregnancy, matched for age and BMI in a 1:2 ratio, were analyzed. All women were invited to undergo reexamination during late pregnancy and 10–12 weeks after delivery.

OGTT
After women had fasted overnight for at least 12 h, a catheter was placed into an antecubital vein, and blood samples for

Table 1—*Baseline characteristics and metabolic parameters in women with GDM and NGT during pregnancy (24th–28th gestational weeks)*

	GDM	NGT	P
BMI (kg/m²)	27.8 ± 4.8	28.0 ± 5.1	NS
Age (years)	33 ± 6	32 ± 6	NS
Parity	1.2 ± 1.26	0.75 ± 1.17	NS
Systolic blood pressure (mmHg)	110.4 ± 12.2	110.4 ± 9.6	NS
Diastolic blood pressure (mmHg)	68.1 ± 6.2	69.0 ± 7.6	NS
Thyroid-stimulating hormone (μU/ml)	1.7 ± 1.0	1.8 ± 1.0	NS
Free triiodothyronine (pg/ml)	2.85 ± 0.37	2.78 ± 0.32	NS
Free thyroxineT4 (ng/dl)	0.94 ± 0.14	0.96 ± 0.15	NS
Osteocalcin (ng/ml)	15.6 ± 6.4	12.6 ± 4.0	0.0146
Triglycerides (mg/dl)	199 ± 70	183 ± 64	NS
Total cholesterol (mg/dl)	265.1 ± 44.3	267.3 ± 43.0	NS
HDL cholesterol (mg/dl)	71.0 ± 15.5	76.8 ± 14.2	NS
LDL cholesterol (mg/dl)	154.3 ± 39.5	154.4 ± 35.2	NS
A1C (%)	4.98 ± 0.39	4.72 ± 0.48	<0.03
hs-CRP (mg/dl)	1.26 ± 3.18	0.53 ± 0.46	NS
Fasting glucose (mg/dl)	85 ± 9	78 ± 5	<0.0001
Basal insulin secretion (pmol $\cdot$ l^{-1} $\cdot$ min^{-1})	40.54 ± 16.59	33.72 ± 12.85	NS
Disposition index (nmol/m³)	22.24 ± 8.22	17.56 ± 7.18	<0.02
AUC_glucose (mmol $\cdot$ l^{-1} $\cdot$ min^{-1})	1.04 ± 0.1	0.79 ± 0.1	<0.0001
AUC_insulin (nmol $\cdot$ l^{-1} $\cdot$ min^{-1})	68.4 ± 27.5	48.4 ± 23.6	0.002
AUC_C-peptide (nmol $\cdot$ l^{-1} $\cdot$ min^{-1})	393.9 ± 111.4	318.4 ± 102.7	0.006

the measurement of glucose, insulin, and C-peptide were taken at baseline as well as at 30, 60, 90, and 120 min after ingestion of 75 g glucose in H_2O solution.

Plasma metabolites
N-MID osteocalcin was measured with an electrochemiluminescent immunoassay (Roche Diagnostics, Mannheim, Germany) using a Roche Modular 170 immunoassay analyzer. Interassay coefficients of variation were 4.2–4.5% and 85 and 169 ng/ml, respectively. Glucose, insulin, C-peptide, C-reactive protein (CRP), hs-CRP, A1C, total cholesterol, LDL cholesterol, and HDL cholesterol were measured with standard kits available in our central laboratory.

Data analysis
The kinetics of glucose, insulin, and C-peptide during the OGTT were analyzed by quantitative methods to obtain metabolic parameters, such as insulin sensitivity through oral glucose insulin sensitivity (OGIS), which describes glucose clearance per unit change of insulin concentration (15). Total insulin secretion (TIS) from C-peptide, its suprabasal component (dynamic TIS), and hepatic insulin extraction (HIE) were obtained with a mathematical model of insulin/C-peptide interactions (16,17). β-Cell function was described as the ability of the β-cell to

adapt insulin secretion to the prevailing insulin resistance and was quantified by the products: OGIS × dynamic AUC insulin (termed disposition index) and OGIS × dynamic TIS (termed adaptation index), where AUC is the area under the insulin concentration curve during the whole test.

Statistical analysis
Comparisons of quantitative variables among groups were performed using ANOVA. Associations between continuous variables are described by the Pearson correlation coefficient. Multiple regression models were used to assess the influence of osteocalcin on metabolic parameters, taking into account potential factors associated with this variable such as BMI and age. Levels of statistical significance were set at $P < 0.05$.

RESULTS

Osteocalcin and metabolic parameters in pregnancy
During pregnancy, plasma concentrations of osteocalcin were significantly higher in women with GDM compared with women with NGT (Table 1). Furthermore, GDM showed significantly elevated levels of glucose at all time points during an OGTT (Fig. 1A). TIS (Fig. 1B), the area under the curve of insulin (AUC_insulin) and the area under
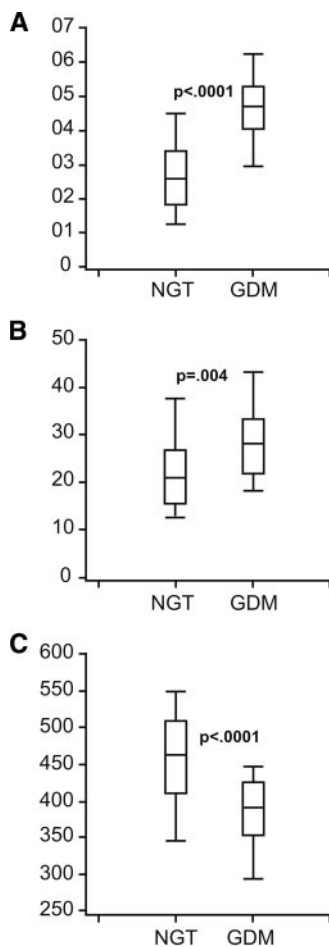
Figure 1—*Comparison of dynamic AUC glucose (A), TIS (B), and insulin sensitivity (OGIS) (C) during pregnancy between women with GDM and women with NGT.*

the curve of C-peptide (AUC_C-peptide) were significantly increased in women with GDM compared with women with NGT (Table 1). Insulin sensitivity (OGIS) was significantly decreased in women with GDM compared with women with NGT (Fig. 1C). The disposition index was significantly increased in women with GDM (Table 1), further indicating good secretory compensation. No differences were detected between women with GDM and NGT for CRP, hs-CRP, and lipid profile (Table 1).

Correlation analysis
Osteocalcin was positively correlated with areas under the curve of glucose, insulin (Fig. 2A), and C-peptide during pregnancy in the whole study group. Furthermore, osteocalcin was significantly correlated with insulin secretion parameters (Table 2) and especially with the disposition index (Fig. 2B) and the adaptation index. Conversely, hepatic in-
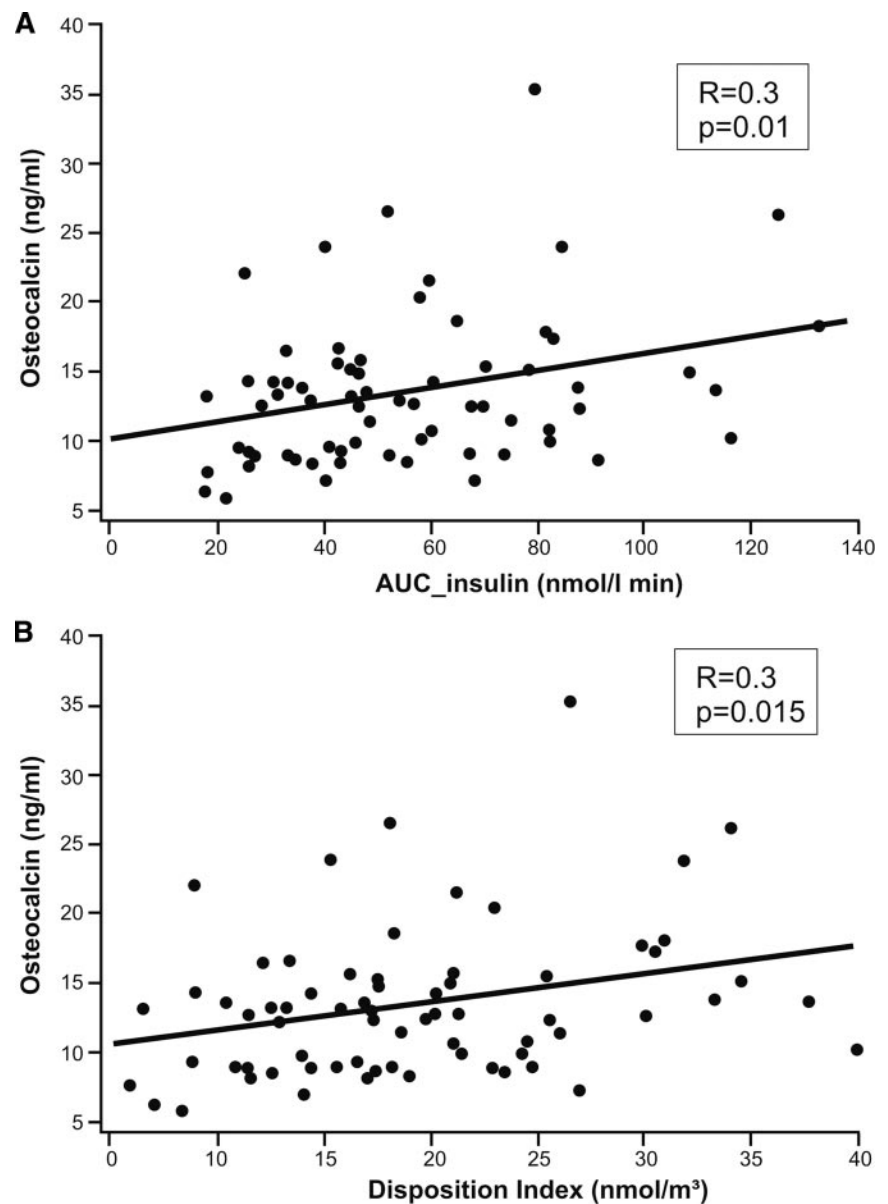

Figure 2—*Correlation analysis during pregnancy in the whole study group: osteocalcin correlates positively with parameters of insulin secretion such as the AUC of insulin (A) and the disposition index (B).*

Table 2—*Correlation analysis: correlations between osteocalcin and parameters of glucose metabolism (Spearman correlation coefficient) during pregnancy in the whole study group*

	Osteocalcin	
	R	*P*
TIS	0.33	<0.005
Dynamic TIS	0.3	<0.008
Basal insulin secretion	0.23	<0.05
AUC_C-peptide	0.3	<0.009
AUC_glucose	0.3	<0.007
HIE	−0.24	0.04

sulin extraction was inversely correlated with osteocalcin (Table 2). No correlation was found between osteocalcin and A1C. Multiple regression models with osteocalcin as the dependent variable and age, BMI, and group as quantitative variables showed that only the group (GDM or NGT) had a significant influence on osteocalcin concentrations.

Longitudinal observation
Twelve women with GDM could also be studied between the 33rd and 38th gestational weeks. Osteocalcin (22.7 ± 9 ng/ml; +39.5%; $P = 0.009$) was significantly increased compared with that at the first

visit (24th–28th gestational weeks) and again did not relate to glycemic control (A1C and ΔA1C); furthermore, osteocalcin was not different between those women with GDM receiving nutrition therapy only and those who were treated with diet and insulin.

Postpartum reexamination

From 78 women investigated during pregnancy, 34 (17 with GDM and 17 with NGT) underwent postpartum fasting plasma sampling, and from these women 17 (10 with GDM and 7 with NGT) also agreed to undergo an OGTT for glucose tolerance reevaluation. At 10–12 weeks postpartum all of the women with prior GDM analyzed by OGTT had already regained NGT. There was no difference between women with prior GDM and women with prior NGT concerning metabolic parameters except in the dynamic AUC_insulin during the OGTT, which was marginally increased in women with prior GDM (35.07 $\pm$ 14.22 vs. 21.05 $\pm$ 10.91 nmol $\cdot$ l^{-1} $\cdot$ min^{-1}; $P = 0.045$). Insulin sensitivity (OGIS: prior GDM 421 $\pm$ 56 ml $\cdot$ min^{-1} $\cdot$ m^{-1} vs. prior NGT 460 $\pm$ 54 ml $\cdot$ min^{-1} $\cdot$ m^{-1}) was not different between the groups at the postpartum visit.

Osteocalcin did not differ between women with prior GDM and prior NGT (36.2 $\pm$ 10.2 vs. 36.2 $\pm$ 13.0 ng/ml) at the postpartum visit but was significantly increased compared with pregnancy values ($P < 0.0001$). No correlations were found between osteocalcin and insulin secretion or AUC of glucose, whereas fasting glucose was inversely correlated with osteocalcin, but this correlation did not reach statistical significance ($R = -0.44$, $P = 0.07$).

CONCLUSIONS — This is the first time that osteocalcin was investigated in GDM, an entity that is characterized by hyperglycemia due to the failure of the pancreatic β-cells to cope with increased insulin demand during the pronounced increase of the physiological insulin resistance period of late pregnancy (13). Furthermore, women with GDM have a high risk for later development of type 2 diabetes (14). Previously, osteocalcin was reported to be decreased in patients with type 2 diabetes (6). In our study, osteocalcin was less restrained in women with GDM than in women with NGT between the 24th and 28th gestational weeks. At the postpartum visit, we found no differences in osteocalcin plasma concentra-

tions between the two groups. In both groups osteocalcin was significantly normalized 12 weeks after delivery, in accordance with findings in previous studies, which showed that osteocalcin levels decline in early pregnancy, start to increase in the third trimester until delivery, and peak during lactation (18–20). Therefore, our longitudinal observations are in accordance with those in previous studies, showing an increase of osteocalcin between the 24th and 28th and 33rd and 38th gestational weeks. The fact that osteocalcin was higher in women with GDM than in women with NGT during pregnancy and was normalized postpartum suggests that osteocalcin declines to a smaller extent in women with GDM.

Recent findings in animal studies have shown that osteocalcin can increase insulin secretion by a direct effect on pancreatic β-cells (5). In our study group osteocalcin levels were positively correlated with parameters of insulin secretion, such as TIS, AUC_insulin, and AUC_C-peptide. The disposition index, which describes the ability to increase insulin secretion to cope with increased insulin resistance and thus represents a parameter of overall glucose tolerance, was positively correlated with osteocalcin. Therefore, it may be hypothesized that the curbed decline of osteocalcin in GDM could act as a compensatory mechanism to cope with pancreatic β-cell dysfunction in these women. In turn, the higher levels of osteocalcin in GDM could also result from increased insulin secretion in these women. In this context insulin can exert anabolic features on bone metabolism via IGF-1 (3,4). Thus, increased insulin secretion to cope with increased insulin resistance could also influence bone metabolism during pregnancy. In addition, because osteocalcin was inversely correlated with hepatic insulin extraction, increased osteocalcin might be related to decreased insulin clearance, further yielding peripheral hyperinsulinemia during an OGTT.

Because osteocalcin is decreased in patients with overt type 2 diabetes, osteocalcin elevation in GDM to cope with increased insulin demand could be seen as an early adaptation mechanism for impaired glucose tolerance, which fails with the onset of overt type 2 diabetes. Studies by Lee et al. (5) showed that osteocalcin can increase insulin sensitivity, assessed by a specific test and a hyperinsulinemic-euglycemic clamp in $Esp^{-/-}$ mice. In humans, we could not find any association

between osteocalcin and insulin sensitivity (OGIS). Our findings that osteocalcin levels were also positively correlated with glucose concentrations during pregnancy are not concordant with the findings in animal studies in which osteocalcin was inversely correlated with hyperglycemia.

Osteocalcin is normally used as a marker of bone formation and thus for the diagnosis and treatment monitoring of osteoporosis (21). To exclude the possible effects of age and BMI on osteocalcin plasma concentrations, our study population was matched for age and BMI in a 1:2 ratio, and multiple regression models confirmed that these variables did not interfere with our results.

In a prior investigation in elderly patients, A1C and hs-CRP were reported to be inversely associated with osteocalcin (9); however, our data could not confirm these findings in pregnant women with or without GDM. Even our longitudinal observations in GDM did not show an association between osteocalcin and glycemic control, assessed by A1C. Furthermore, osteocalcin was not different between those women with GDM who were receiving nutrition therapy only and those who were treated with diet and insulin. However, we must also point out that our longitudinally investigated group was quite small.

At 10–12 weeks postpartum, women with prior GDM had already regained NGT, and their metabolic parameters did not differ from those of the women with NGT, except that dynamic AUC of insulin was higher, although marginally. This finding can be interpreted as chronic hyperinsulinemia in women with prior GDM because of increased insulin resistance reported for women with prior GDM. However, postpartum insulin sensitivity was not different between the two groups in our study population, which can be ascribed to the smaller number of postpartum participants and the fact that both groups were well matched for the degree of overweight.

In both groups we found significant differences between pregnancy and postpartum values for all metabolic parameters (OGIS, disposition index, AUC_insulin, HIE, A1C, and osteocalcin). Considering the findings that women with prior GDM showed increased insulin secretion parameters compared with women with NGT during pregnancy and a significant decline in glucose, insulin, and C-peptide levels postpartum, we conclude that insulin secretion may be impaired in these women but can still be transiently increased in periods

of pronounced insulin resistance. Perhaps additional mechanisms that have yet not been considered enhance hyperglycemia in these women during pregnancy.

In summary, GDM is associated with a milder decline in plasma concentrations of osteocalcin during the 24th–28th gestational weeks. In view of the positive association of osteocalcin with insulin secretion parameters, this could reflect a compensatory mechanism in insulin-resistant young women in periods of pronounced insulin resistance such as late pregnancy to cope with increased insulin demand, which cannot be accomplished because of a pancreatic β-cell defect in these women.

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