Osteocalcin is related to enhanced insulin secretion in gestational diabetes

Yvonne Winhofer MD¹, Ammon Handisurya MD¹, Andrea Tura PHD², Christina Bittighofer¹, Katharina Klein MD³, Barbara Schneider PHD⁴, Christian Bieglmayer MD⁵, Oswald F. Wagner MD⁵, Giovanni Pacini DSC², Anton Luger MD¹, Alexandra Kautzky-Willer MD¹

¹Medical University of Vienna, Department of Internal Medicine III, Division of Endocrinology and Metabolism, Vienna, Austria
² Metabolic Unit, Institute of Biomedical Engineering, National Research Council, Padova, Italy
³ Medical University of Vienna, Department of Obstetrics and Gynecology, Vienna, Austria
⁴ Medical University of Vienna, Institute of Medical Statistics, Vienna, Austria
⁵ Clinical Institute for medical and chemical laboratory diagnostic, General Hospital of Vienna, Vienna, Austria

Corresponding author:
Alexandra Kautzky-Willer, MD
Email: alexandra.kautzky-willer@meduniwien.ac.at

Submitted 7 July 2009 and accepted 9 September 2009.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://care.diabetesjournals.org.
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. Since gestational diabetes mellitus (GDM) can serve as a model of pre-type 2 diabetes, the aim of this study was to investigate osteocalcin in gestational diabetes.

Research design and methods: Osteocalcin measurement and an oral glucose tolerance test were performed in 78 pregnant women (26 women were diagnosed with gestational diabetes (GDM) and 52 women had normal glucose tolerance during pregnancy (NGT); matched for age and BMI) and in 34 women postpartum.

Results: During pregnancy osteocalcin was significantly higher in GDM compared to NGT (15.6±6.4 vs. 12.6±4.0 ng/ml; p<0.015), while no difference was observed between the 2 groups 12 weeks postpartum (36.2±10.2 vs. 36.2±13.0 ng/ml), when osteocalcin was found to be increased compared to the pregnant state in all women (+145±102% in GDM; +187±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).

Conclusion: In gestational diabetes osteocalcin was higher and thus less curbed 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 can not be excluded.
Evidence is growing about reciprocal interaction between bone and energy metabolism (1). The findings that adipose tissue via leptin can regulate bone metabolism (2) and that insulin, as anabolic hormone, influences bone metabolism via insulin like growth factor-1 (IGF-1) (3,4) raised the idea that bone in turn could also affect energy metabolism. Osteocalcin, an osteoblast derived protein locally acting on bone formation, is suspected to be involved in the regulation of glucose and fat metabolism. Since animal models showed that mice lacking osteocalcin display insulin resistance and obesity, bone moved into the centre of interest concerning endocrine action like adipose tissue did when adipocytokines were detected. It has been demonstrated that osteocalcin can stimulate insulin secretion, acting directly on proliferation and secretion of the pancreatic beta 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 which encodes for a receptor-like protein called OST-PTP, which is thought to influence the bioactivity of osteocalcin, show increased energy expenditure, 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, glycated haemoglobin (HbA1C), insulin resistance (assessed by HOMA-R), high sensitive CRP (hsCRP) and body mass index (BMI), and increases 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 remodelling, partly by a reduction of visceral fat (9). Furthermore, weight loss after bariatric surgery has been reported to be associated with a significant increase of osteocalcin (10). These findings again emphasize the connection between osteocalcin and substrate metabolism.

Since gestational diabetes, 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 occurred about osteocalcin action and regulation in this entity, which affects about 10 % of pregnant women in the middle European population. Besides pronounced insulin resistance compared to women with normal glucose tolerance during pregnancy (12), pancreatic beta cell dysfunction has been described as the main metabolic characteristic in women with gestational diabetes (13). Despite increased insulin release, women with gestational diabetes fail to cope with the increased insulin demand during the physiological insulin resistance of late pregnancy, resulting in hyperglycemia. The majority of women with gestational diabetes regain normal glucose tolerance 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 gestational diabetes both during pregnancy and after delivery.

**STUDY POPULATION 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 gynaecology to
the outpatient clinic for routine glucose tolerance testing between 24-28th gestational weeks, were invited to take part in this investigation. None of these women had a high risk for GDM (e.g. history of GDM or obstetric complications in a previous pregnancy, any history of impaired glucose tolerance, 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 and fasting plasma sampling for the measurement of osteocalcin, HbA1C, hsCRP and lipid profile. We used the ADA-criteria for 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 1 value was pathological. All women with GDM received dietary counselling and were requested to measure their blood glucose levels four times daily. If the aim to achieve blood glucose levels below 90 mg/dl at fasting and 140 mg/dl one hour after meal could not be achieved by nutrition therapy, additional insulin therapy was started.

Data were analysed from 26 women diagnosed with gestational diabetes (GDM) and 52 women with normal glucose tolerance (NGT) during pregnancy matched for age and body mass index (BMI) in a 1:2 ratio. All women were invited to undergo re-examination during late pregnancy and 10-12 weeks after delivery.

Oral glucose tolerance test. After an overnight fast for at least 12 hours, a catheter was placed into an antecubital vein and blood samples for the measurement of glucose, insulin and C-peptide were taken at baseline as well as 30, 60, 90 and 120 minutes after ingestion of 75 g glucose in H2O solution.

Plasma metabolites. N-mid osteocalcin was measured with an electrochemiluminescent immunoassay (ECLIA) purchased from Roche Diagnostics (Mannheim, Germany) by a Roche Modular 170 immunoassay analyser. Interassay coefficients of variation were 4.2 to 4.5 % a 85 and 169 ng/ml, respectively. Glucose, insulin, C-peptide, CRP, hsCRP, HbA1C, total cholesterol, LDL and HDL were measured with standard available kits in our central lab.

Data analysis. The kinetics of glucose, insulin and C-peptide during OGTT were analyzed by quantitative methods to obtain metabolic parameters, such as insulin sensitivity through OGIS, that describes glucose clearance per unit change of insulin concentration (15). Total insulin secretion from C-peptide (TIS), its supra-basal component (dynamic TIS) and hepatic insulin extraction (HIE) were obtained with a mathematical model of insulin/C-peptide interactions (16,17). Beta-cell function was described as the ability of the beta 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 Pearson’s 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 like 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
ostecalcin were significantly higher in women with gestational diabetes compared to pregnant women with normal glucose tolerance (Table 1). Furthermore, GDM showed significantly elevated levels of osteocalcin were significantly higher in women with gestational diabetes compared to pregnant women with normal glucose tolerance (Table 1). Furthermore, GDM showed significantly elevated levels of glucose at all time points during OGTT (Fig. 1A). Total insulin secretion (TIS, Fig. 1B), the area under the curve of insulin (AUC_insulin) and the area under the curve of C-peptide (AUC_C-peptide) were significantly increased in GDM compared to NGT (Table 1). Insulin sensitivity (OGIS) was significantly decreased in GDM compared to NGT (Fig. 1C). The Disposition Index was significantly increased in GDM (Table 1), further indicating good secretory compensation. No differences were detected between GDM and NGT concerning CRP, hsCRP 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. Hepatic insulin extraction was instead inversely correlated with osteocalcin (Table 2). No correlation was found between osteocalcin and HbA1C.

Multiple regression models with osteocalcin as dependent and age, BMI and group as quantitative variables showed that only the group (GDM, NGT) had a significant influence on osteocalcin concentrations.

**Longitudinal Observation.** 12 GDM could also be studied between 33-38th gestational weeks. Osteocalcin (22.7±9 ng/ml; +39.5%; p=0.009) was significantly increased compared to first visit (24-28th gestational weeks) and again did not relate to glycemic control (HbA1C, ΔHbA1C); furthermore osteocalcin was not different between those GDM on nutrition therapy only and those who were treated with diet and insulin.

**Postpartum re-examination.** From 78 women investigated during pregnancy, 34 (17 GDM, 17 NGT) underwent postpartum fasting plasma sampling and from these women 17 (10 GDM, 7 NGT) also agreed to undergo an oral glucose tolerance test for glucose tolerance re-evaluation. At 10-12 weeks postpartum all of the prior GDM (pGDM) analysed by OGTT had already regained normal glucose tolerance. There was no difference between pGDM and priorNGT (pNGT) concerning metabolic parameters except of the dynamic area under the curve of insulin (dynamicAUC_insulin) during OGTT, which was marginally increased in pGDM (35.07±14.22 vs. 21.05±10.91 nmol/l min; p=0.045). Insulin sensitivity (OGIS: pGDM: 421±56, pNGT: 460±54 ml/min m²) was not different between the groups at postpartum visit.

Osteocalcin did not differ between pGDM and pNGT (36.2±10.2 vs. 36.2±13.0 ng/ml) at postpartum visit but was significantly increased compared to pregnancy values (p<.0001). No correlations were found between osteocalcin and insulin secretion as well as AUC of glucose, while fasting glucose was inversely correlated to osteocalcin, but this correlation did not reach statistical significance (R= -0.44, p=0.07).

**DISCUSSION**

This is the first time osteocalcin is investigated in gestational diabetes, an entity which is characterized by hyperglycemia due to the failure of the pancreatic beta cells to cope with increased insulin demand during the pronounced increase of the physiological insulin resistance period of late pregnancy (13), and furthermore characterizing women with high risk for later development of type 2 diabetes (14). Previously, osteocalcin has been reported to be decreased in patients with type 2 diabetes (6). In our study, osteocalcin was less curbed in women with gestational diabetes compared to women with normal
glucose tolerance between 24-28th gestational weeks. At postpartum visit, we found no differences in osteocalcin plasma concentrations between the 2 groups. In both groups osteocalcin was significantly normalized 12 weeks after delivery, which is in accordance with previous studies, showing that osteocalcin levels decline in early pregnancy, start to increase in the third trimester until delivery and peak during lactation (18, 19, 20). Therefore our longitudinal observations are in accordance with previous studies, showing an increase of osteocalcin between 24-28th and 33-38th gestational weeks. The fact that osteocalcin was higher in GDM compared to NGT during pregnancy and was normalized postpartum suggests that osteocalcin declines to a smaller extent in women with gestational diabetes.

Recent findings in animal studies have shown that osteocalcin can increase insulin secretion by a direct effect on pancreatic beta cells (5). In our study group osteocalcin levels were positively correlated with parameters of insulin secretion, like TIS (Total insulin secretion) and the areas under the curve of insulin and C-peptide. The Disposition index, which describes the ability to increase insulin secretion in order 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 gestational diabetes could act as a compensatory mechanism in order to cope with pancreatic beta cell dysfunction in these women. In turn, the higher levels of osteocalcin in gestational diabetes 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 in order to cope increased insulin resistance could also influence bone metabolism during pregnancy. Additionally, because osteocalcin was inversely correlated with hepatic insulin extraction, increased osteocalcin might be related to a decreased insulin clearance further yielding peripheral hyperinsulinemia during OGGT.

Since osteocalcin is decreased in patients with overt type 2 diabetes, osteocalcin elevation in gestational diabetes in order to cope with increased insulin demand could be seen as an early adaptation mechanism for impaired glucose tolerance, which fails as soon as overt type 2 diabetes has onset.

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 to glucose concentrations during pregnancy are not concordant with the findings in animal studies where 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 possible effects of age and body mass index 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, HbA1C and hsCRP were reported to be inversely associated with osteocalcin (9), however, our data could not confirm these findings in pregnant women with or without gestational diabetes. Even our longitudinal observations in GDM could not observe an association between osteocalcin and glycemic control, assessed by HbA1C. Furthermore, osteocalcin was not different between those GDM on 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 pGDM had already regained normal glucose tolerance and the metabolic parameters did not differ from those of NGT, except the dynamic AUC of insulin that was higher, though marginally, than in NGT. This can be interpreted as chronic hyperinsulinemia in pGDM due to increased insulin resistance as reported for women with prior gestational diabetes. 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 in all metabolic parameters (OGIS, Disposition Index, AUC_insulin, HIE, HbA1C, and osteocalcin). Considering the findings that pGDM showed increased insulin secretion parameters compared to NGT during pregnancy and a significant decline in glucose, insulin and C-peptide levels postpartum leads us to conclude that insulin secretion might be impaired in these women but can still be transiently increased in periods of pronounced insulin resistance. Maybe additional mechanisms enhance hyperglycemia in these women during pregnancy which have yet not been considered.

In summary, gestational diabetes is associated with a milder decline in plasma concentrations of osteocalcin during 24-28\textsuperscript{th} gestational weeks. Considering 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 like late pregnancy in order to cope with increased insulin demand which cannot be accomplished due to a pancreatic beta cell defect in these women.

ACKNOWLEDGEMENTS:
The authors have no relevant conflict of interest to disclose.
This work was supported by a grant of the Jubilaeumsfond of the Austrian Nationalbank to A.K.-W. (OeNB 13244) and an unrestricted grant of Takeda to A.K.-W.

Figure legend:

Figure 1: Comparison of dynamic AUC glucose (A), total insulin secretion (B) and insulin sensitivity (OGIS; C) during pregnancy between GDM and NGT.

Figure 2: Correlation analysis during pregnancy in the whole study group: Osteocalcin correlates positively with parameters of insulin secretion like the area under the curve of insulin (A) and the disposition index (B).
REFERENCES:
4. Hock JM, Centrella M, Canalis E. Insulin-like growth factor I has independent effects on bone matrix formation and cell replication. Endocrinology 1988; 122(1): 254-60
of glucose metabolism and endothelial function in type 2 diabetes subjects under metformin nd thiazolidinedione. Diabetes Obes Metab 2006; 8(5):561-7
Table 1: Baseline characteristics and metabolic parameters in women with gestational diabetes (GDM) and normal glucose tolerance (NGT) during pregnancy (24-28th gestational weeks); ns=not significant

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GDM</th>
<th>NGT</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>27.8±4.8</td>
<td>28.0±5.1</td>
<td>ns</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33±6</td>
<td>32±6</td>
<td>ns</td>
</tr>
<tr>
<td>Parity</td>
<td>1.2±1.26</td>
<td>0.75±1.17</td>
<td>ns</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>110.4±12.2</td>
<td>110.4±9.6</td>
<td>ns</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>68.1±6.2</td>
<td>69.0±7.6</td>
<td>ns</td>
</tr>
<tr>
<td>TSH (uU/ml)</td>
<td>1.7±1.0</td>
<td>1.8±1.0</td>
<td>ns</td>
</tr>
<tr>
<td>fT3 (pg/ml)</td>
<td>2.85±0.37</td>
<td>2.78±0.32</td>
<td>ns</td>
</tr>
<tr>
<td>fT4 (ng/dl)</td>
<td>0.94±0.14</td>
<td>0.96±0.15</td>
<td>ns</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>15.6±6.4</td>
<td>12.6±4.0</td>
<td>0.0146</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>199±70</td>
<td>183±64</td>
<td>ns</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>265.1±44.3</td>
<td>267.3±43.0</td>
<td>ns</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>71.0±15.5</td>
<td>76.8±14.2</td>
<td>ns</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>154.3±39.5</td>
<td>154.4±35.2</td>
<td>ns</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>4.98±0.39</td>
<td>4.72±0.48</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>hsCRP (mg/dl)</td>
<td>1.26±3.18</td>
<td>0.53±0.46</td>
<td>ns</td>
</tr>
<tr>
<td>Fasting Glucose (mg/dl)</td>
<td>85±9</td>
<td>78±5</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Basal Insulin Secretion (pmol/l/min)</td>
<td>40.54±16.59</td>
<td>33.72±12.85</td>
<td>ns</td>
</tr>
<tr>
<td>Disposition Index (nmol/m³)</td>
<td>22.24±8.22</td>
<td>17.56±7.18</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>AUC_Glucose (mmol/l min)</td>
<td>1.04±0.1</td>
<td>0.79±0.1</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>AUC_Insulin (nmol/l min)</td>
<td>68.4±27.5</td>
<td>48.4±23.6</td>
<td>0.002</td>
</tr>
<tr>
<td>AUC_C-Peptide (nmol/l min)</td>
<td>393.9±111.4</td>
<td>318.4±102.7</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Table 2: Correlation analysis: Correlations between osteocalcin and parameters of glucose metabolism (Spearman’s correlation coefficient) during pregnancy in the whole study group.

<table>
<thead>
<tr>
<th></th>
<th>Osteocalcin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td>Total Insulin Secretion (TIS)</td>
<td>0.33</td>
</tr>
<tr>
<td>Dynamic Total Insulin Secretion (dynTIS)</td>
<td>0.3</td>
</tr>
<tr>
<td>Basal Insulin Secretion (BSR)</td>
<td>0.23</td>
</tr>
<tr>
<td>AUC_C-Peptide</td>
<td>0.3</td>
</tr>
<tr>
<td>AUC_Glucose</td>
<td>0.3</td>
</tr>
<tr>
<td>Hepatic Insulin Extraction (HIE)</td>
<td>-0.24</td>
</tr>
</tbody>
</table>
Figure 2

A

Osteocalcin (ng/ml)

R = 0.3
p = 0.01

B

Osteocalcin (ng/ml)

R = 0.3
p = 0.015