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Plasma lipocalin-2 levels in pregnancy

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Abstract

Objective. To evaluate plasma levels of lipocalin-2, which is a novel adipokine associated with obesity and insulin resistance, in pregnant women. *Design.* Prospective case–control study. *Setting.* University hospital. *Population.* Pregnant women with pre-pregnancy body mass index >25kg/m^2 (overweight; n=29) and body mass index <25kg/m^2 (n=27), whose gestational ages were between 24 and 28weeks, as study groups and nonpregnant control women with body mass index <25kg/m^2 (n=29). *Methods.* Plasma lipocalin-2 levels, fasting plasma glucose and fasting plasma insulin levels; homeostasis model assessment insulin resistance index and fasting plasma glucose/fasting plasma insulin ratio were measured for each subject. *Main Outcome Measures.* Comparisons among the groups and correlations for lipocalin-2 and the parameters of insulin resistance. *Results.* Plasma lipocalin-2 levels among the pregnant women were significantly higher than those of the control group (p<0.001 for both group comparisons). Lipocalin-2 levels were significantly higher in the group with pre-pregnancy body mass index >25kg/m^2 compared with the group with pre-pregnancy body mass index <25kg/m^2 (p=0.003). Lipocalin-2 levels were positively correlated with homeostasis model assessment insulin resistance index and fasting plasma insulin and negatively correlated with fasting plasma glucose/fasting plasma insulin ratio in both pregnant groups. *Conclusions.* Lipocalin-2 was found to be higher in pregnant women, especially when pre-pregnancy body mass index was >25kg/m^2, and it was correlated with markers of insulin resistance.

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HOMA-IR, homeostasis model assessment insulin resistance index; LCN-2, lipocalin-2.

Introduction

The prevalence of obesity in developing countries is rapidly increasing. Obesity and high body mass index (BMI) values increase the risk of pregnancy complications, including preeclampsia, congenital malformations, preterm birth, cesarean delivery, first trimester miscarriage, stillbirth and neonatal death (1–3). Pre-pregnancy BMI >30kg/m^2 has been shown to be a significant risk factor for the development of gestational diabetes mellitus (4).

Insulin resistance increases progressively throughout pregnancy, and the underlying mechanisms are not clearly understood. Although insulin resistance in pregnancy is associated with placental hormones, including human placental lactogen, human placental growth hormone and progesterone, as well as prolactin and cortisol, adipose tissue and placenta-derived adipokines and/or cytokines may also play important roles in this process (5). Insulin resistance may be practically evaluated with fasting insulin levels, the fasting glucose/fasting insulin ratio or by the homeostatic assessment model indices (HOMA-IR).

Lipocalin-2 (LCN-2) is a member of a protein family whose common feature is the presence of six- or eight-stranded β-barrels in their tertiary structure, and it is abundantly expressed in adipose tissue and liver (6). This novel adipokine is associated with obesity, obesity-related inflammatory processes and insulin resistance. Lipocalin-2 was shown to be highly expressed by fat cells in vivo and in vitro, and expression of LCN-2 was elevated by agents that promote insulin resistance and reduced by thiazolidinediones that decrease insulin resistance (7). There are a number of studies that have investigated the relation between LCN-2, obesity, insulin

resistance and proinflammatory processes (8–11). In humans, circulating LCN-2 concentrations were positively correlated with adiposity, hypertriglyceridemia, hyperglycemia and the insulin resistance index, but negatively correlated with high-density lipoprotein cholesterol. There was also a strong positive association between LCN-2 concentrations and high-sensitivity C-reactive protein, independent of age, sex and adiposity (8).

As pregnancy is a risky period in terms of insulin resistance and the underlying mechanisms are not completely clear, we aimed to evaluate LCN-2 levels and correlations between LCN-2 and parameters of insulin resistance in pregnant women without any systemic diseases or pregnancy complications. As LCN-2 is highly expressed in adipose tissue, we involved two separate groups of pregnant women categorized according to pre-pregnancy BMI (as BMI >25kg/m^2 and BMI <25kg/m^2) and a nonpregnant control group with BMI <25kg/m^2.

Material and methods

The study group was constituted from consecutive 56 pregnant women between 24 and 28weeks of gestation with an age range of 18–35years, who were seen in the outpatient clinics of the Department of Obstetrics and Gynecology in our institution for a routine pregnancy follow-up visit, between November 2008 and March 2009. The control group consisted of nonpregnant women within the same age range and with a BMI <25kg/m^2 who only had gynecological complaints related to vaginitis or cervicitis ($n = 29$). The exclusion criteria were endocrinological disorders such as overt or gestational diabetes mellitus, hyperthyroidism, hypothyroidism or Cushing's disease, chronic inflammatory disorders such as rheumatoid arthritis, Behçet's disease or Crohn's disease, collagen tissue disorders, renal, liver or lung diseases, cardiovascular diseases such as atherosclerosis and chronic hypertension, thromboembolic diseases, infectious diseases, high-risk pregnancies and any major obstetric diseases such as hypertensive disorders, multiple pregnancy, preterm labor or obstetrical hemorrhage.

The study population were all informed about the study and a written consent obtained from each women. A full Institutional Review Board approval was obtained from Kirikkale University School of Medicine Ethical Committee (approval no. 2009/007).

Demographic parameters, height, weight and pre-pregnancy weight were recorded for each woman. Body mass indices were calculated according to the formula weight (in kilograms) divided by height (in metres) squared. Pregnant women were categorized into two groups according to pre-pregnancy BMI as pre-pregnancy BMI >25kg/m^2 (overweight; $n = 29$) and pre-pregnancy BMI <25kg/m^2 ($n = 27$).

Venous blood was drawn after an overnight 12-hour fast to determine the levels of LCN-2, fasting plasma glucose (FPG) and fasting plasma insulin (FPI). Insulin resistance was calculated using HOMA-IR [fasting plasma glucose (mg/dl) $\times$ FPI (μIU/ml)/405]. All pregnant women were screened for gestational diabetes mellitus with a one-hour 50g oral glucose loading test. If patients had positive test results (plasma glucose >7.5mmol/L) or risk factors (history of macrosomic infant or first-degree relatives with diabetes mellitus), gestational diabetes mellitus was ruled out with a 100g oral glucose tolerance test.

To measure plasma LCN-2 levels, 5ml venous blood samples were obtained from subjects, and plasma was separated and stored at -20°C. Plasma samples were studied at the same time with sandwich ELISA (BioTek Instruments, Highland Park, VT, USA), IgG was biotinylated with BioVendor Research and Diagnostic Products Human Lipocalin-2/Lipocalin-2 ELISA reagent kit (BioVendor, Candler, NC, USA). The LCN-2 levels were measured at a wavelength of 450nm (absorbance) on a spectrophotometer, and LCN-2 concentrations were calculated according to a standard curve.

Statistical analysis

All statistical and post hoc power calculations were performed with the SPSS 11.0 statistical software package (SPSS Inc., Chicago, IL, USA). We used the Shapiro–Wilk test to test variables for normality. As the variables were not distributed normally, we used the Kruskal–Wallis test to compare groups and the Mann–Whitney *U*-test for dual comparisons. Results of descriptive analysis are presented as means$\pm$SD. Pearson correlation coefficients were used to establish the relation between LCN-2 concentrations, FPG, FPI and HOMA-IR. In all statistical comparisons, a p-value ≤ 0.05 was used to indicate a significant difference. A post hoc power analysis was performed for the Kruskal–Wallis test concerning lipocalin-2 levels. Initially, observed power was calculated for the univariate general linear model (analysis of variance), taking lipocalin-2 as the dependent variable. Next, the power for the Kruskal–Wallis test was estimated by multiplying the observed power for analysis of variance by 0.955, based on the notion of asymptotic relative efficiency (12).

Results

Demographic characteristics of the groups are shown in Table 1. There was no significant difference between mean age in the three groups. As expected, the overweight pregnant women showed a higher BMI compared with the lean women and the control group. No significant difference was detected in gestational ages between the pregnant groups. Although there was significant difference between body weight means of the pregnant groups at 24–28weeks gestation, weight gain

Table 1. Demographic characteristics of the participating women.

	Pre-pregnancy BMI >25kg/m^2 (n=29)	Pre-pregnancy BMI <25kg/m^2 (n=27)	Control group (n=29)	p-Value
Age (years)	24.4±4.1	24.5±4.8	25.9±5.2	0.402
Body weight, pre-pregnancy level (kg)	73.3±10.2	54.3±5.5	55.5±6.1	<0.001
Body weight at 24–28weeks (kg)	79.7±10.0	61.6±6.5	–	<0.001
Weight gain during pregnancy (kg)	6.4±2.7	7.3±3.5	–	0.558
Height (m)	1.59±0.07	1.61±0.05	1.62±0.05	0.015
Pre-pregnancy BMI (kg/m^2)	29.28±3.64	20.83±1.82	20.86±1.93	<0.001
Gestational age (weeks)	25.8±1.6	25.3±1.5	–	0.175
Gravida	3.3±1.7	2.0±1.3	0.2±0.8	<0.001
Parity	1.6±1.2	0.8±0.9	0.1±0.5	<0.001

Abbreviation: BMI, body mass index

during the pregnancy was similar. There were significant differences among the groups in terms of gravidity and parity.

Oral glucose loading and oral glucose tolerance test results of the individuals among the three groups were all within normal limits.

Significant differences were found among groups in terms of FPG, FPI, LCN-2, HOMA-IR and FPG/FPI values (Table 2). Although there was no difference between FPG levels of pregnant groups (p=0.43), FPG levels of the group with the pre-pregnancy BMI >25kg/m^2 and the group with the pre-pregnancy BMI <25kg/m^2 were significantly lower than that of the control group (p<0.001 and p<0.001, respectively). The FPI levels in the pregnant group with higher BMI were significantly higher than those of the other pregnant group (p<0.001). The FPI levels of each pregnant group were significantly higher than those of the control group (p<0.001 for both comparisons). The FPG/FPI ratios of the group with pre-pregnancy BMI<25kg/m^2 were significantly higher than those of the group with pre-pregnancy BMI >25kg/m^2 (p<0.001). The FPG/FPI ratios of the pregnant groups were significantly lower than those of the control group (p<0.001 for both comparisons). The HOMA-IR levels of the group with pre-pregnancy BMI >25kg/m^2 were significantly higher than in the other pregnant group (p<0.001). The HOMA-IR levels of pregnant groups were also higher compared with the control group (p<0.001 for both comparisons).

Plasma LCN-2 levels were significantly different among the groups (p<0.001). The post hoc power rate was 0.79 for the Kruskal–Wallis test concerning LCN-2 levels. Plasma LCN-2 levels were significantly higher in the group with higher pre-pregnancy BMI compared with the group with the normal pre-pregnancy BMI (p=0.003). When the two pregnant groups were compared with the control group, LCN-2 levels in the control group were significantly lower than those of the pregnant groups (p<0.001 for both comparisons).

The correlations between LCN-2 levels and the other parameters are shown in Table 3. There was no correlation between LCN-2 and FPG levels in the group with pre-pregnancy BMI >25kg/m^2. In the same group, LCN-2 levels were positively correlated with FPI, HOMA-IR and BMI, but negatively correlated with the FPG/FPI ratio. In the pregnant group with BMI <25kg/m^2, LCN-2 levels were positively correlated with FPI and HOMA-IR. There was a negative correlation between LCN-2 and FPG/FPI ratio. In the control group, LCN-2 levels were positively correlated with FPI.

Table 2. Comparisons of FPG, FPI, FPG/FPI, HOMA-IR and lipocalin-2 among the study groups.

	Pre-pregnancy BMI >25kg/m^2 (n=29)	Pre-pregnancy BMI <25kg/m^2 (n=27)	Control group (n=29)	p-Value
FPG (mg/dl)	76.17±3.96	77.59±5.26	86.03±7.29	<0.001
FPI (μIU/ml)	13.82±4.47	8.14±2.11	5.53±1.29	<0.001
FPG/FPI	5.08±1.79	10.35±3.49	16.57±4.63	<0.001
HOMA-IR	2.59±0.84	1.54±0.44	1.18±0.28	<0.001
Lipocalin-2 (ng/ml)	110.69±58.1	42.8±20.1	29.54±14.35	<0.001

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HOMA-IR, homeostatic assessment model indices.

Table 3. Correlations of lipocalin-2 with FPG, FPI, FPG/FPI, HOMA-IR and BMI in each group.

	Pre-pregnancy BMI >25kg/m^2 lipocalin-2 (n=29)		Pre-pregnancy BMI <25kg/m^2 lipocalin-2 (n=27)		Control group lipocalin-2 (n=29)	
	r	p-Value	r	p-Value	r	p-Value
FPG	0.118	0.540	0.338	0.085	0.239	0.212
FPI	0.938	<0.001	0.676	<0.001	0.412	<0.001
FPG/FPI	−0.840	<0.001	−0.527	0.005	−0.341	0.070
HOMA-IR	0.940	<0.001	0.757	<0.001	0.485	0.080
BMI	0.529	0.003	0.319	0.105	−0.057	0.770

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HOMA-IR, homeostatic assessment model indices.

Discussion

Recently, lipocalin-2 has been classified as an adipokine and/or proinflammatory cytokine. In a study including healthy individuals, it was shown that LCN-2 levels in the circulation and secretion of LCN-2 in ex vivo adipocytes increased via hyperinsulinemic induction (13). Lipocalin-2 levels were increased by agents which cause insulin resistance (7). A study on both humans and animals has shown that expression of LCN-2 was higher in adipose tissue and liver of *db*/*db* obese diabetic mice (a model for type 2 diabetes and for fluctuating insulin and glucagon ratios) compared with lean littermates, and LCN-2 expression was normalized by rosiglitazone; in humans, levels of LCN-2 in the circulation were positively correlated with adiposity, C-reactive protein, BMI, FPI and HOMA-IR (8). Some other members of the adipokine group associated with obesity and insulin resistance, such as tumor necrosis factor-α, interleukin-6 and resistine, behave in a manner to reduce the effect of insulin, while adiponectin and leptin increase insulin sensitivity (14,15). Interferon-γ alone cannot induce LCN-2 up-regulation, but increases up-regulation by interleukin-1β (16,17). Additionally, LCN-2 production is strongly induced by leptin (18). Obesity in pregnancy leads to worsening of the metabolic, vascular and inflammatory regulatory processes. It is known that adipokine and/or cytokine levels relate to obesity and insulin resistance during pregnancy (19).

Debate about gestational diabetes mellitus screening methods (selective screening or universal screening) continues. It has been suggested that the 'selective screening' approach may lead to underdiagnosis and that glucose intolerance in pregnancy must be evaluated within the continuity of the measured glucose levels (20,21). In our study, none of the pregnant women had gestational diabetes mellitus.

Progressive changes in glucose tolerance have been reported even in the first trimester. The reduction of FPG levels through the pregnancy is related to possible factors such as dilutional effect, increased consumption and low levels of liver glucose production (22). In the present study, there was no significant difference between FPG levels of the two groups of pregnant women. However, their FPG levels were lower than in the control group. This finding is consistent with data reported in the literature that FPG shows progressive reduction during pregnancy.

During pregnancy, a decrease in FPG levels is accompanied by an increase in FPI to protect pregnant women from hypoglycemia (5). In this study, it has been found that FPI levels of pregnant women with pre-pregnancy BMI >25kg/m^2 were higher than among those with a normal BMI. In this context, higher FPI levels of those with a higher BMI may be due to the additional insulin resistance seen in such a condition, besides the aggravating effects of pregnancy on carbohydrate metabolism (23).

The HOMA-IR is a model using FPI and FPG levels, and its sensitivity and specificity is comparable to the euglycemic–hyperinsulinemic clamp method (24,25). The HOMA-IR values of the two pregnant groups and the control group were different. The FPG, FPI and HOMA-IR are mathematically related. In this context, it is not surprising that pregnant women with a higher pre-pregnancy BMI had higher HOMA-IR levels than the other two groups.

Human and animal studies have demonstrated that LCN-2 concentrations in the circulation of obese humans and animals are higher than among those of normal weight (26,27). Positive correlations have been found between plasma LCN-2 levels and BMI, plasma insulin and HOMA-IR (8). We found that LCN-2 levels of pregnant women with pre-pregnancy BMI >25kg/m^2 were positively correlated with FPI, HOMA-IR and BMI. The LCN-2 levels of the pregnant women with pre-pregnancy BMI <25kg/m^2 were also positively correlated with FPI and HOMA-IR. There was no correlation between LCN-2 levels and BMI in the same group. It seems, therefore, that LCN-2 levels correlate with insulin resistance parameters in pregnant women regardless of pre-pregnancy BMI.

Based on the findings from this study and data obtained from reports mentioned above, it can be proposed that higher

LCN-2 values in pregnancy may be related to increased insulin resistance and be associated with inflammatory processes induced by pre-pregnancy excessive weight in addition to pregnancy-dependent physiological changes.

A limitation of our study design was that we did not enroll a nonpregnant control group with BMI >25kg/m^2. It might also be valuable to measure and compare LCN-2 levels of individuals both before and during pregnancy. Further studies are needed to clarify the relation between LCN-2 levels and insulin resistance in pregnant women.

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