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Association of Adiponectin and Placental Growth Factor in Amniotic Fluid with Second Trimester Fetal Growth

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Abstract. *Background: We investigated the associations between second trimester amniotic fluid (AF) levels of human adiponectin and placental growth factor (PLGF) in small for gestational age (SGA), large for gestational age (LGA) and appropriate for gestational age (AGA) fetuses. Materials and Methods: Adiponectin and PLGF levels were determined by enzyme immunoassay in AF of 21 SGA, 13 LGA and 44 AGA fetuses between 15-22 weeks of gestation, derived from pregnant women who underwent amniocentesis. Results: Adiponectin and PLGF levels were detectable in AF. Median (25th-75th percentile) adiponectin levels were 16.1 (10.9-32.3) ng/ml in SGA, 19.5 (15.1-30.9) ng/ml in AGA, and 18.2 (14.7-30.8) ng/ml in LGA fetuses. Median (25th-75th percentile) PLGF levels were 24.2 (19.9-34.9) pg/nl in SGA, 26.4 (20.9-33.8) pg/ml in AGA and 33.5 (21.8-40.4) pg/ml in LGA fetuses. The differences were not statistically significant. Nevertheless, indication of differentiation of levels existed when SGA and LGA fetuses in the extremes of distribution were considered. Specifically, very severely SGA fetuses (≤2.5th percentile) tended to have high levels of adiponectin and reduced levels of PLGF in AF. Conclusion: This is the first study presenting adiponectin and PLGF concentrations in early second trimester amniotic fluid in AGA, SGA and LGA fetuses. The altered concentrations of adiponectin and PLGF in very severely SGA fetuses possibly result from the growth-promoting effect of these factors through the metabolic route and the vascular integrity of the placenta, respectively.*

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Amniotic fluid (AF), which is composed not only of fetal urine and lung excretions but also contains molecules and factors that facilitate fetal growth (1, 2), changes during pregnancy to provide a dynamic environment for the fetus.

Small for gestational age (SGA) generally refers to fetuses of a weight below the 10th percentile for the specific gestational age (3). By contrast, fetuses are considered large for gestational age (LGA) if their birth weight is greater than the 90th percentile for that gestational age (4).

SGA fetuses have a greater risk of perinatal and later complications (5, 6). The underlying mechanism of the SGA fetus remains undetermined, while reliable measures for prediction have not as yet been established. This raises the urgent need for the identification of biomarkers that will help to better understand the mechanisms of the pathology so as to develop effective strategies for early detection, prevention and treatment (7, 8).

Human adiponectin, a collagen-like protein, is mainly synthesized in adipose tissue and is induced during adipocyte differentiation (9). The properties of adiponectin as an insulin sensitizer were observed when attention was focused on adipose tissue as a contributor to the insulin resistant state (10). It has in fact been confirmed that adiponectin plays an essential role in energy homeostasis, as well as in the metabolism of carbohydrates and lipids (10).

Placental growth factor (PLGF), a member of the angiogenic vascular endothelial growth factor (VEGF) family, is produced by trophoblasts which stimulate proliferation, migration and activation of endothelial cells (11, 12). In pregnancy, PLGF controls the invasion of the maternal spiral arteries in trophoblast. The occurrence of inadequate trophoblast invasion in the presence of

Table I. *Demographic data of participating mothers and fetuses (appropriate for gestational age, AGA; small for gestational age, SGA and large for gestational age, LGA). Mean, standard deviations (in parenthesis) and percentages (in parentheses) for quantitative variables are given.*

	AGA (n=44)	SGA (≤10th percentile) (n=21)	LGA (≥90th percentile) (n=13)	*p*-Values*
Maternal age (years)	35.7 (3.1)	34.4 (3.7)	32.5 (5.4)	0.075
Maternal BMI (kg/m^2)	23.2 (3.8)	26.2 (6.6)	22.8 (3.4)	0.356
Parity[x]				
0	12 (30%)	6 (31.6%)	3 (25%)	0.923
1+	28 (70%)	13 (68.4%)	9 (75%)	
Duration of pregnancy (weeks)	38.9 (0.8)	37.9 (2.5)	38.2 (1.2)	0.116
Natural birth				
Yes	20 (45.5%)	12 (57.1%)	9 (69.2%)	0.284
No	24 (54.5%)	9 (42.9%)	4 (30.8%)	
Birth weight (g)	3339.8 (216.9)	2509.8 (531.5)	3798.5 (368.4)	≤0.001
Gender of offspring[a]				
Male	21 (50%)	12 (63.2%)	3 (25%)	0.116
Female	21 (50%)	7 (36.8%)	9 (75%)	
Maternal smoking[a]				
Yes	1 (2.3%)	4 (20%)	0 (0%)	0.020
No	42 (97.7%)	16 (80%)	12 (100%)	

*Overall comparison of groups using the Kruskal-Wallis test for quantitative variables and $\times^2$ for qualitative variables. [x]Some factors have missing values.

insufficient vascular growth results in spiral arteries that lead to a reduced functioning of the vascular network. This causes low perfusion of the placenta, a state which is implicated in SGA fetuses (13, 14).

Given the metabolic and angiogenic importance of adiponectin and PLGF, respectively, their relationship in fetal growth and maturation in second trimester AF is of particular importance. Our study is the first, to our knowledge, aimed at documenting the presence of adiponectin and PLGF in early second trimester AF samples, as well as at the evaluation of their concentrations. We furthermore sought evidence of potential differences among the SGA, AGA and LGA fetuses as a result of their different metabolic state.

Materials and Methods

Our study group consisted of 300 women who underwent an equal number of amniocenteses in the early second trimester of pregnancy. At 15-22 gestational weeks, a routine mid-trimester amniocentesis was carried out for various indications, including advanced maternal age, abnormal nuchal translucency screening, past history of genetic disorder, or identification of an abnormality on the second trimester ultrasound screening. Twin pregnancies or pregnancies with major congenital anomalies were excluded. The study was approved by the Ethics Committee of our teaching hospital and informed consent was obtained.

All patients were Caucasians. A questionnaire was completed concerning maternal age, weight, height, parity, cigarette smoking, medical and obstetrical-gynecological history. Gestational age was calculated both by the first day of the last menstrual period and the crown rump length of the embryos, as determined in the first trimester ultrasound examination. The duration of pregnancy, mode of delivery, neonatal birth weight/gender and the outcome were recorded. The AF samples, having been collected in pyrogen-free tubes and immediately centrifuged, were kept frozen at –80˚C until the determination of adiponectin and PLGF concentrations.

In order to allocate the centile of each fetus at delivery, a gestation-related optimal weight (GROW) computer-generated program was used (15). Fetuses below the 10th customized percentile were characterized as SGA and those above the 90th customized percentile as LGA. Our study sample consisted of 21 SGA fetuses and 13 LGA fetuses, which were matched for gestational age, sex, maternal height and weight with 44 AGA fetuses that composed the control group. We additionally compared the concentrations of adiponectin and PLGF in AGA fetuses to those in fetuses with more extreme somatometric characteristics, namely severely SGA/LGA fetuses as defined by the ≤5th and ≥95th percentiles, respectively, and very severe SGA/LGA fetuses as defined by the ≤2.5th and ≥97.5th percentiles, respectively.

The measurement of adiponectin and PLGF concentrations was performed by enzyme immunoassay ELISA (Quantikine Human Adiponectin/Acrp30 Immunoassay; R&D Systems, Minneapolis, United States Of America. PLGF ELISA; DRG Diagnostics, Marburg, Germany). The minimum detectable concentration (MDC) for adiponectin ranged from 0.079-0.891 ng/ml, with a mean MDC of 0.246 ng/ml, and the MDC of the PLGF assay was <1 pg/ml.

Due mainly to the small sample sizes within each group, the distribution of the measured analytes, as well as the mothers' characteristics, deviates from the normality assumption. We used the Kruskal-Wallis test for comparison of the concentrations of substances between the three groups. We also applied logistic regression to investigate the risk of SGA *vs*. AGA and LGA *vs*. AGA associated with adiponectin and PLGF concentrations. To control for possible confounding, we adjusted the models for

Table II. *Distribution of measured adiponectin (ng/ml) in the different fetal size groups. Median levels (25th-75th percentile) are presented.*

Fetus	n	Median	25-75th percentile	*p*-Values
AGA	44	19.5	15.1-30.9	
SGA				
≤10th percentile	21	16.1	10.9-32.3	0.620
≤5th percentile	12	13.7	10.9-39.7	0.585
≤2.5th percentile	7	32.5	11.3-49.6	0.258
LGA				
≥90th percentile	13	18.2	14.7-30.8	0.620
≥95th percentile	8	16.6	12.4-29.1	0.585
≥97.5th percentile	1	8.7		0.258

p-Values were derived from the Kruskal-Wallis test for the comparison of adiponectin between AGA, SGA and LGA group.

Table III. *Distribution of measured placental growth factor (pg/ml) in the different fetal size groups. Median levels (25th-75th percentile) are presented.*

Fetus	n	Median	25-75th percentile	*p*-Values
AGA	44	26.4	20.9-33.8	
SGA				
≤10th percentile	21	24.2	19.9-34.9	0.570
≤5th percentile	12	25.0	19.1-34.4	0.256
≤2.5th percentile	7	23.9	20.9-37.1	0.293
LGA				
≥90th percentile	13	33.5	21.8-40.4	0.570
≥95th percentile	8	34.9	23.0-42.4	0.256
≥97.5th percentile	1	55.8		0.293

p-Values were derived from the Kruskal-Wallis test for the comparison of placental growth factor between AGA, SGA and LGA groups.

maternal age (continuously, in years), body mass index (BMI, categorically <25, 25-29, 30+ kg/m^2), duration of gestation (continuously, in weeks), gender of offspring (boy *vs*. girl), smoking during pregnancy (yes *vs*. no, for the association between SGA and AGA) and parity (parous *vs*. nulliparous).

Results

Table I presents the descriptive characteristics of mothers and fetuses. There were no statistically significant differences in maternal age, maternal BMI, parity, duration of gestation, way of delivery or gender of offspring between the three groups. Nevertheless, there was a statistically significant difference in size status as related to maternal smoking habits (p=0.020), since none of the mothers with LGA fetuses smoked. The correlation between the two compounds in the amniotic fluid was negative (Spearman r for the whole sample=–0.14, p=0.238). The negative (non-statistically significant) correlation was observed also within groups defined by different sizes of the fetuses (for example, in the AGA group r=–0.114, p=0.460).

Table II presents the comparison of the distribution of adiponectin by fetal size. None of the comparisons for the concentrations of adiponectin and PLGF between AGA, SGA and LGA were statistically significant. The median adiponectin concentrations by different fetal size group indicate a decreasing trend in adiponectin concentrations by increase in the LGA group. Adiponectin concentrations in AF were not statistically significantly different by fetal size, neither when we considered SGA fetal sizes as usually defined (0th-10th percentile definitions), nor when we took into account severe sizes compared with AGA (p=0.620 between the three groups in the former case and p=0.585 in the latter). However, concentrations were increased in women who had very severely SGA fetuses (p=0.258) for the comparison of the three groups.

Table III presents the comparison of PLGF distribution by fetal size. PLGF concentrations in AF were lower in women with SGA fetuses and higher in women with LGA fetuses, irrespectively of the cutoff percentile for the definition of size, although the overall comparisons were not statistically significant (p=0.570, p=0.256 and p=0.293, respectively, for 90th-100th, 95th-100th and 97.5th-100th percentile definition). There seems to be an indication of a trend in the PLGF concentrations, where the smaller the fetus (lower SGA), the lower the concentrations, while on the other hand, LGA fetuses had higher concentrations.

Multiple-logistic regression results for the risk of SGA *vs*. AGA indicated a greater risk among SGA fetuses for higher adiponectin concentrations (odds ratio, OR=1.53, 95% confidence intervals CI=0.74-3.17, p=0.255) and lower concentrations of PLGF (OR=0.72, 95% CI=0.34-1.53, p=0.394) in the second trimester AF per one standard deviation increase in the concentrations of the corresponding compounds. For LGA fetuses there is a reduced risk for increased concentrations of adiponectin (OR=0.63, 95% CI=0.15-2.70, p=0.532) and a greater risk for increased PLGF concentrations (OR=1.13, 95% CI=0.47-2.71, p=0.779).

Discussion

We examined the association between adiponectin and PLGF concentrations in SGA/LGA fetuses, taking into account that AF composition seems to be similar to that of fetal plasma during the first half of pregnancy (16). This is the first study to show the presence of both factors, namely one metabolic (adiponectin) and one angiogenic factor (PLGF) in second trimester AF. Adiponectin and PLGF were present in the AF of all fetuses examined. The small sample sizes could possibly have limited our ability to detect a statistically significant association, if such exists.

Adipose tissue (and particularly adipocytes) is the primary tissue in which adiponectin is produced (17). It functions not only as an inactive fat storage tissue but also as an endocrine organ where bioactive molecules are secreted and regulate body metabolism (18). Adiponectin, which is formed from 247 amino acids consisting of four domains, was initially identified as an adipose-tissue peptide found to be dysregulated in obesity (19). Under specific circumstances it can also be produced in other tissues, such as skeletal muscle cells and cardiomyocytes (20). Documentation of its ability to promote the expression of endothelial adhesion molecules (21), and to soothe smooth muscle cell proliferation (22), established its nature as an anti-atherogenic factor. In tissues sensitive to insulin, adiponectin takes part in the route of modulation of glucose and lipid metabolism (23). Free fatty acid uptake and β-oxidation in muscle and the hepatic action of insulin is also promoted by adiponectin, thus ameliorating the insulin resistant state (24). Recently, hypoadiponectinemia has been found to be closely associated with obesity and type 2 diabetes mellitus (25). Higher adiponectin concentrations in SGA fetuses in second trimester AF, as shown in our study, are thought to reflect the important action of adiponectin on tissue growth and metabolism. The higher adiponectin concentrations in fatless fetuses could be explained by a negative feedback mechanism (26, 27).

The concentrations of adiponectin found in SGA fetuses in our study are in agreement with those of other studies in later life determining that there is an inverse association of adiponectin concentrations with body weight or BMI (28). Adiponectin decreases in obesity, while body weight reduction or energy restriction increases its concentrations (29). Moreover, the association of adiponectin with fetal growth has been confirmed *via* documentation of its control of lipid and insulin metabolism (19) and the reduction of body fat mass which is observed in SGA fetuses. Additionally, a reduced adiponectin level in SGA fetuses has a fundamental role in the development of insulin resistance and metabolic syndrome (30). The higher adiponectin concentrations observed in this study in the very severely SGA fetuses could be a component of the biological mechanism leading to development of SGA fetuses.

The relationship between adiponectin and LGA fetuses is not well known. As stated earlier in our study, adiponectin concentrations were not statistically different between AF from LGA and AGA fetuses. However, other studies have reported statistically significantly lower or higher serum adiponectin concentrations, later in life, in LGA neonates (19, 27), or abnormalities in insulin sensitivity (31, 32). Reduced adiponectin concentrations due to adiposity substantiate the insulin-sensitizing role of adiponectin (33). It has been shown that LGA newborns not only have a large amount of adipose tissue but also larger adipocytes (34), while hypertrophic adipocytes exert negative feedback with regard to adiponectin production and the correlation with birth weight is disturbed (35). Thus, the concomitant fetal body weight and large number and size of adipocytes in LGA fetuses would seem to explain the lower adiponectin concentrations found in AF in our study.

The quantity of adipose tissue is extremely high in LGA fetuses, with the negative feedback attaining a point that lowers the concentrations of adiponectin. Fetal adiposity characterizes this group (36), thus there is an increasing risk for both neonatal and childhood complications (9). The lower but not statistically significant concentrations of adiponectin presented in LGA fetuses that were found in this study are also in accordance with this hypothesis. Moreover, the hypoadiponectinemia that has been associated later in infancy and early childhood with weight and fat gain also concords with this hypothesis (37). PLGF is a dimeric glycoprotein and a member of the cysteine-knot family of growth factors, with significant amino acid homology to the VEGF family (38); it promotes vessel development within the amnion, while it also impacts the permeability of the microvessels perfusing both fetal and placental surfaces. The main source of PLGF during pregnancy is the placental trophoblast, where it is chiefly produced (11, 12, 39). Vasorelaxation of human placental vessels is induced by PLGF; thus, placental/fetal vascular flow in early pregnancy may be promoted through PLGF production by trophoblasts.

In the SGA fetuses studied, we observed lower PLGF concentrations in AF in the second trimester as compared with AGA fetuses, which however did not reach statistical significance. Previously published results are inconclusive, with some studies showing that increased PLGF concentrations in the first trimester serum of pregnant women resulted in SGAs lower than the 5th centile (40, 41). Other researchers found that PLGF concentrations between SGA pregnancies and controls (AGA) were not significantly different (42). A third set of studies concluded that reduced first trimester maternal PLGF concentrations were related to a higher risk for an SGA fetus (43, 44).

The factors that regulate PLGF have not yet been determined to a satisfactory degree. Oxygen is thought to regulate PLGF function and production (45), with high concentrations of pO2 encouraging PLGF expression and low concentrations down-regulating it (39, 45). Other researchers concur with this view, their studies having reported that PLGF expression increases due to an increase in oxygenation within the intervillous space (46), while a relative decrease in pO_2 inhibits the PLGF expression in trophoblast (39, 45, 47). Additionally, diminished fetal growth associated with maternal hypoxemia (48) supports the hypothesis of PLGF up-regulation by oxygen.

Although fetal growth is strongly related to normal

placental function (49), very little is known about the role of placental function in determining macrosomia. Our study has revealed an increase in the concentration of PLGF in fetuses above the 90th percentile. Other studies of macrosomic fetuses investigated whether or not an increased growth is caused by an augmentation of maternal oxygen and nutritient provision through placenta. Even though there is little information about circulatory mechanisms in LGA fetuses, what is certain is that blood flow in the umbilical vein is higher in macrosomic fetuses as compared with that in the control group (50). Consequently, oxygenation and nutrient delivery will be higher in LGA fetuses (51). A higher oxygenation level may result in greater production of molecules that are involved in vessel integrity and placental function (51). The fact that PLGF production is elevated at high pO_2 concentrations might be the reason for increased concentrations of PLGF in LGA fetuses, as were found in our study.

Even more significantly, our results in the AF are in agreement with previously published studies investigating maternal and neonatal serum measurements of adiponectin and PLGF (41, 52). Although no statistical correlation was found between adiponectin and PLGF, a relationship has been documented in the literature, as alterations in adiponectin concentrations are associated with impaired endothelium-dependent vasodilatation. Moreover, adiponectin maintains endothelial functional integrity and its deficiency may lead to endothelial dysfunction, which in turn impacts upon PLGF expression (53, 54).

Adiponectin and PLGF are present in second trimester AF and their concentration was estimated. This study did not demonstrate statistically significant differences in adiponectin concentrations among SGA fetuses, LGA fetuses and AGA controls. However, in SGA fetuses below the 2.5th customized percentile for birth weight, there were higher concentrations of adiponectin in comparison with those of AGA and LGA fetuses. Furthermore, PLGF concentrations were not statistically significantly different among SGA, AGA and LGA fetuses. By contrast, PLGF concentrations were higher in LGA fetuses, possibly due to a placental functional effect. This highlights the potential role of the placenta in releasing angiogenic factors and stresses the role of an angiogenic imbalance of placental origin in LGA. The present study elucidates the weak associations between adiponectin and PLGF concentrations in AF with the extremes of birth weight fetal percentiles. To our knowledge, this is the first study to investigate adiponectin and PLGF concentrations in second trimester AF in relation to the size of fetuses. Further studies potentially validating our results will lead to greater understanding of these conditions and their implications, thus providing new avenues of research for preventive and therapeutic interventions earlier in pregnancy.

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