Relationship between maternal bone biomarkers and fetal adiposity through normal pregnancy

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Abstract

Purpose

To examine the association of maternal bone markers (sclerostin, sRANKL, osteocalcin, $25OHD_3$) with fetal intra-abdominal and subcutaneous adipose tissue deposition and birthweight during normal pregnancy.

Methods

One hundred pregnant women (aged 30.4±5.6 years, mean±SD) with pre-pregnancy BMI=24.1±4.6 kg/m² were seen prospectively during each trimester. At each visit they were submitted to anthropometric measurements, a fasting blood sampling, a 75gr oral glucose tolerance test (OGTT) and a fetal ultrasonogram. At birth, neonates had birth weight measurement.

Results

In the 2nd trimester maternal sclerostin concentrations correlated positively with fetal abdominal circumference and birth weight; maternal sRANKL concentrations correlated positively with fetal abdominal subcutaneous fat thickness, sagittal abdominal diameter and abdominal circumference. Fetuses born to mothers with greater (>254 ng/mL) compared to fetuses born to mothers with lower (≤254 ng/mL) sRANKL concentrations had greater abdominal circumference, sagittal diameter and abdominal subcutaneous fat thickness. Maternal serum sclerostin concentrations were the best positive predictors of birth weight. In the 3rd trimester maternal sclerostin concentrations correlated positively with fetal sagittal abdominal diameter; maternal sRANKL concentrations positively correlated with fetal abdominal circumference and fetal abdominal sagittal diameter.

Conclusions

Maternal bone markers sclerostin and sRANKL may relate with fetal intra-abdominal adipose tissue deposition through direct or indirect unknown as yet mechanisms contributing thus, to birthweight.

keywords: sclerostin, sRANKL, birthweight, fetal intra-abdominal fat, pregnancy

Introduction

Pregnancy is characterized by insulin resistance (particularly in the second and third trimesters) which facilitates the transfer of maternal glucose into the fetus. In the past, we and others have shown that during normal pregnancy of nonobese women there is a progressive increase of insulin resistance together with an increase of first and second phase insulin secretion (1,2,3). Transfer of glucose from the maternal circulation into the fetal is one of the main mechanisms determining fetal growth and fat mass distribution. Higher maternal glucose concentrations may lead to increased fetal adipose growth and fetal abdominal adipose tissue deposition (4). Fetal abdominal fat deposition, is evaluated by ultrasound (U/S) measured fetal abdominal circumference and sagittal diameter. The latter is a good measure of intra-abdominal fat as well as metabolic risk as we have shown (5, 6). Furthermore, studies have found U/S measured subcutaneous tissue thickness to correlate positively with postnatal caliper skinfold measurements as well as birth weight (7). Thus, it seems that U/S measured fetal subcutaneous tissue thickness is a reliable method to determine fetal subcutaneous fat thickness (8, 9). Overall, maternal metabolism and several maternal endocrine organs such as pancreas, placenta and adipose tissue interfere directly and/or indirectly with fetal growth (10, 11, 12) while birthweight and neonatal body fat mass distribution are determinants of metabolic disorders during adult life (13, 14).

Bone metabolism is characterized by a highly coordinated process responsible for bone resorption and formation, which is necessary to repair damaged bone and to maintain mineral homeostasis. Maternal bone turnover markers such as sclerostin, soluble receptor activator of nuclear factor- kappa B ligand (sRANKL), and osteocalcin participate in this process. Sclerostin has anti-anabolic effects on bone formation (15). Study in mice shows that sclerostin exerts an endocrine action influencing body composition and white adipose tissue by regulating catabolic and anabolic metabolism in adipocytes (16). Furthermore, sRANKL

is involved in the bone resorption process. Age-related bone marrow adipogenesis is linked to increased expression of sRANKL in bone marrow cells at the pre-adipocyte stage (17). Recently Matsuo et al. have shown sRANKL to increase energy expenditure by inducing beige adipocyte differentiation in preadipocytes (18). In addition, in mice maternal oral administration of uncarboxylated osteocalcin protects from high fat- and high sucrose- fed female offspring from metabolic disorders, such as adipose tissue inflammation and body weight, induced by maternal obesity (19). Furthermore, in a prospective human study of 922 mother-child pairs, authors found that 10 nmol/L increase in maternal 25-hydroxyvitamin D concentration at 15 weeks gestation was associated with a 2% decrease in offspring percentage body fat but not with child BMI z-score at the age 5-6 years old (20)

Changes in maternal bone density and markers during pregnancy suggest that the latter is associated with deterioration of maternal bone mass (21). Indeed, increased maternal osteoclast activity during pregnancy has been suggested to facilitate the building of fetal bones (22). However, the relationship of maternal bone markers such as sclerostin, sRANKL, osteocalcin, 25OHD3 with fetal intra-abdominal and subcutaneous adipose tissue deposition as well as birthweight have not been fully studied in humans during pregnancy.

The aim of this study was to examine the association of maternal bone markers such as sclerostin, sRANKL, osteocalcin and $25OHD_3$ with fetal intra-abdominal and subcutaneous adipose tissue deposition as well as birthweight during normal pregnancy.

Subjects and Methods

Subjects

One hundred and five primigravidae women of Caucasian origin (age range of 20-38 years old) and no medical history of obesity or diabetes, were recruited randomly with no preference of fetal sex, during the 1st trimester of pregnancy in an antenatal outpatient clinic of Aretaieion University Hospital between May 2016 and December 2018. To avoid bias,

women were recruited based on a computer software random numbers generator. *A posteriori*, one hundred women (age, mean±SD: 30.4±5.6 years; pre-pregnancy BMI: 24.1±4.6kg/m²) were included in the study protocol due to the exclusion of five women who developed gestational diabetes mellitus (GDM). Exclusion criteria included: non-caucasian origin (in an effort to study a homogeneous population regarding insulin resistance traits); pre-pregnancy BMI>30 kg/m^2; history of type 1 or type 2 diabetes mellitus, and glucose intolerance; presence of GDM; multiple pregnancy; major vaginal bleeding; hypertension; preeclampsia; urinary tract infection; fetal-placental abnormalities, such as congenital anomalies; placenta previa; placental abruption; nephropathy; liver disease; current smoking; alcohol intake. Babies born small (SGA, birth weight below the 10th percentile for gestational age) or large (LGA, birth weight above the 90th percentile for gestational age) for gestational age (23) or premature were also excluded. The study was approved by the institutional ethical committee, functioning according to the 4th edition of the Guidelines on the Practice of Ethical Committees in Medical Research issued by the Royal College of Physicians of London (Royal College of Physicians, 2007). Written consent has been obtained from each pregnant woman after full explanation of the purpose and nature of all procedures used.

Protocol

Women were seen once during each of the three trimesters of their pregnancy between 10-12th, 24th-26th and 34th-36th week of gestation, respectively. Pregnant women had a basic dietetic advice at the beginning without regular dietetic follow up. At each visit they were submitted to anthropometric measurements; a fasting blood sampling at 8 am for measurement of bone metabolism markers (sclerostin, sRANKL, osteocalcin, vitamin D); a 75gr oral glucose tolerance test (OGTT) with blood samples drawn at 0, 30, 60, 90 and 120 min time-points for measurement of glucose and insulin concentrations and a fetal

Ultrasound. Diagnosis of GDM was based on the OGTT according to the diagnostic criteria set by WHO (24). Five of the recruited women, as mentioned earlier, were diagnosed with GDM and were excluded from the study. Age and pre-pregnancy BMI of the remaining one hundred women who participated in the study were 30.4±5.6 years and 24.1±4.6kg/m^2, respectively. Pregnancies gave birth to 52 and 48 male and female neonates, respectively. Mean gestational age at birth (in gestational weeks) was 39th week**.** At birth, neonates were submitted to birth weight measurement while at the third post-partum day they were submitted to waist measurement. Blood samples for measurement of bone markers were collected in tubes with EDTA. After blood collection, Millipore's serine protease inhibitor was added. Tubes were inverted several times to mix and they were centrifuged immediately. After centrifugation plasma was collected, aliquoted and stored at –70° C until assayed.

Indices of carbohydrate metabolism

Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR= [insulin at baseline, pmol/L x glucose at baseline, mmol/L]/135) (25, 26)

Anthropometric measurements

All measurements of pregnant women were carried out by a single observer. For all women pre-pregnancy weight was measured to the nearest 0.1 kg using a beam balance, while height was measured to the nearest mm using a stadiometer. At each visit, weight without shoes and light clothing was measured and BMI in kg/m^2 was calculated**.** Birthweight was measured in kilograms with a portable digital electronic scale (Seca GMBH and Co. kg Germany, model 834) accurate to the nearest 10 gr, without clothing or diapers. Neonatal waist was measured at third post-partum day using a measuring tape over the iliac crest.

Fetal ultrasound measurements

During U/S fetal measurements (estimated weight, abdominal circumference, head circumference, biparietal diameter) were recorded by a single observer. Ultrasound measured abdominal sagittal diameter was estimated by the anteroposterior diameter of the fetus at the umbilical level. Abdominal subcutaneous fat thickness was evaluated by measuring the thickness of the anterior abdominal subcutaneous tissue on the same axial image as that used for abdominal circumference measurement. Ultrasonograms were performed by employing a Philips HD11 ultrasonographer.

Blood chemistry and hormone assays

Blood chemistry including measurements of serum glucose as well as insulin, osteocalcin and $25OHD_3$ concentrations were performed using the Cobas 501 and Cobas e411 analyzers (Roche Diagnostics, Basel, CH). sRANKL was performed with immunoenzymatic assay (ELISA) (BioVendor - Laboratorni medicina a.s. Brno, Czech Republic) with intra- and inter- assay CVs ranging from 7.2-11.5% and 11.2-12.8%, respectively. Sclerostin was measured by ELISA (R&D Systems, Inc. Minneapolis, MN , USA) with intra- and inter- assay CVs ranging between 1.8-2.1% and 8.2-10.8%, respectively.

Statistical analysis

Data are presented as mean ± SD or median and interquartile range (25^{th}-75^{th} percentile) for data normally and not normally distributed, respectively. To test the change of each variable during the three trimesters of pregnancy the one-way repeated measures ANOVA test was used in case of normally distributed variables and the non parametric Friedman ANOVA test in case of non-normally distributed variables. To test for correlations between different variables the Spearman correlation analysis was performed. Stepwise multiple regression analysis was undertaken to define second and 3^{rd} trimester predictive

variables. A *p-value* of <0.05 was considered to be significant. The SPSS 21 statistical software was used for statistical analysis (27).

Results

1. Change of maternal and fetal anthropometric variables, maternal bone metabolism variables and carbohydrate variables during pregnancy (Table 1).

Maternal weight values increased significantly from the 1st to the 2nd and 3rd trimesters ($p<0.05$) with a median increase of 14.5kg between 1st and 3rd trimester. Ultrasound fetal measurements of estimated weight, abdominal circumference, sagittal diameter and subcutaneous fat thickness, head circumference and biparietal diameter increased significantly from 2nd to 3rd trimester ($p<0.05$). From the 1st to the 2nd to the 3rd trimester, maternal HOMA-IR increased significantly ($p<0.05$). Maternal fasting sclerostin and sRANKL concentrations decreased significantly from the 1st to the 2nd trimester ($p=0.01$ and $p=0.041$, respectively) and remained statistically unchanged between the 2nd to the 3rd trimester ($p>0.05$) (Table 1).

2. Correlations of maternal bone markers with fetal and neonatal anthropometric variables at each trimester (Table 2).

In the 2nd trimester maternal sclerostin concentrations correlated positively with U/S measured fetal abdominal circumference ($r=0.361$, $p=0.03$) and birth weight ($r=0.632$, $p<0.001$) (Figure 1) measurements; maternal sRANKL concentrations correlated positively with U/S measured fetal abdominal subcutaneous fat thickness ($r=0.357$, $p=0.034$), sagittal diameter ($r=0.502$, $p=0.004$) and circumference ($r=0.416$, $p=0.015$) measurements; maternal $25OHD_3$ concentrations correlated positively with U/S measured fetal abdominal subcutaneous fat thickness ($r=0.287$, $p=0.032$) and neonatal abdominal circumference ($r=0.344$, $p=0.407$) measurements at birth.

In the 3rd trimester maternal sclerostin concentrations correlated positively with U/S measured abdominal diameter (r=0.763, p=0.019) measurements; maternal sRANKL concentrations positively correlated with U/S measured fetal abdominal circumference (r=0.328, p=0.044) and sagittal abdominal diameter (r=0.402, p=0.038) measurements.

3. **Comparison of U/S fetal adiposity markers divided according to higher and lower maternal sRANKL concentrations through the 2nd trimester (Table 3)**

Ultrasonography fetal abdominal adiposity markers were compared in the 2nd trimester between women with sRANKL circulating concentrations greater and lower than 254 ng/ml in the same trimester, respectively. Value 254 ng/ml was the median value of circulating maternal sRANKL concentrations in the 2nd trimester. It was chosen arbitrarily to distinguish the 50% of greater concentrations (n=50) from the 50% of lower concentrations (n=50). Mothers with greater sRANKL concentrations (sRANKL>254 ng/mL) carried fetuses with greater U/S measured abdominal circumference (p<0.003), U/S measured sagittal diameter (p<0.001) and subcutaneous abdominal thickness (p<0.0002) compared to fetuses of mothers with lower sRANKL concentrations (sRANKL ≤254 ng/mL) (Table 3).

4. Predictors

Best maternal bone marker predictors of birthweight: In the 2nd trimester, multiple regression analysis showed that maternal serum sclerostin concentrations were the best positive predictors of birthweight (dependent variable) (p=0.003, standardized coefficient beta=0.568, adjusted R^2=0.264) in a model that included maternal BMI, HOMAR values, sRANKL, sclerostin, 25OHD3 and osteocalcin concentrations (all taken as independent variables) (Table 4). The results of the multivariate regression model between birthweight values and maternal 2nd trimester sclerostin concentrations did not change when the model was corrected either for 2nd trimester insulin concentrations or gestational age or sex of the infant.

Discussion

In a cohort of non-obese, non-diabetic pregnant women studied during the three trimesters of pregnancy, we found that, in the 2nd trimester, maternal sclerostin concentrations correlated positively with birthweight and were its best positive predictors, among the rest of maternal bone markers studied and HOMA-IR values, an insulin resistance marker. Furthermore, maternal sclerostin concentrations in the 2nd and 3rd trimesters correlated positively with U/S fetal intra-abdominal fat measurements (abdominal circumference and sagittal diameter, respectively). It is not clear as yet whether maternal sclerostin communicates with the human fetal compartment and modulates fetal adiposity in a direct or indirect fashion (21). In a recent study, in 1,325 children and adolescents, sclerostin concentrations were 29.3% greater in obese than in normal-weight children and correlated positively with BMI SDS and waist-to-hip ratio SDS as well as area-under-the-curve (AUC) insulin/AUC glucose (28). Furthermore, in postmenopausal women serum sclerostin concentrations correlate positively with percentages of abdominal and gynoid fat (29). Sclerostin is secreted by osteocytes and some chondrocytes, inhibits bone formation by osteoblasts and has anti-anabolic effect on bone formation *via* inhibition of the *Wnt* signaling pathway (30). However, recently, sclerostin has emerged as an endocrine factor regulating fat metabolism. Recently when mice which overproduce sclerostin, as a result of adeno-associated virus expression in their liver, were fed a high-fat diet, fat mass, serum insulin concentrations, and pancreatic β-cell area per tissue area and islet size were increased as compared to control wild animals (31). In that study it was shown that sclerostin promotes differentiation of precursor cells into white adipocytes and negatively regulates browning of white adipocytes into beige adipocytes. Thus, the authors concluded that sclerostin, in mice, regulates adipocyte hypertrophy while it is not produced by adipocytes. Indeed, in mice, sclerostin modulates bone marrow adipose tissue differentiation by inhibiting Wnt signaling

in pre-adipocytes and by promoting thus, adipose tissue formation in the bone marrow (32). Although there are already experimental and clinical studies showing the positive association between sclerostin and adipose tissue, in the present study, for the first time to our knowledge, an endocrine effect of maternal sclerostin into fetal intra-abdominal adipose tissue deposition during pregnancy is suggested.

Maternal sRANKL is a regulator of osteoclastogenesis and an osteoclast activator (17). In this study maternal sRANKL concentrations were lower in the 2nd than the 1st and 3rd trimesters. Interestingly, in this study, maternal sRANKL concentrations correlated positively with U/S measured intra-abdominal fetal fat markers such as fetal abdominal circumference, sagittal diameter and subcutaneous fat thickness measurements during the 2nd and 3rd trimesters of pregnancy. Furthermore, in the 2nd trimester, fetuses characterized by increased maternal sRANKL serum concentrations were found to have increased U/S measured abdominal circumference and sagittal diameter measurements, markers of fetal intra-abdominal fat deposition (5, 6). Research in mice revealed that there is a communication regarding sRANKL passage between fetal and maternal compartments promoting maternal-fetal tolerance (33). In addition, studies in pups have demonstrated that the fetal phenotype associated with sRANKL invalidation is attenuated through compensation by maternal sRANKL suggesting that the latter could compensate into fetal physiology (34). This molecule has also been associated with the regulation of glucose metabolism. Indeed, inhibition of sRANKL signaling has been suggested to improve hepatic insulin sensitivity and to have a role in ß cells replication in mouse models (35) while in patients with osteoporosis, its inhibition improves muscle strength and insulin sensitivity (36). Thus, it appears that sRANKL contributes to the development of insulin resistance which may contribute indirectly to deposition of adipose tissue. Taken together, it would appear that as maternal sRANKL passes into the fetal circulation this could lead to fetal fat deposition for

unknown pathophysiological reasons yet. In this study, sRANKL does associate with fetal adiposity markers in the 2nd trimester but not with final birthweight The exact mechanisms of physiology connecting maternal bone markers with fetal adiposity is not known as yet. Fetal weight depends on several other than fetal adiposity factors, either endogenous or exogenous, especially at the 3rd trimester. In addition, neonatal body composition consists of different tissues, the growth of which depends on different physiologic factors.

In summary, although the exact pathophysiological mechanism is not yet clear, it appears that maternal bone markers sclerostin and sRANKL are endocrine factors associated directly or indirectly in fetal intra-abdominal adipose tissue deposition, during pregnancy. Maternal sclerostin has at yet not been proven to pass into fetal circulation but it seems that it is a strong predictor of birthweight independent of maternal insulin resistance. It seems that in pregnancy, maternal bone metabolism is associated with fetal adipose tissue deposition during the process of fetal growth. Regarding the limitations of the study, it would be desirable to have studied pregnancies in bigger numbers from a different ethnic and racial spectrum. The results of the study apply to a Caucasian origin population in normal pregnancy. Generalized conclusions regarding different ethnic groups or pregnancies with gestational diabetes or obesity cannot yet be drawn. Another limitation is the lack of neonatal body fat composition at birth.

In conclusion, it appears that the studied maternal bone markers sclerostin and sRANKL during pregnancies of non-obese, non-diabetic women may modulate fetal intra-abdominal adipose tissue deposition and birthweight through direct or indirect unknown as yet mechanisms. Maternal bone seems to be an important organ into fetal intra-abdominal adipose tissue development and growth *via* mechanisms not fully understood yet. In accordance to the previous findings maternal bone metabolism could be an important factor contributing to fetal adipose tissue deposition and thus affecting possibly the process of

endometrial programming during fetal growth in pregnancy. The future direction would be to conduct human prospective multinational multicenter studies focusing into the effects of maternal bone metabolism markers, such as sclerostin and sRANKL, into fetal adipose tissue deposition, neonatal body composition and beyond. In addition, studies should look into fetal bone metabolism and fetal adipose tissue metabolism in normal and complicated (i.e. with gestational diabetes or obesity) pregnancies. Furthermore, it would be interesting to conduct translational research studies to unravel mechanisms of physiology relating maternal bone metabolism with fetal adipose tissue deposition.

Data Availability: Some or all dataset generated or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request**.**

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Author contribution paragraph: G.V. developed the concept, interpreted data, wrote and revised the manuscript; D.M., S.K. and A.B. researched data; C.S. and I.P. measured hormones, T.M.B. and S.K. interpreted data and revised the manuscript. G.F. performed ultrasonograms and N.V. revised the manuscript; G.M. interpreted data and revised the manuscript

Table 1: Maternal anthropometric, hormone, and metabolic variables during pregnancy. Variables are expressed as mean (±SD) or median (25th–75th interquartile range); the asterisk (*) denotes statistically significant difference from the 1st trimester (P<0.05); (#) denotes statistically significant difference (P<0.05) from the 2nd trimester.

N=100			
MATERNAL VALUES			
Pre-pregnancy BMI (kg/m²)	24.1±4.6		
Age (years)	30.4± 5.6		
PREGNANCY	**1st trimester**	**2nd trimester**	**3rd trimester**
Weight (kg)	67.3 (±13.8)	73.7 (±14.8)*	79.5 (±16.6) *#
Glucose (mg/dL)	79.3 (±9.5)	77.6 (±8.9)	74.2 (±3.9)
Insulin (µIU/mL)	4.2 (2.3-6.5)	6 (3.2-11.3)*	7.6 (4.1-12.8) *#
HOMA-IR	0.9(0.5-1.4)	1.2(0.7-2.5)*	1.4 (0.8-3.6) *#
25OHD3 (ng/mL)	13.3 (8.8-26.2)	14.3 (7.4-21.9)	13.7 (3.9-28.9)
Osteocalcin (µg/L)	14 (10.7-19.4)	11.7 (8.8-14.6)	18.9(16.9-32.5)#
sRANKL (ng/mL)	292.4 (214.4-582.5)	254.1 (163-441.2)*	271.2 (177.9-530.5)
Sclerostin (ng/mL)	58.4 (35.5-94)	39.9 (25.7-61.6)*	35.5(27.3-66.5)
FETAL U/S VALUES			
Abdominal circumference (mm)		179.6 (±16.5)	305.8 (±35.5)#
Sagittal abdominal diameter (mm)		53.4 (±5.1)	97.3 (±9.5) #
Subcutaneous depth thickness (mm)		2.7 (±0.8)	4 (±0.8) #
Head circumference (mm)		218.6 (±20.7)	308.5 (±14.9)#
Estimated weight (gr)		650 (503–815)	2270 (1800–2413)#

Biparietal diameter (mm)		58.6 (±5.1)	84.3 (±5.4) [#]
NEONATAL Anthropometry			
Gestational age (week)	39th		
Birthweight (g)	3201.7 (2075-4470)		
Birth waist (cm)	29.7 (±3)		

Table 2: Correlations (r-values) of maternal sclerostin, sRANKL and $25OHD_3$ concentrations with fetal U/S abdominal (circumference, saggital diameter, subcutaneous fat thickness) and neonatal (birthweight, birth waist) anthropometric variables in the 2nd and 3rd trimesters. Statistical significance was set at $p<0.05$. (r and p of significant correlations are in bold characters).

	sclerostin	**sRANKL**	**25OHD3**	**osteocalcin**
2nd trimester				
Abdominal circumference	**r=0.361** **p=0.032**	**r=0.416** **p=0.015**	r=0.137 p=0.248	r=0.106 p=0.300
Sagittal diameter	r=0.199 p=0.160	**r=0.502** **p=0.004**	r=0.122 p=0.271	r=0.103 p=0.304
Subcutaneous fat thickness	r=-0.122 p=0.272	**r=0.357** **p=0.034**	r=0.291 p=0.070	**r=0.327** **p=0.048**
birthweight	**r=0.632** **p=0.000**	r=-0.252 p=0.112	r=0.066 p=0.376	r=0.235 p=0.129
Birth waist circumference	r=0.308 p=0.142	r=0.219 p=0.226	r=0.344 p=0.070	r=-0.009 p=0.488
3rd trimester				
Abdominal circumference	r=-0.500 p=0.196	r=-0.328 p=0.052	r=0.000 p=0.500	r=-0.700 p=0.094
Sagittal diameter	**r=-0.763** **p=0.019**	r=-0.402 p=0.019	r=-0.100 p=0.446	r=-0.500 p=0.196
Subcutaneous fat thickness	r=-0.400 p=0.252	r=-0.100 p=0.436	r=-0.600 p=0.142	r=-0.100 p=0.436

birthweight	r=-0.100 p=0.436	r=-0.600 p=0.142	r=0.100 p=0.436	r=-0.600 p=0.142
Birth waist circumference	r=-0.600 p=0.200	r=-0.600 p=0.200	r=0.200 p=0.400	r=-0.800 p=0.100

Table 3: Comparison of fetal abdominal adiposity markers by ultrasonography (U/S) in the 2nd trimester between women with sRANKL circulating concentrations greater and lower than 254 ng/ml in the same trimester, respectively. Value 254 ng/ml was the median value of circulating maternal sRANKL concentrations in the 2nd trimester. It was chosen arbitrarily to distinguish the 50% of greater concentrations (n=50) from the 50% of lower concentrations (n=50). The asterisk (*) denotes statistically significant difference at the level of $p<0.05$.

Fetal U/S abdominal adiposity markers	**Maternal sRANKL ≤254 ng/mL**	**Maternal sRANKL >254 ng/mL**
circumference (mm)	169.2±15.1	189.1±14.2*
sagittal diameter (mm)	51.2±4.9	56.3±5.2*
subcutaneous fat thickness (mm)	2.3±0.9	3.1±0.9*

Table 4: In the 2nd trimester, multiple regression analysis showed that maternal serum sclerostin concentrations were the best positive predictors of birthweight (dependent variable) (p=0.003, beta=0.568, adjusted R^2=0.264) in a model that included maternal sclerostin, 25OHD3, sRANKL and osteocalcin concentrations, and BMI and HOMAR values (all taken as independent variables)

Model	Standardized coefficient beta	Significance p-value
sclerostin	0.568	**0.003**
25OHD3	0.068	0.725
sRANKL	-0.273	0.105
osteocalcin	-0.298	0.176
BMI	-0.049	0.809
HOMAR	0.274	0.173

Legend to the Figure 1

Figure 1: Correlation between maternal sclerostin concentrations at the 2nd trimester and birthweight (r=0.632, p=0.000)

Figure 1