Title: Gender determines the actions of adiponectin multimers on fetal growth and adiposity.

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Disclosure of interests

The authors report no potential conflict of interest.

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Condensation

Gender determines the actions of cord blood adiponectin multimers on fat accretion and fetal growth in the third trimester of pregnancy

Brief Title: adiponectin multimers and neonatal fat mass

Abstract

Objective: To analyze the role of cord blood adiponectin and its multimeric forms in neonatal adiposity and fetal growth (FGV) during the third trimester of pregnancy according to fetal gender.

Study Design: Prospective analytical observational study conducted at the Diabetes and Pregnancy unit, University Hospital Joan XXIII, Tarragona. Ninety-six healthy pregnant women were included in the early third trimester and were followed up until delivery. Maternal blood was obtained upon recruitment, and cord blood was obtained at delivery. Serial fetal ultrasounds were performed during the third trimester to assess fetal growth velocity. Skinfolds were measured after birth to assess neonatal adiposity. Adiponectin multimers were determined in maternal and cord blood.

Results: In female neonates, adiposity and fetal growth velocity in the late third trimester were correlated positively with cord blood insulin (cbinsulin) (r:0.343; p=0.015 and r:0.430;p=0.002, respectively) and maternal pregravid BMI (r:597;p<0.001 and r:0.428;p=0.002, respectively), and negatively with maternal High Molecular Weight (HMW)/total adiponectin ratio (r:-0.269;p=0.035 and r:-0.387;p=0.005, respectively), but in the stepwise multiple regression model, the main determinants were cbinsulin, pregravid BMI and cord blood HMW adiponectin. Otherwise, in male neonates, adiposity and fetal growth were correlated with cord blood low molecular weight adiponectin (LMW) (r:0.486;p=0.003 and r:0.394;p=0.020, respectively), and it was this multimeric form that emerged as an independent determinant in the stepwise regression model.

Conclusion: Adiponectin seems to determine fetal growth and adipose tissue accretion, and LMW is more specifically implicated in males, whereas the HMW isoform may be more important in females.

Keywords: adiponectin, neonatal adiposity, fetal growth

Introduction

Fetal growth is the result of integrated interplay between genetic, nutritional and endocrine factors. Birth weight (BW) is considered a marker of fetal well-being and it has been correlated with body composition in neonates. However, sometimes weight alone is not a sufficiently sensitive parameter to determine appropriate fat deposits. Fat mass (FM) has an huge capacity for altering this body compartment as a result of intrauterine growth and is a more sensitive indicator of the fetal nutritional status than lean body mass, which has a greater genetic influence[1,2]. Recently there has been growing interest in the study of factors that modify intrauterine growth and the effect it could have on the appearance of diseases later in life[3]. Obstetric ultrasound is a good tool to assess prenatal nutritional status and fetal growth pattern[4], and the use of longitudinal data in the third trimester in relation to metabolic and clinical parameters can allow us to identify potential markers of fetal wellbeing and adipose tissue accretion.

Insulin is one of the best known key regulators of fetal growth, but recently some adipokines have also emerged as a link between maternal metabolism, insulin resistance and fetal growth[5]. In this context, adiponectin, a protein with insulin sensitizing actions involved in energy homeostasis in adults, could be expected to have a significant effect on fetal growth and development[6]. Adiponectin circulates in different sized complexes: trimers (LMW), hexamers (MMW) and high-molecular-weight (HMW) multimers containing 18 monomeric subunits or more. The distribution and the amount of the different multimeric forms seem to be essential for its biological effects[7,8]. Adiponectin is present during fetal life and its levels increase gradually from mid-gestation until delivery[9] and for this reason it has been suggested that it could play a role in fetal growth and adipose tissue deposition[5]. However, data in the literature are scarce and

sometimes contradictory[10-16]. The information about the distribution of its multimeric forms in cord blood and its relationship with neonatal weight and adiposity are also greatly limited[17]. Recently, we have described a close relationship with ponderal index, a surrogate marker of adiposity, suggesting a role of these multimeric forms in fetal adiposity[18]. In this line, Basu et al. reported a correlation between the HMW isoform and neonatal adiposity in female neonates, but not in their male counterparts[17].

Gender dimorphism in fetal growth and fetal mass accretion has been observed previously. Female fetuses increase their percentage body fat to a greater extent than their male counterparts in the third trimester of pregnancy[19] and also male infants seem to be more vulnerable to undernutrition, as evidenced by a greater risk of later cardiovascular disease in the offspring of women exposed to famine[20].

The aim of this work was to study in greater depth the relationship of umbilical adiponectin and its multimeric forms with fetal growth and neonatal body composition in a cohort of newborn infants of healthy pregnant women and to determine whether adipose tissue accretion in the third trimester of pregnancy could be related with these parameters. We hypothesized that the concentration of the multimeric forms and their distribution could be determinants of neonatal body composition and fetal growth and that this relationship could be modified by gender.

Methods

This is a prospective analytical observational study of neonates delivered to women who were invited to participate in this study conducted at the Joan XXIII University Hospital. It was approved by the centre's Research Ethics Board and all subjects provided their written informed consent. Three hundred and seventy-seven pregnant Caucasian women were recruited at the time of antepartum screening for GDM between the 26th and 30th weeks of pregnancy. Women with an abnormal 1 h 50 g glucose

challenge test, underwent a 3 h 100 g oral glucose tolerance test. Maternal blood samples (10 mL in a silicone and 10 mL in an EDTA tube) were obtained at the time of recruitment. Cord blood samples (10 mL in silicone and 10 mL in an EDTA tube) were collected at the time of delivery from the umbilical vein before placental separation. Blood samples were centrifuged at 5000 x g for 15 minutes. Serum and plasma samples were frozen and kept at -70º in a GMD biobank collection until assay. In this study we included 96 women who recorded a 3 h 100 g oral glucose tolerance test (OGTT), according to NDDG criteria[21] and that fulfilled the following criteria at the end of pregnancy: 1) a singleton pregnancy, 2) accurate gestational age confirmed by an ultrasound examination before 20 weeks of gestation, 3) the absence of fetal anomalies identified at birth, 4) at least two ultrasound explorations, one at recruitment and another in the middle of the third trimester, 5) cord blood sample obtained at delivery, and 6) neonatal biometry within 48 hours of delivery.

Clinical and Demographic Data

Upon inclusion, demographic and historical information was collected by an interviewer administering a questionnaire that included patient demographics, personal medical information and information regarding the current and previous pregnancies. Also, height, and weight before pregnancy and at the end of pregnancy were recorded. BMI was calculated using the formula BMI = weight (in kilograms)/height (in meters)^2. Increase in BMI was calculated by the formula BMI Gain= [final BMI]-[pregravid BMI].

Newborn infants (neonates) were evaluated in the first 48 hours of life. Neonatal length and weight were determined using a measuring board to the nearest 0.1 cm and a calibrated scale to the nearest of 10 g. Triceps, biceps, subscapular, and flank skinfold thickness were assessed with a Holtain skinfold caliper (Chasmors Ltd, London UK).

158 Each skinfold was measured at least three times until a consistent and stable reading
159 was obtained. All skinfold measurements were taken from the left side by the same
160 physician experienced in the technique (AM). The sum of the four skinfolds (SSF) was
161 used to estimate neonatal adiposity. Birth weight was transformed into a standard
162 deviation score using gender-specific references of fetal growth[22].

163 *Fetal ultrasound*

164 One examiner performed all ultrasound examinations using color Doppler ultrasound
165 equipment (RAB 4-8L, Voluson 730 Expert, General Electrics Medical Systems,
166 Austria) incorporating hybrid mechanical and curved array abdominal ultrasonic
167 transducers. Fetal weight estimations were calculated at approximately 28 (range, 27–
168 30) and 35 (range, 34–36) weeks' (wk) gestation from femur length, biparietal diameter,
169 and abdominal circumference using Hadlock's equation[23]. The fetal weight estimates
170 were transformed into standard deviation scores (SDS) using gender-specific references
171 of fetal growth[22]. Fetal Growth velocity (FGV) in the early third trimester (FGV_E) was
172 determined by linear regression between the two ultrasound measurements[24] and FGV
173 in the late third trimester (FGV_L) was also calculated by linear regression between the
174 last ultrasound and neonatal weight at delivery. Both measurements were expressed as
175 ΔSDS per week. All ultrasound examinations were performed by the same experienced
176 obstetrician (MB).

177 *Laboratory measurements*

178 Glucose levels were determined in an ADVIA 2400 (Siemens AG, Munich, Germany)
179 autoanalyzer using the standard enzyme methods. Fasting plasma insulin and C-peptide
180 were determined by immunoassay in an ADVIA Centaur System (Siemens AG,
181 Munich, Germany). This assay shows a cross-reactivity of lower than 0.1 % to intact

182 human proinsulin and the primary circulating split form (Des31, 32 proinsulin).
183 Homeostasis model assessment of insulin resistance (HOMA-IR) was determined
184 according to the formula proposed by Matthews et al[25].

185 Serum adiponectin levels were determined using a human ELISA kit (Multimeric
186 Adiponectin ELISA Kit; Bühlmann, Schönenbuch, Switzerland). The intra- and inter-
187 assay CVs were lower than 15%, and assay sensitivity was 0.08 ng/ml. We calculated
188 the ratio of HMW/total adiponectin levels (S_A) for maternal and cord blood adiponectin
189 concentrations, mS_A and cbS_A respectively.

190 Statistics

191 All statistical analyses were performed using SPSS 13.0 software (SPSS, Chicago, IL).
192 We performed the 1-sample Kolmogorov-Smirnov test to verify the normal distribution
193 of the quantitative variables. Normally distributed data were expressed as the mean ±
194 SD, whereas variables with a skewed distribution were represented as the median
195 (interquartile range). Categorical variables were reported as numbers (percentages).
196 Student's t test analysis was used to compare the mean value of normally distributed
197 continuous variables. For variables with skewed distributions, we used the Mann-
198 Whitney U test. To analyze the differences in nominal variables between groups we
199 performed the χ^2 test. Linear associations between variables were assumed after
200 checking them by scatter plots (not shown). Pearson's correlation coefficient was used
201 to analyze the bi-variate correlation between FGV and neonatal anthropometric
202 parameters with clinical and metabolic parameters, including maternal and neonatal
203 adiponectin concentrations. Finally, to identify the parameters that best predicted FGV,
204 birth weight standard deviation score (BW SDS) and the SSF we performed a multiple
205 regression analysis using the stepwise option of the SPSS including these variables as

dependent variables. The significance to drop (Probability OUT) was 0.1 and to add (Probability IN) was 0.5. Cord blood insulin, maternal insulin and HOMA-IR were log transformed before bivariate and multivariate analysis. A p-value of less than 0.05 was considered significant.

Results

Maternal and newborn infant characteristics by gender are shown in table 1. Forty-three 43 male and 53 female neonates were included in the study. The clinical and metabolic characteristics of both groups were similar except for BMI gain, which was higher in the mothers of female neonates (p=0.013) compared to mothers of male neonates.

Univariate analysis of birth weight and neonatal body composition with clinical and metabolic parameters.

Whole Group

The SSF and BW SDS were positively related with pregravid BMI (r:0.349; p=0.001 and r:0.209; p=0.041, respectively) and cord blood insulin (cbinsulin) (r:0.284; p=0.006 and r:0.395; p<0.001, respectively), and negatively correlated with mSA (r:-0.251; p=0.015 and r: -0.299; p=0.003, respectively). In addition, the SSF was positively related with cord blood Adiponectin (cbAdiponectin) (r: 0.230; p=0.026), cord blood LMW (cbLMW) (r: 0.233; p=0.035), and fasting maternal glucose levels (r: 0.204; p=0.049). SSF and BW SDS were unrelated with gestational age at delivery, cord blood HMW (cbHMW), MMW (cbMMW) or any of the other clinical or metabolic maternal parameters considered.

Female neonates

Both, BW SDS and the SSF were positively associated with cbinsulin (r:0.431; p=0.001 and r:0.343; p=0.015, respectively), pregravid BMI (r:403; p=0.003 and r:0.597; p<0.001, respectively) and negatively related to mSA (r:-0.407; p=0.002 and r: -0.269; p=0.035, respectively). The strength of these associations increased compared to the whole group. The SSF was also positively correlated with maternal glucose (r:0.319; p=0.023) and HOMA-IR (r:0.309; p=0.029) (see table 2). No correlation was observed between cbdiponectin and BW SDS or neonatal adiposity.

Male neonates

CbLMW was positively correlated with BW SDS (r:0.400; p=0.017) and SSF (r; 0.486; p=0.003). cbinsulin was also positively correlated with BW SDS (r: 0.325; p=0.038) but unrelated with SSF. No other relationship was observed (see table 2).

Univariate analysis of fetal growth velocity with clinical and metabolic parameters.

Whole Group

Fetal Growth velocity in the early and in the latter third trimester was positively correlated with cbinsulin (r: 0.230; p=0.029 and r: 0.386; p= <0.001, respectively) and inversely associated with mSA (r:-0.263; p=0.011 and r: -0.277; p=0.007, respectively). We also observed that FGV_E was positively correlated with maternal LMW (mLMW) concentrations (r:0.258; p=0.013), but this association was not found in the latter third trimester. In contrast, cbLMW was-positively associated with FGV_L (r: 0.219; p=0.048) (see table 3).

Female Neonates

Pregravid BMI was positively related with FGV_E and FGV_L (r:0.393 and r:0.428, respectively; p<0.01, for all). Also, FGV_L was positively and strongly related with

cbinsulin levels (r:0.430; p=0.002) and negatively related with mSA (r:-0.387; p=0.005).

BW SDS and the SSF were strongly and positively related with both FGV_E (r: 0.588 and r. 0.629, respectively; p<0.001 for all) and FGV_L (r:0.883 and r: 0.997, respectively; p<0.01, for all). Correlation coefficients are shown in table 3.

Male Neonates

FGV_E was positively correlated with maternal Adiponectin (mAdiponectin) (r;0.356; p=0.046), mLMW (r:0.356; p=0.021) and cbLMW (r:0.348; P=0.040) whereas, FGV_L was positively related with cbinsulin (r:0.318; p=0.043) and cbLMW (r:0.392; p=0.020). No relationship with pregravid BMI or BMI gain was observed.

Birth weight and the SSF were also strongly and positively related with both FGV_E (r:0.606 and r:0.638, respectively, p<0.001 for all) and FGV_L (r: 0.831 and r:0.997; respectively; p<0.01, for all). Data are presented in table 3.

Multiple Regression Analysis.

To further determine the variables that better explained birth weight and neonatal adiposity, we constructed a stepwise multiple linear regression model in which BW SDS and the SSF were introduced as dependent variables, and maternal age, pregravid BMI, HOMA-IR, mSA, glucose, cord blood insulin and cord blood adiponectin multimers were the variables introduced for selection. Cbinsulin and cbLMW emerged as positive, independent determinants of BW SDS in male neonates, explaining 28.3 % of the birth weight variation. cbLMW was the sole determinant of the SSF in the male group explaining an approximately 22% of the variance. An increase in 1 unit of

cbLMW corresponded to a 0.258 SD increase (95% CI 0.025 to 0.490) in BW SDS and a 0.826 mm increase (95% CI 0.269-1.383) in the SSF value (see table 4).

On the other hand, in the female group, cord blood insulin, pregravid BMI and cbMMW adiponectin emerged as positive predictors of BW SDS. Adiposity in this group was also positively associated with cbinsulin and pregravid BMI, but in this case the most relevant multimer was the HMW isoform. These models reconciled 43.1% and 57.7% of the variance of BW SDS and SSF, respectively (see table 4).

We also explored the independent relationships between umbilical adiponectin multimers and FGV in the third trimester. Similar stepwise regression analyses were performed with dependent variables FGV_E and FGV_L. In male neonates, for dependent variables FGV_E and FGV_L, cbLMW and cbinsulin emerged as independent determinants. These models explained 19.1% of the variance of FGV_E and 27.1% of the variance of FGV_L. In female neonates, maternal age and pregravid BMI were the independent predictors of FGV_E. This model reconciled 23.3% of the variance of FGV_E. For dependent variable FGV_L, mS_A emerged as an independent and negative determinant, while cbinsulin, pregravid BMI and cbHMW emerged as positive, independent determinants, with this model explaining the 47.8% variance of FGV in this period. See table 5.

Comment

In this study we report that fetal growth and adipose tissue accretion during fetal life may be mediated by cord blood adiponectin with marked gender dimorphism regarding molecular isoforms, with LMW in males and HMW in females being the main isoforms involved. Furthermore, fetal insulin levels and maternal metabolic status seem to play a more important role in female than male infants.

297 Despite the fact that several pieces of evidence have suggested a role of adiponectin in
298 fat mass accretion and growth regulation during fetal life[17], the relationship of maternal
299 or umbilical adiponectin with BW have been inconsistent[10-16]. In our cohort, maternal
300 adiponectin concentrations were unrelated with neonatal anthropometric measures, but,
301 in agreement with a previous report, mS_A was found to be inversely related with birth
302 weight and fetal adiposity[26]. On the other hand, cord blood adiponectin, and in
303 particular the LMW isoform, were positively related with neonatal adiposity,
304 reinforcing experimental data that show a potential role of this adipokine in fetal
305 adipogenesis[27]. These results are in line with some previous reports in which total
306 cbAdiponectin was associated with BW and skinfold thicknesses in Asiatic
307 populations[10,11] and with neonatal adiposity measure by anthropometric methods at birth
308 in the female neonates of Caucasian women[17].

309 It is worth mentioning that circulating multimeric forms influence biological activity of
310 adiponectin[8,27,28]. Thus, total adiponectin levels may not adequately reflect the effects of
311 this adipokine on metabolic events. Interestingly, in our study we have observed that its
312 multimeric forms exert a different influence on FM accretion during fetal life depending
313 on neonate gender. The cbLMW isoform emerged as the main determinant of
314 subcutaneous fat mass in male neonates, whereas cord blood insulin and the HMW
315 isoform were more relevant in female neonates. This gender dimorphism of the
316 adiponectin multimeric forms on neonatal FM has also been described by Basu et al.[17].
317 In their report, the HMW isoform was associated with neonatal adiposity only in female
318 neonates, but no other isoforms were determined. These observations lead us to
319 speculate that fetal gender dimorphism could determine multimeric adiponectin
320 distribution and some differences in its metabolic events (mainly insulin sensitizer
321 activity) influencing fat deposition during fetal life.

Recently, it has become apparent that cord blood C-peptide concentrations, as a surrogate marker of insulin levels, are higher in female than in male neonates determining a slower growth velocity during the first year of life[29]. In our cohort, we have also observed a gender dimorphism regarding cbinsulin levels and adiposity. Insulin and LMW concentrations in cord blood insulin were independent predictors of fetal growth velocity in male neonates. In female neonates, in the early third trimester, maternal factors, such as pregravid weight and age were the most important determinants of fetal growth, whereas in the latter third trimester, fetal insulin and HMW concentrations became relevant. Furthermore, the HMW to total adiponectin ratio in maternal serum seems only to be a negative determinant of fetal growth in females, suggesting that lower mS_A might be associated with a higher transfer of nutrients across the placenta.

Our findings highlight the role of this adipokine in fetal growth and adipose tissue accretion during pregnancy and should be considered in future studies searching for biomarkers with predictive value in some chronic diseases in adult life such as type 2 diabetes or obesity.

Little is known about the regulation of adiponectin secretion and the role of its molecular forms in fetal life. In adults, these forms are prevalently active in peripheral tissues and in the central nervous system. One is tempted to extrapolate the effect of these isoforms observed in adult life to the fetal being, hypothesizing a possible contribution in the programming of adipose tissue *intrautero*. In fact, umbilical adiponectin has been related with the degree of adiposity later in childhood[30].

We are aware of some of the limitations of this study that must be acknowledged. Body composition was assessed by anthropometric measurements, a non-invasive method

validated as a measure of body fat in neonates and children[31]. This method allows us to evaluate only subcutaneous fat mass, omitting information with regard to the amount of visceral fat.

Conclusions

In summary, we report that fetal adiponectin multimers are related with fetal growth and neonatal body composition in the neonates of healthy pregnant women, and that the relevance of the multimeric form depends on the gender of the neonate. Besides, maternal factors such as pregravid weight and metabolic markers seem to be more important determinants of adiposity in females than in males. As there is increasing evidence that newborn body composition may be related with the appearance of diseases later in life, such as obesity, metabolic syndrome and type 2 diabetes, we believe that greater knowledge of hormonal factors, particularly adipokines, and metabolic factors involved in the regulation of fetal growth and fat deposition is mandatory.

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1 Table 1. Maternal, fetal and neonatal characteristics of the population studied.

	Male (43)	Female (53)	P
Maternal age (years)	31.00±4.69	31.87±5.08	0.391
Multiparous n (%)	19 (44.19)	25 (47.17)	0.838
Tobacco n (%)	4 (9.30)	11 (20.75)	0.162
Pregravid BMI (kg/m^2)	25.57±5.90	24.41±4.92	0.296
Gain in BMI (kg/m^2)	4.35±2.03	5.41±2.03	0.013
Maternal insulin (mU/L)	9.13 (6.52-14.34)	7.05 (5.50-13.27)	0.088
HOMA-IR	1.94 (1.25-2.88)	1.46 (1.10-2.76)	0.240
mAdiponectin (µg/mL)	5,88±2.32	5.87±2.18	0.975
mHMW (µg/mL)	3.35±1.60	3.31±1.66	0.905
mMMW (µg/mL)	1.25±0.57	1.20±0.47	0.632
mLMW (µg/mL)	1.28±1.00	1.39±0.85	0.580
mSA	0.55±0.13	0.55±0.12	0.747
cbAdiponectin (µg/mL)	17.61±5.44	18.45±6.45	0.495
cbHMW (µg/mL)	12.39±0.45	13.00±5.05	0.534
cbMMW (µg/mL)	3.47±1.58	3.12±1.64	0.288
cbLMW (µg/mL)	2.51±1.60	2.60±1.90	0.832

cbSA	0.70±0.12	0.70±0.07	0.891
Gestational week at delivery (weeks)	39.14±1.78	39.70±1.51	0.100
Birth weight (g)	3255.58±535.25	3319.42±487.59	0.543
SSF	15.40±2.77	15.83±2.81	0.469
BW SDS (SDS)	-0.031±1.149	0.280±1.076	0.175
cbInsulin (mU/L)	4.27 (2.15-6.26)	4.34 (2.47-8.12)	0.289
FGV_E ΔSDs/week	0.62±1.07	0.90±2.03	0.217
FGV_L ΔSDs/week	-0.04±1.27	0.25±1.19	0.259
Fetal weight estimate 28-30 wks (SD)	0.94±0.76	1.10±0.73	0.393
Fetal weight estimate 34-36 wks (SD)	0.67±0.87	0.88±0.80	0.229

Data are presented as mean ± SD or median and interquartile range, unless otherwise indicated. SSF: Sum four skinfolds. BW SDS: Birth weight standard deviation score. SD: Standard deviation. ΔSDs change in Standard deviation. FGV_E: fetal growth velocity in the early third trimester. FGV_L: fetal growth velocity in the latter third trimester.

Table 2. Significant Pearson's Correlation Coefficients observed between BW SDS and/or SSF with some maternal and fetal parameters

	Whole Group (96)		Males (43)		Females (53)	
	BW SDS	SSF	BW SDS	SSF	BW SDS	SSF
Pregravid BMI[a]	**0.209 (0.041)**	**0.349 (0.001)**	0.043	0.110	**0.403 (0.003)**	**0.597 (<0.001)**
Glucose	0.157	**0.204 (0.049)**	0.045	0.060	0.228	**0.319 (0.023)**
mSA	**-0.299 (0.003)**	**-0.251 (0.015)**	-0.178	-0.196	**-0.407 (0.002)**	**-0.269 (0.035)**
HOMA-IR[a]	0.073	0.156	-0.084	0.006	0.245	**0.309 (0.029)**
cbAdiponectin	0.148	**0.230 (0.026)**	-0.045	0.290	0.227	0.185
cbLMW	0.194	**0.233 (0.035)**	**0.400 (0.017)**	**0.486 (0.003)**	0.060	0.078
cbInsulin[a]	**0.395 (<0.001)**	**0.284 (0.006)**	**0.325 (0.038)**	0.190	**0.431 (0.001)**	**0.343 (0.015)**

BW SDS: Birth Weight Standard Deviation Scores. SSF: Sum Four Skinfolds.

[a] Log transformed before analysis. For pregravid BMI, only in the whole group analysis.

15 Table 3. Significant Pearson's Correlation Coefficients observed between Fetal Growth

16 Velocity in the early and latter third trimester and some maternal and fetal parameters

	Whole group		**Female**		**Male**	
	FGV_E	**FGV_L**	**FGV_E**	**FGV_L**	**FGV_E**	**FGV_L**
Pregravid BMI[a]	0.111	0.033	**0.393 (0.004)**	**0.428 (0.002)**	0.119	0.080
mAdiponectin	0.106	-0.002	-0.070	-0.063	**0.356 (0.021)**	0.062
mLMW	**0.258 (0.013)**	0.120	0.148	0.181	**0.356 (0.021)**	0.049
mSA	**-0.263 (0.011)**	**-0.277 (0.007)**	-0.272	**-0.387 (0.005)**	-0.245	-0.163
cbLMW	0.051	**0.219 (0.048)**	0.148	0.181	**0.348 (0.040)**	**0.392 (0.020)**
Cb insulin[a]	**0.230 (0.029)**	**0.386 (<0.001)**	0.177	**0.430 (0.002)**	0.284	**0.318 (0.043)**
Birth weight	**0.598 (<0.001)**	**0.855 (<0.001)**	**0.588 (<0.001)**	**0.883 (<0.001)**	**0.606 (<0.001)**	**0.831 (0.001)**
BW SDS	**0.639 (<0.001)**	**0.997 (<0.001)**	**0.629 (<0.001)**	**0.997 (<0.001)**	**0.638 (<0.001)**	**0.997 (<0.001)**

SSF	**0.441 (<0.001)**	**0.606 (<0.001)**	**0.389 (0.006)**	**0.547 (0.001)**	**0.493 (0.001)**	**0.666 (<0.001)**

FGV_E: fetal growth velocity in the early third trimester. FGV_L: fetal growth velocity in the latter third trimester. BW SDS: Birth Weight standard deviation score. SSF: sum four skinfolds

[a] Log transformed before analysis. For pregravid BMI only in the whole group analysis.

24 Table 4. Stepwise Linear Regression for Birth Weight, SDS and SSF in male and female neonates.

	Dependent variables	**Independent variable**	**B (95% CI)**	**P**
Males	**BW SDS (r^2:0.283)[a]**	cbInsulin[b]	1.282 (0.206-2.357)	0.021
		cbLMW	0.258 (0.025-0.490)	0.031
	SSF (r^2:0.222)	cbLMW	0.826 (0.269-1.383)	0.032
Females	**BW SDS (r^2:0.431)**	cbInsulin	0.939 (0.303-1.576)	0.005
		Pregravid BMI	0.098 (0.043-0.153)	0.001
		cbMMW	0.196 (0.030-0.362)	0.031
	SSF (r^2:0.557)	Pregravid BMI	0.388 (0.260-0.516)	<0.001
		cbInsulin	1.859 (0.451-3.268)	0.011
		cbHMW	0.145 (0.033-0.258)	0.016

B (95% CI): unstandardized B coefficient and 95% Confidence interval. Beta: standardized Beta coefficient. BW SDS: Birth Weight Standard Deviation Scores. SSF: Sum four Skinfolds. [a] r^2 : model r^2. [b] cbinsulin log transformed for analysis.

37 Table 5. Stepwise multiple regression model for Fetal Growth Velocity in the early and latter third trimester in male and female neonates.

Males	**Dependent variables**	**Independent variable**	**B (95% CI)**	**P**
	FGV_E (r^2:0.239)[a]	cbLMW	0.241 (0.012-0.470)	0.039
		cbInsulin[b]	1.061 (0.001-2.121)	0.049
	FGV_L (r^2:0.271)	cbInsulin	1.389 (0.191-2.588)	0.025
		cbLMW	0.277 (0.018-0.536)	0.037
Females	**FGV_E (r^2:0.233)**	Pregravid BMI	0.090 (0.031-0.148)	0.003
		Age	-0.060 (-0.116- -0.003)	0.039
	FGV_L (r^2:0.478)	cbInsulin	1.246 (0.474-2.018)	0.002
		Pregravid BMI	0.080 (0.16-0.145)	0.017
		cbHMW	0.064 (0.009-0.119)	0.024

		mSA	-3.067 (-5.825- -0.289)	0.031

B (95% CI): unstandardized B coefficient and 95% Confidence interval .Beta: standardized Beta coefficient. FGV_E: fetal growth velocity in the early third trimester. FGV_L: fetal growth velocity in the latter third trimester.

[a] r^2 : model r^2. [b] cbinsulin log transformed for analysis.