RESEARCH ARTICLE

Levels of Adipokines in Amniotic Fluid and Cord Blood Collected from Dichorionic-Diamniotic Twins Discordant for Fetal Growth

Seung Mi Lee¹, Joong Shin Park^{1*}, Errol R. Norwitz², Sitthysack Panyavatthanasinh³, Sun Min Kim^{1,4}, JoonHo Lee¹, Chan-Wook Park¹, Byoung Jae Kim^{1,4}, Jong Kwan Jun¹

1 Department of Obstetrics & Gynecology, Seoul National University College of Medicine, Seoul, Korea, **2** Department of Obstetrics & Gynecology, Tufts University School of Medicine, Boston, Massachusetts, United States of America, **3** Department of Obstetrics & Gynecology, University of Health Sciences, Vientiane, Lao PDR, **4** Department of Obstetrics & Gynecology, Seoul Metropolitan Government Seoul National University Boramae Medical Center, Seoul, Korea

* jsparkmd@snu.ac.kr

OPEN ACCESS

Citation: Lee SM, Park JS, Norwitz ER, Panyavatthanasinh S, Kim SM, Lee JH, et al. (2016) Levels of Adipokines in Amniotic Fluid and Cord Blood Collected from Dichorionic-Diamniotic Twins Discordant for Fetal Growth. PLoS ONE 11(5): e0154537. doi:10.1371/journal.pone.0154537

Editor: Oreste Gualillo, SERGAS (Servizo Galego de Saude) and IDIS (Instituto de Investigación Sanitaria de Santiago), the NEIRID Lab, Research Laboratory 9, Santiago University Clinical Hospital. Santiago de Compostela, SPAIN, SPAIN

Received: January 30, 2016

Accepted: April 14, 2016

Published: May 2, 2016

Copyright: © 2016 Lee et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper. (All the necessary data [median, interquartile ranges, and the p values] are included in the tables.)

Funding: This study was supported by a grant of the Korea Health R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HI12C00240300).

Abstract

Objective

To compare the concentrations of adipokines in amniotic fluid (AF) and cord blood collected from discordant dichorionic-diamniotic (DCDA) twin fetuses.

Study Design

The study population included DCDA twins discordant for fetal growth (birth weight difference >10%) who either underwent mid-trimester amniocentesis for routine clinical indication (Cohort 1) or whose amniotic fluid was collected at the time of delivery (Cohort 2). In both cohorts, cord blood was collected at delivery.

Results

A total of 92 twin pairs were enrolled (n = 49 in Cohort 1; n = 43 in Cohort 2). In Cohort 1, the concentrations of adiponectin (median, 68.5 ng/mL vs 61.4 ng/mL; p<0.05) and leptin (median, 13.9 ng/mL vs 11.2 ng/mL; p<0.1) in mid-trimester AF were significantly higher in smaller compared with larger twins. In Cohort 2, the concentration of serpin E1 (median, 246.0 ng/mL vs 182.8 ng/mL; p<0.01) in AF at delivery was significantly higher in smaller twins, but no difference was noted in adiponectin and leptin concentrations. Levels of adiponectin (median, 10425.5 ng/mL vs 11552.0 ng/mL; p<0.005) and leptin (median, 2.1 ng/mL vs 2.6 ng/mL; p<0.005) were significantly lower in the cord blood of smaller twins whereas cord blood concentrations of serpin E1 (median, 15.5 ng/mL vs 13.3 ng/mL; p<0.05) was higher in the smaller twins.

Competing Interests: The authors have declared that no competing interests exist.

Conclusion

In discordant DCDA twin pairs, concentrations of adiponectin, leptin, and serpin E1 in mid-trimester AF, AF at delivery, and cord blood at birth vary significantly but predictably between the smaller and larger twins.

Introduction

The intrauterine environment is known to be a critical determinant of adult health. For example, fetal growth restriction (FGR) is associated with the development of adult metabolic diseases, such as obesity, diabetes, hypertension, and coronary heart disease [1]. In the setting of a suboptimal intrauterine environment, the growth restricted fetus undergoes a series of developmental adaptations including a permanent change in its hypothalamic-pituitary-adrenal axis and in insulin-glucose metabolism. It is these changes that appear to be responsible for the increased risk of metabolic diseases in adulthood [2–4].

Adipose tissue is an endocrine organ capable of modulating metabolic and inflammatory processes and contributing to the development of metabolic complications [5]. It has been suggested that adipose tissue may provide the critical link between FGR and subsequent adult diseases [6–9]. Fetuses with growth restriction have a reduced overall body fat mass, but a disproportionate increase in visceral fat tissue [7]. This central obesity has been associated with rapid catch-up growth and the early development of insulin resistance [6–8].

Adipose tissue affects metabolism and energy homeostasis throughout the body primarily through the secretion of adipokines, such as leptin and adiponectin. Given the critical role that adipose tissue plays in fetal growth and the risk of subsequent metabolic diseases, it is important to understand the association between adipokine concentrations within the various intrauterine compartments and fetal growth, especially in the setting of FGR. Unfortunately, the literature on this topic is conflicting. Several investigators have reported a decrease in cord blood concentrations of leptin and adiponectin in growth-restricted fetuses [10–14], whereas others found no association [15–17]. In addition, there are few studies on adipokine concentrations in the amniotic fluid (AF) or on longitudinal changes throughout pregnancy.

In this study, we compare the concentrations of a series of adipokines in AF and cord blood at birth between the larger and smaller fetuses in a cohort of dichorionic-diamniotic (DCDA) twin pairs discordant for fetal growth. By focusing on twins, we hope to abrogate the effects of maternal and intrauterine variations on fetal growth. In addition, we measured adipokine concentrations in mid-trimester AF to examine the longitudinal change in AF adipokine concentrations.

Materials and Methods

Study design

This retrospective cohort study consisted of consecutive twin pregnant women cared for at Seoul National University Hospital with the following inclusion criteria: (1) DCDA twins with birth weight discordancy (birth weight difference greater than 10% between the larger and smaller twin); (2) AF retrieved at mid-trimester or at birth; (3) cord blood collected at birth; and (4) who delivered in our institution. Cases were classified into Cohort 1 or Cohort 2 depending on the timing of AF retrieval. Cohort 1 included women who underwent clinically-indicated mid-trimester amniocentesis between 15–20 weeks of gestation for fetal karyotyping;

Cohort 2 consisted of women whose AF and cord blood was collected at the time of cesarean delivery. Cases with early preterm delivery (<32 weeks of gestation), major fetal anomalies, or intrauterine fetal death were excluded.

Clinically-indicated mid-trimester amniocentesis was performed on each twin under ultrasound guidance after informed consent had been obtained. Amniocentesis at the time of cesarean delivery was performed just before amniotomy by inserting a needle into the fetal membranes of each twin under direct visualization after performing the hysterotomy. Cord blood was collected after delivery of the neonates by withdrawing blood from the umbilical vein of each twin. Retrieved AF and cord blood was centrifuged, aliquoted, and stored at -70°C until assayed.

The Institutional Review Board at Seoul National University Hospital approved the study. Patients provided written consent for the collection and use of these samples and their accompanying clinical information for research purposes.

Measurement of adipokines

Stored AF (mid-trimester AF in Cohort 1; AF at birth in Cohort 2) and cord blood samples were analyzed for multiple adipokines and obesity-related biomarkers (Complement Factor D/Adipsin, Serpin E1/PAI-1, Adiponectin/Acrp30, C-Reactive Protein, CCL2/MCP-1, Leptin, Resistin) using the Luminex Performance Assay kit (R&D Systems, Inc., Minneapolis, MN, USA). For multiplex assays, the analyte-specific antibodies were pre-coated onto color-coded microspheres and the immobilized antibodies were allowed to bind the analyte of interest. After adding a biotinylated antibody cocktail specific to the analytes of interest, streptavidin-phycoerythrin conjugate bound to the captured biotinylate antibodies were read with the Luminex analyzer. The magnitude of the phycoerythrin-derived signal is directly proportional to the amount of analyte bound. For analysis, values below the lower limit of detection for each analyte were recorded as the lower limit of quantification (LLOQ).

Statistical methods

The clinical characteristics between the large and small twin pairs were compared with Wilcoxon signed rank test for continuous variables and with McNemar test for categorical variables. The concentrations of analytes in AF and cord blood between large and small twins were also compared with Wilcoxon signed rank test. A generalized estimating equation (GEE) was adopted for the adjustment of confounding variables in the relationship between twin birth weight order and analyte concentrations. GEE can be used in multivariate analysis from the same subject [18,19] and in family-based association studies [20]. Statistical analyses were conducted using the IBM SPSS version 20. $P < 0.05$ was considered significant.

Results

Clinical characteristics

A total of 92 twin pairs met the inclusion criteria, including 49 cases in Cohort 1 and 43 cases in Cohort 2. Table 1 shows the clinical characteristics of the twin pairs. The fetal biometric measurements at the time of mid-trimester amniocentesis were not significantly different between the twin destined to be smaller at birth and the twin destined to be larger at birth, although birth weights were significantly different between twin pairs in both cohorts.

Table 1. Clinical characteristics of the study population.

Cohort 1 (Mid-trimester amniocentesis; n = 49)			
	Small twin	Large twin	p
Maternal age at amniocentesis (years)		34 (33–36)	
Nulliparity (n)		40 (82%)	
Gestational age at amniocentesis (weeks)		17 (16.4–17.8)	
Indication for amniocentesis (n)			
Advanced maternal age		36 (74%)	
Abnormal serum screening		10 (20%)	
Maternal request		3 (6%)	
Biparietal diameter at amniocentesis (cm)*	3.7 (3.6–3.95) (n = 41)	3.8 (3.5–4.05) (n = 41)	NS
Femur length at amniocentesis (cm)*	2.15 (2.0–2.4) (n = 42)	2.2 (2.0–2.4) (n = 42)	NS
Gestational age at delivery (weeks) *	37.1 (36.0–37.9)	37.1 (36.0–37.9)	NS
Presenting twin (n) [†]	26 (53%)	23 (47%)	NS
Neonatal sex (male) (n) [†]	20 (41%)	27 (55%)	NS
Birth weight (grams) *	2240 (1875–2490)	2660 (2310–2935)	<0.001
Small for gestational age at delivery (n) [†]	31 (63%)	5 (10%)	<0.001
Cesarean delivery (n) [†]	35 (71%)	35 (71%)	NS
Cohort 2 (Amniocentesis at delivery; n = 43)			
	Small twin	Large twin	p
Maternal age at amniocentesis (years)		32 (30–35)	
Nulliparity (n)		35 (81%)	
Gestational age at amniocentesis (weeks)	37.0 (35.3–37.7)	37.0 (35.3–37.7)	NS
Gestational age at delivery (weeks) *	37.0 (35.3–37.7)	37.0 (35.3–37.7)	NS
Presenting twin (n) [†]	12 (28%)	31 (72%)	NS
Neonatal sex (male) (n) [†]	19 (44%)	25 (58%)	NS
Birth weight (grams) *	2150 (1850–2500)	2690 (2340–3040)	<0.001
Small for gestational age at delivery (n) [†]	30 (70%)	0 (0%)	(-)
Cesarean delivery (n) [†]	43 (100%)	43 (100%)	NS

Data are presented as median and interquartile range; NS, not significant

* Analyzed with Wilcoxon signed rank test

[†] Analyzed with McNemar test

doi:10.1371/journal.pone.0154537.t001

Concentrations of biomarkers in AF and cord blood

[Table 2](#) compares the concentrations of adipokines and obesity-related biomarkers in AF and cord blood between the small and large twin pairs. In mid-trimester AF, the concentrations of adiponectin, leptin, and C-reactive protein of twins destined to be smaller at birth were significantly higher than twins destined to be larger. The difference in concentrations of adiponectin and leptin between twin pairs remained significantly different after adjustment for fetal sex and birth order. However, the concentrations of adiponectin, leptin, and C-reactive protein in AF collected at the time of birth were not significantly different between twin pairs. The median concentration of serpin E1 in the AF of smaller twins were significantly higher than that in the AF collected from the larger twin, and this difference remained significant after adjustment ([Table 2](#)).

In all 92 twin pairs from both cohorts, the concentrations of adiponectin and leptin in the cord blood collected from the smaller twins were significantly lower than that collected from the larger twin, whereas the cord blood concentrations of C-reactive protein and serpin E1 in

Table 2. Concentrations of select chemokines in smaller versus larger twins.

Mid-trimester AF, Cohort 1 (Mid-trimester amniocentesis; n = 49)			
	Small twin	Large twin	P value*
Adiponectin (ng/mL)	68.5 (40.1–106.6)	61.4 (41.7–77.7)	<0.05†
Leptin (ng/mL)	13.9 (9.9–25.3)	11.2 (7.5–18.9)	<0.01†
C-Reactive Protein (ng/mL)	25.2 (12.5–57.4)	18.1 (6.4–54.9)	<0.005
Complement factor D (ng/mL)	1256.2 (927.9–1793.3)	1190.7 (872.8–1653.5)	NS
Serpin E1 (ng/mL)	174.3 (105.9–230.2)	161.3 (110.6–250.1)	NS
CCL2/MCP-1 (ng/mL)	0.57 (0.45–0.77)	0.54 (0.38–0.71)	NS
Resistin (ng/mL)	12.7 (7.3–23.6)	14.3 (6.9–25.1)	NS
AF at the time of delivery, Cohort 2 (Amniocentesis at delivery; n = 43)			
	Small twin	Large twin	P value*
Adiponectin (ng/mL)	49.7 (36.9–74.7)	56.7 (39.4–75.5)	NS
Leptin (ng/mL)	2.0 (1.3–3.1)	2.0 (1.0–2.6)	NS
C-Reactive Protein (ng/mL)	26.3 (9.8–62.7)	25.1 (11.1–73.7)	NS
Complement factor D (ng/mL)	878.3 (656.2–1163.5)	868.0 (650.6–1150.9)	NS
Serpin E1 (ng/mL)	246.0 (167.7–290.9)	182.8 (150.1–249.9)	<0.01†
CCL2/MCP-1 (ng/mL)	0.32 (0.21–0.44)	0.33 (0.26–0.50)	NS
Resistin (ng/mL)	12.3 (9.1–20.2)	12.3 (8.7–21.3)	NS
Cord blood at the time of delivery, Cohort 1 and 2 (n = 92)			
	Small twin	Large twin	P value*
Adiponectin (ng/mL)	10425.5 (7378.4–13183.0)	11552.0 (9351.7–14287.8)	<0.005†
Leptin (ng/mL)	2.1 (1.1–4.2)	2.6 (1.4–4.8)	<0.005†
C-Reactive Protein (ng/mL)	26.6 (16.4–52.5)	20.1 (14.9–44.6)	<0.001
Complement factor D (ng/mL)	5183.8 (4427.5–5888.1)	5364.0 (4653.2–6260.5)	0.083
Serpin E1 (ng/mL)	15.5 (9.7–34.0)	13.3 (9.7–22.5)	<0.05†
CCL2/MCP-1 (ng/mL)	0.13 (0.08–0.17)	0.12 (0.10–0.17)	NS
Resistin (ng/mL)	69.3 (38.6–193.0)	84.0 (35.5–163.6)	NS

Data are presented as median and interquartile range; NS, not significant

* Analyzed with Wilcoxon signed rank test

† Significant after adjustment for fetal sex and birth order

doi:10.1371/journal.pone.0154537.t002

the smaller twin were significantly higher than that of larger twin (Table 2). This difference in cord blood concentrations of adiponectin, leptin and serpin E1 remained significant after adjustment.

Discussion

Main findings

The principal findings of this study were: (1) concentrations of adiponectin and leptin in mid-trimester AF of twins destined to be smaller at birth were significantly higher than twins destined to be larger; (2) concentrations of adiponectin and leptin in AF at the time of delivery were not significantly different between smaller and larger twin pairs, but the concentration of serpin E1 in AF at the time of delivery was significantly higher in smaller twins than in larger twins; and (3) levels of adiponectin and leptin in cord blood of smaller twins were significantly lower than that in the cord blood of the larger twins, whereas the concentration of serpin E1 in cord blood of smaller twins was higher than that in larger twins.

Leptin and adiponectin in cord blood of growth restricted fetuses

Leptin is a circulating hormone that affects energy homeostasis by regulating energy expenditure and food intake [21]. Levels of leptin are elevated in the circulation of adults with obesity, metabolic syndrome, and cardiovascular disease [22, 23]. In contrast, adiponectin appears to have anti-atherogenic and anti-inflammatory properties, likely mediated by its effects on glucose and lipid metabolism. In adults, circulating levels of adiponectin are inversely correlated with body weight or body fat mass [24, 25].

Prior studies investigating the association between leptin/adiponectin and FGR have shown conflicting results. Most reported that neonates with FGR had lower leptin concentrations than their appropriately grown controls [10–12,26], but others found no difference [15,16]. Similarly, some studies have shown a positive correlation between cord blood adiponectin concentrations and birth weight, with growth-restricted fetuses having lower adiponectin levels [13,14], but other study found no such association [17]. These inconsistent results may be due to the effect of a number of variables which could affect the cord blood concentrations of adipokines, including the gestational age at delivery, maternal obesity, or maternal diet [27]. In an effort to abrogate these maternal and intrauterine variables, we compared the concentrations of adipokines in cord blood between the larger and smaller fetuses in DCDA twin pairs discordant for growth. Our findings confirm prior reports [10–14,26] that both leptin and adiponectin concentrations are decreased in the cord blood of smaller fetuses. Cord blood leptin is derived from a number of sources, including maternal adipose tissues, fetal adipose tissue, and the placenta [28]. Lower concentrations of adipokines in growth-restricted fetuses may be attributed to reduced fat mass or reduced placental production. The studies by Kamoda et al. [29] and Takaya et al. [30] showed that growth-restricted neonates had lower concentrations of adiponectin and that this was associated with the future development of insulin resistance and metabolic complications.

Serpin E1 in fetal growth restriction

Serpin E1, also known as plasminogen activator inhibitor-1 (PAI-1), regulates fibrinolysis and is associated with endothelial dysfunction. Serpin E2 is derived from endothelial cell and adipose tissue, and is believed to regulate insulin resistance. The literature suggests that, in adults, high levels of serpin E1 is associated with dyslipidemia and metabolic syndrome [31]. Serpin E1 levels are known to be elevated in the maternal circulation of pregnancies with FGR and uteroplacental insufficiency [32], but there are few studies on the relationship between serpin E1 and growth restriction in the fetus or neonate. In the study of Boutsikou et al. [33], circulating levels of serpin E1 were not significantly different between growth-restricted neonates and neonates with normal growth. In the current study, smaller fetuses had higher concentrations of serpin E1 in both cord blood and AF at delivery, which is consistent with the result of studies in adults with metabolic syndrome [31].

Adipokines in amniotic fluid of growth-restricted fetuses

In the current study, the pattern of adipokine concentrations were different between AF collected in the mid-trimester and AF collected at delivery. In the mid-trimester, AF leptin and adiponectin concentrations were higher in the twin destined to be smaller at birth than in the twin destined to be larger, and the concentration of serpin E1 was not significantly different between the twins. However, at the time of birth, the AF leptin and adiponectin concentrations were not different between the twin pairs discordant for growth, whereas the concentration of serpin E1 was higher in the smaller twins than the larger twins.

To our knowledge, this is the first study to compare the concentrations of adipokines in twin AF at the time of birth and also the first study to investigate the temporal change in AF adipokine concentrations during pregnancy and its association with fetal growth. There are two possible explanations for the temporal change in AF adipokine concentration. First, the precise source of AF adipokines is still unknown, and may be different in the mid-trimester and at the time of birth. In the mid-trimester, fetal urine is a less important constitute of AF and it is possible that the placenta or fetal membranes might be the major source of AF proteins at this time [34–36]. In support of this explanation, prior studies have shown excessive expression of leptin in placentas collected from women with preeclampsia [37,38] and elevated concentrations of leptin and adiponectin in mid-trimester AF have been measured before the development of FGR or preeclampsia in singleton pregnancies [36,39]. In third trimester, the source of AF adipokines may include both the fetus and the placenta, which may explain why AF leptin and adiponectin concentrations were not different at delivery, while serpin E1 levels were increased in the smaller twins. The second possible explanation is that placental and/or fetal expression of adipokines may be different between the mid-trimester and third trimester, resulting in temporal changes in AF concentrations. This temporal change in placental and/or fetal expression of adipokines could be only demonstrated by longitudinally sampling fetal cord blood or placental tissues or AF serially in the same patient from the mid-trimester to term, which would be difficult to do in humans.

In conclusion, concentrations of adiponectin, leptin, and serpin E1 vary significantly between smaller and larger twins, and show different patterns of change in mid-trimester AF, AF at delivery, and cord blood at birth in DCDA twin pairs discordant for growth. Additional studies are needed to determine whether these changes are causally related to the development of FGR and/or to the subsequent development of metabolic complications known to occur at a higher incidence in growth-restricted fetuses.

Acknowledgments

The authors would like to thank Sohee Oh, PhD of the Department of Biostatistics in Seoul Metropolitan Government Seoul National University Boramae Medical Center for her statistical advice.

Author Contributions

Conceived and designed the experiments: SML JSP. Performed the experiments: SML JSP. Analyzed the data: SML JSP ERN SP SMK. Contributed reagents/materials/analysis tools: SML JSP SMK JHL CWP BJY JKJ. Wrote the paper: SML JSP ERN.

References

1. Barker DJ. Adult consequences of fetal growth restriction. *Clin Obstet Gynecol.* 2006; 49: 270–283. PMID: [16721106](#)
2. Dörner G, Plagemann A. Perinatal hyperinsulinism as possible predisposing factor for diabetes mellitus, obesity and enhanced cardiovascular risk in later life. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et métabolisme.* 1994; 26: 213–221. PMID: [8076902](#)
3. Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia.* 1992; 35: 595–601. PMID: [1644236](#)
4. Fowden AL, Forhead AJ. Endocrine mechanisms of intrauterine programming. *Reproduction.* 2004; 127: 515–526. PMID: [15129007](#)
5. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. *Clin Chem.* 2004; 50: 1511–1525. PMID: [15265818](#)

6. Jaquet D, Deghmoun S, Chevenne D, Collin D, Czernichow P, Levy-Marchal C. Dynamic change in adiposity from fetal to postnatal life is involved in the metabolic syndrome associated with reduced fetal growth. *Diabetologia*. 2005; 48: 849–855. PMID: [15834547](#)
7. Yajnik CS, Lubree HG, Rege SS, Naik SS, Deshpande JA, Deshpande SS, et al. Adiposity and hyperinsulinemia in Indians are present at birth. *J Clin Endocrinol Metab*. 2002; 87: 5575–5580. PMID: [12466355](#)
8. Jaquet D, Gaboriau A, Czernichow P, Levy-Marchal C. Insulin resistance early in adulthood in subjects born with intrauterine growth retardation. *J Clin Endocrinol Metab*. 2000; 85: 1401–1406. PMID: [10770173](#)
9. Ibanez L, Lopez-Bermejo A, Suarez L, Marcos MV, Diaz M, De Zegher F. Visceral adiposity without overweight in children born small for gestational age. *J Clin Endocrinol Metab*. 2008; 93: 2079–2083. doi: [10.1210/jc.2007-2850](#) PMID: [18334595](#)
10. Pighetti M, Tommaselli GA, D'elia A, Di Carlo C, Mariano A, Di Carlo A, et al. Maternal serum and umbilical cord blood leptin concentrations with fetal growth restriction. *Obstet Gynecol*. 2003; 102: 535–543. PMID: [12962938](#)
11. Yildiz L, Avci B, Ingec M. Umbilical cord and maternal blood leptin concentrations in intrauterine growth retardation. *Clin Chem Lab Med*. 2002; 40: 1114–1117. PMID: [12521228](#)
12. Jaquet D, Leger J, Levy-Marchal C, Oury JF, Czernichow P. Ontogeny of leptin in human fetuses and newborns: effect of intrauterine growth retardation on serum leptin concentrations. *J Clin Endocrinol Metab*. 1998; 83: 1243–1246. PMID: [9543149](#)
13. Tsai PJ, Yu CH, Hsu SP, Lee YH, Chiou CH, Hsu YW, et al. Cord plasma concentrations of adiponectin and leptin in healthy term neonates: positive correlation with birthweight and neonatal adiposity. *Clinical endocrinology*. 2004; 61: 88–93. PMID: [15212649](#)
14. Pardo IM, Geloneze B, Tambascia MA, Barros-Filho AA. Hyperadiponectinemia in newborns: relationship with leptin levels and birth weight. *Obesity research*. 2004; 12: 521–524. PMID: [15044670](#)
15. Kyriakakou M, Malamitsi-Puchner A, Milioti H, Boutsikou T, Margeli A, Hassiakos D, et al. Leptin and adiponectin concentrations in intrauterine growth restricted and appropriate for gestational age fetuses, neonates, and their mothers. *European journal of endocrinology / European Federation of Endocrine Societies*. 2008; 158: 343–348. doi: [10.1530/EJE-07-0692](#) PMID: [18299467](#)
16. Shekhawat PS, Garland JS, Shivpuri C, Mick GJ, Sasidharan P, Pelz CJ, et al. Neonatal cord blood leptin: its relationship to birth weight, body mass index, maternal diabetes, and steroids. *Pediatr Res*. 1998; 43: 338–343. PMID: [9505271](#)
17. Lindsay RS, Walker JD, Havel PJ, Hamilton BA, Calder AA, Johnstone FD, et al. Adiponectin is present in cord blood but is unrelated to birth weight. *Diabetes care*. 2003; 26: 2244–2249. PMID: [12882843](#)
18. Khairy P, Ouyang DW, Fernandes SM, Lee-Parritz A, Economy KE, Landzberg MJ. Pregnancy outcomes in women with congenital heart disease. *Circulation*. 2006; 113: 517–524. PMID: [16449731](#)
19. Van Nimwegen FA, Penders J, Stobberingh EE, Postma DS, Koppelman GH, Kerkhof M, et al. Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. *J Allergy Clin Immunol*. 2011; 128: 948–955 e941–943. doi: [10.1016/j.jaci.2011.07.027](#) PMID: [21872915](#)
20. Xu WL, Atti AR, Gatz M, Pedersen NL, Johansson B, Fratiglioni L. Midlife overweight and obesity increase late-life dementia risk: a population-based twin study. *Neurology*. 2011; 76: 1568–1574. doi: [10.1212/WNL.0b013e3182190d09](#) PMID: [21536637](#)
21. Ahima RS, Flier JS. Leptin. *Annual review of physiology*. 2000; 62: 413–437. PMID: [10845097](#)
22. Krechowec SO, Vickers M, Gertler A, Breier BH. Prenatal influences on leptin sensitivity and susceptibility to diet-induced obesity. *The Journal of endocrinology*. 2006; 189: 355–363. PMID: [16648302](#)
23. Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, Gaw A, et al. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). *Circulation*. 2001; 104: 3052–3056. PMID: [11748099](#)
24. Schondorf T, Maiworm A, Emmison N, Forst T, Pfutzner A. Biological background and role of adiponectin as marker for insulin resistance and cardiovascular risk. *Clinical laboratory*. 2005; 51: 489–494. PMID: [16285470](#)
25. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun*. 1999; 257: 79–83. PMID: [10092513](#)
26. Lepercq J, Challier JC, Guerre-Millo M, Cauzac M, Vidal H, Hauguel-De Mouzon S. Prenatal leptin production: evidence that fetal adipose tissue produces leptin. *J Clin Endocrinol Metab*. 2001; 86: 2409–2413. PMID: [11397832](#)
27. Kajantie E, Hytinen T, Hovi P, Andersson S. Cord plasma adiponectin: a 20-fold rise between 24 weeks gestation and term. *J Clin Endocrinol Metab*. 2004; 89: 4031–4036. PMID: [15292345](#)

28. Alexe DM, Syridou G, Petridou ET. Determinants of early life leptin levels and later life degenerative outcomes. *Clinical medicine & research*. 2006; 4: 326–335.
29. Kamoda T, Saitoh H, Saito M, Sugiura M, Matsui A. Serum adiponectin concentrations in newborn infants in early postnatal life. *Pediatr Res*. 2004; 56: 690–693. PMID: [15371566](#)
30. Takaya J, Yamato F, Higashino H, Kaneko K. Intracellular magnesium and adipokines in umbilical cord plasma and infant birth size. *Pediatr Res*. 2007; 62: 700–703. PMID: [17957162](#)
31. Bastard JP, Pieroni L, Hainque B. Relationship between plasma plasminogen activator inhibitor 1 and insulin resistance. *Diabetes/metabolism research and reviews*. 2000; 16: 192–201. PMID: [10867719](#)
32. Sheppard BL, Bonnar J. Uteroplacental hemostasis in intrauterine fetal growth retardation. *Seminars in thrombosis and hemostasis*. 1999; 25: 443–446. PMID: [10625199](#)
33. Boutsikou T, Mastorakos G, Kyriakakou M, Margeli A, Hassiakos D, Papassotiriou I, et al. Circulating levels of inflammatory markers in intrauterine growth restriction. *Mediators Inflamm*. 2010; 2010: 790605. doi: [10.1155/2010/790605](#) PMID: [20614004](#)
34. Cleveland MG, Bakos MA, Pyron DL, Rajaraman S, Goldblum RM. Characterization of secretory component in amniotic fluid. Identification of new forms of secretory IgA. *J Immunol*. 1991; 147: 181–188. PMID: [2051019](#)
35. Touboul C, Boulvain M, Picone O, Levailant JM, Frydman R, Senat MV. Normal fetal urine production rate estimated with 3-dimensional ultrasonography using the rotational technique (virtual organ computer-aided analysis). *Am J Obstet Gynecol*. 2008; 199: 57 e51–55.
36. Wang CN, Chang SD, Peng HH, Lee YS, Chang YL, Cheng PJ, et al. Change in amniotic fluid levels of multiple anti-angiogenic proteins before development of preeclampsia and intrauterine growth restriction. *J Clin Endocrinol Metab*. 2010; 95: 1431–1441. doi: [10.1210/jc.2009-1954](#) PMID: [20080845](#)
37. Haugen F, Ranheim T, Harsem NK, Lips E, Staff AC, Drevon CA. Increased plasma levels of adipokines in preeclampsia: relationship to placenta and adipose tissue gene expression. *Am J Physiol Endocrinol Metab*. 2006; 290: E326–333. PMID: [16144822](#)
38. Mise H, Sagawa N, Matsumoto T, Yura S, Nanno H, Itoh H, et al. Augmented placental production of leptin in preeclampsia: possible involvement of placental hypoxia. *J Clin Endocrinol Metab*. 1998; 83: 3225–3229. PMID: [9745432](#)
39. Chan TF, Su JH, Chung YF, Hsu YH, Yeh YT, Yuan SS. Elevated amniotic fluid leptin levels in pregnant women who are destined to develop preeclampsia. *Acta Obstet Gynecol Scand*. 2006; 85: 171–174. PMID: [16532910](#)