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# Neonatal birth waist is positively predicted by second trimester maternal active ghrelin, a pro-appetite hormone, and negatively associated with third trimester maternal leptin, a pro-satiety hormone

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ABSTRACT

*Introduction:* In pregnancy physiological mechanisms activated by maternal appetite contribute to adequate energy intake for the mother and for the fetus. The role of maternal appetite-related peptides and their possible association with neonatal energy stores and glucose metabolism have not been investigated as yet. The aim was to investigate, during pregnancy, the association of fasting maternal appetite-related hormones levels [ghrelin (active), GLP1 (active), total PYY and leptin] with neonatal waist, percent total body fat and insulin levels at birth.
*Methods:* Forty-two normal and thirty eight overweight women (mean ± SD; age: 26.9 ± 2.5 years; pre-pregnancy BMI 26 ± 2.2 kg/m$^2$) were seen during each of the three trimesters, had blood sampling and a 75 g oral glucose tolerance test. At birth, neonates underwent anthropometry and cord blood sampling for c-peptide, glucose, insulin.
*Results:* During all three trimesters maternal weight correlated positively with percent total neonatal body fat while during the second and third trimesters it correlated positively with birth weight. The second trimester maternal active ghrelin levels correlated positively with neonatal waist and were its best positive predictor. The third trimester maternal active ghrelin levels correlated positively with neonatal waist and negatively with percent total neonatal body fat, fetal cord blood insulin levels and were the best negative predictor of the latter. The third trimester maternal leptin levels correlated negatively with neonatal waist.
*Conclusions:* During pregnancy circulating maternal active ghrelin, a pro-appetite hormone, is associated with neonatal visceral energy storage (as expressed by neonatal waist). By inhibiting glucose-driven maternal insulin secretion, ghrelin might ensure adequate fasting glucose and nutrient supplies to the fetus while limiting overall fetal adipose tissue deposition.



## 1. Introduction

Maternal metabolism during pregnancy affects directly and/or indirectly birth weight and neonatal metabolism [1–3]. Physiological mechanisms activated by maternal appetite contribute to adequate energy intake for the mother and, indirectly, for the fetus [4,5]. Ghrelin, GLP-1 and PYY are gut-secreted peptides characterized by their orexigenic (ghrelin) or by their anorexigenic [PYY, GLP-1] effects [6,7]. In addition to active ghrelin, the adipocytokine leptin (which at high levels produces satiation) is an essential element of the appetite control system. They both "inform" the corresponding hypothalamic centers about the nutritional status and the level of energy storage [8–10]. Ghrelin exists in two forms: active or acyl ghrelin, which activates its growth hormone secretagogue receptor (GHSR), and to inactive ghrelin. Ghrelin is orexigenic when administered centrally or peripherally and is inversely correlated with leptin levels [11,12]. GHSR is present in the arcuate and ventromedial hypothalamic nuclei suggesting its central mediation that results in positive energy balance via food intake increase [13,14]. Negative energy balance is associated with increase of plasma ghrelin, of hypothalamic AMPK and of food intake whereas leptin leads to suppressed hypothalamic AMPK activity and contributes to restriction of food intake [15]. On the other hand, ghrelin is expressed in pancreatic cells and inhibits glucose-driven insulin release in mice, rats and humans thus resulting in increased circulating glucose levels [16,17]. In addition, studies in pregnant rats showed maternal active ghrelin to play an important role in rat fetal development [18,19]. In human pregnancy the role of these appetite-related peptides and their

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possible association with neonatal energy stores and glucose metabolism has not as yet been investigated.

Neonatal waist circumference, a measure of liver volume and visceral adiposity, reflects the adipose-energy deposits of the neonate and is predictive of its future growth and metabolic health [20–22]. Waist circumference in newborns has been used in past studies in the literature as a measure of visceral fat deposition [23,24]. The validity of this technique as a measure of visceral adiposity in the newborn is highly reliable [25]. In the Bogalusa Heart Study in children aged 5 to 17 years, abdominal fat distribution as indicated by waist circumference was associated with adverse concentrations of triacylglycerol, HDL cholesterol, LDL cholesterol and insulin [26]. However, little is known about the physiological significance of waist circumference at birth. Suboptimal nutrition at early stages of gestation has been linked to adverse effects on fetal growth [27].

Pregnancy is a state of physiological hyperphagia which is maintained, despite progressive maternal weight gain, only during its duration. In addition maternal appetite during pregnancy is a signal for acquisition of the necessary nutrients for maternal and fetal metabolism and could be crucial for the neonatal fat mass, birth weight and energy deposits (as expressed by waist circumference), glucose metabolism and its future metabolic development. This study aimed at investigating whether in normal weight and overweight, non-diabetic pregnancies studied longitudinally during the three trimesters of pregnancy the maternal fasting levels of appetite- related gut-derived hormones ghrelin (active), GLP1(active), total PYY and adipocytokine leptin could be associated with neonatal waist circumference and percent total body fat as well as insulin levels at birth.

## 2. Materials and methods

### 2.1. Participants

The investigation was approved by the Local Ethics Committee, functioning according to the 3rd edition of the Guidelines on the Practice of Ethics Committees in Medical Research issued by the Royal College of Physicians of London. Consent was obtained from each patient after full explanation of the purpose and nature of all procedures used. Eighty-five pregnant primigravidae Caucasian women (mean ± SD; age: 26.7 ± 2.3 years; pre-pregnancy BMI 26.5 ± 2.4 kg/m$^2$) were recruited during the first trimester of pregnancy from an Obstetrics and Gynecology outpatient clinic of a university hospital between January 2011 and September 2012. Exclusion criteria included non-Caucasian origin, BMI > 30 kg/m$^2$ before pregnancy, history of type 1 or type 2 diabetes mellitus or gestational diabetes (GDM), multiple pregnancy, major vaginal bleeding, hypertension, preeclampsia, urinary tract infection, fetal–placental abnormalities such as congenital anomalies, placenta previa, placental abruption, remarkable previous medical, surgical and gynecological history and current smoking or alcohol intake. To avoid bias, women were recruited based on a computer software random number generator.

### 2.2. Protocol

The women were seen once during each of the three trimesters of their pregnancy in the 10th–12th, 24th–26th and 34th–36th week. Pregnant women received basic dietetic advice at the beginning without regular dietetic follow-up. At each visit they were submitted to anthropometric measurements, a fasting blood sampling for measurement of hormones (active ghrelin; total PYY; active GLP-1, insulin, leptin) and a 75 g 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 levels. Diagnosis of GDM was based on the OGTT according to the diagnostic criteria proposed in the HAPO study [28]. Five of the recruited women were diagnosed with GDM and were excluded from the study. Age and BMI of the remaining 80 women who participated in the study were 26.9 ± 2.5 years and 26 ± 2.2 kg/m$^2$ (38 overweight 25 < BMI < 30 kg/m$^2$ and 42 normal weight BMI ≤ 25 kg/m$^2$) respectively (Table 1). At birth, neonates were submitted to anthropometric measurements while cord blood was sampled for c-peptide, glucose and insulin measurements. On the third postnatal day neonates were submitted to skinfold measurements. Skinfold measurements were taken three days after birth by a specialist neonatologist so that subcutaneous edemas presented usually at birth are resolved. Blood samples for measurement of hormones were collected in tubes with EDTA as anticoagulant. After blood collection, Millipore's serine protease inhibitor for active ghrelin was added. Tubes were inverted several times to mix and they were centrifuged immediately. Following centrifugation plasma was collected, aliquoted and stored at −70 °C until assayed.

### 2.3. Anthropometric measurements

All measurements of pregnant women were carried out by the same investigator. For all women weight before pregnancy was retrieved from their records and height was measured to the nearest mm using a stadiometer. At each visit weight without shoes with light clothing was measured in kilograms to the nearest 0.1 kg on a beam balance and BMI was calculated. At birth, weight and waist circumference of the neonates were measured by a single observer–neonatologist (Table 2). Birth weight was measured in kilograms with a portable digital electronic scale (seca GmbH and Co. KG Germany, model 834) accurate to the nearest 10 g, without clothing or diapers. Waist circumference was measured with an inextensible tape measure (in mm) midway between the costal margin and the iliac crest, during expiration [24,29]. On the 3rd day after delivery triceps, biceps, suprailiac, and subscapular skinfold thickness (SFTs) of the neonate were measured in triplicate on the left side of the body under standard conditions by using a standard skinfold caliper (Holtain Ltd, Croswell, Crymych, United Kingdom) operated with a constant pressure of 10 g/mm$^2$. While the neonate was supine and the arm was slightly abducted and extended, biceps SFT was measured 1 cm proximal to the skin crease of the elbow. Next the newborn was turned onto the right side. Triceps SFT was then measured parallel to the long axis of the arm midway between the acromion and the olecranon, with the arm slightly flexed. Suprailiac SFT was carefully measured along the midaxillary line just above the iliac crest. The subscapular SFT was measured below the inferior angle of the left scapula at a diagonal in the natural cleavage of the skin. The caliper was left in place until a constant reading was obtained. Triplicate measurements were performed, and the mean was calculated. All measurements were made by the same observer. To estimate the percent body fat the equations proposed by Slaughter et al. for males [1.21 × (triceps + subscapular) − 0.008 × (triceps + subscapular)$^2$ − 1.7] and females [1.33 × (triceps + subscapular) − 0.013 × (triceps + subscapular)$^2$ − 2.5] and validated in newborns by Schmelzle et al. were employed [30,31].

### 2.4. Blood chemistry and hormone assays

All measurements were performed in maternal and cord blood plasma. Glucose levels were measured with the Siemens Advia 1800 Clinical Chemistry System (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Insulin levels were measured with an electrochemiluminescence immunoassay with the Cobas e411 immunochemistry analyzer (Roche Diagnostics, Basel, CH) with intra- and inter-assay coefficients of variation (CV) at 2.0% and 2.8%, respectively, and sensitivity limit (SL) at 0.2 mIU/L. Cord blood C-peptide levels were measured with a solid phase two-site chemiluminescent immunometric assay, using the Immulite 2000 Chemiluminescence autoanalyzer (Siemens Healthcare Diagnostics, Los Angeles, CA., USA) with intra- and inter-assay CVs at 3.5% and 6.2%, respectively, and SL at 0.09 ng/mL. Active ghrelin, total

**Table 1**
Maternal anthropometric, hormone and metabolic variables during pregnancy. Variables expressed as mean (SD) or median (25th–75th interquartile range).

| N | 80 | | |
|---|---|---|---|
| BMI before pregnancy (kg/m$^2$) | 26 ± 2.2 | | |
| Pregnancy | 1st trimester | 2nd trimester | 3rd trimester |
| Maternal weight (kg) | 68.2 (13.3) | 75 (12.4)* | 81.9 (14.8)*,# |
| Fasting active ghrelin (pg/mL) | 33.1 (19–42.3) | 41.9 (29–50.9)* | 43.8 (38.2–55)* |
| Fasting total PYY (pg/mL) | 92.1 (67.4–126.6) | 95.3 (72.3–140.1) | 97 (93–119.2) |
| Fasting active GLP-1 (pg/mL) | 154.7 (83.5–212) | 170.3 (127.2–206) | 208.3 (169–245.5)*,# |
| Leptin (pg/L) | 27.9 (19.6–52.8) | 46.6 (29.5–72.1) | 56 (40–63) |
| Fasting glucose (mmol/L) [mg/dL] | 4.2 (0.4) [75.6 (7.2)] | 4.5 (0.3) [81 (5.4)] | 4.6 (0.4) [82.8 (7.2)] |
| Fasting insulin (pmol/L) [uU/mL] | 55.3 (41.5–70) [7.7 (5.7–9.75)] | 58 (48–120.3) [8.1 (6.7–16.7)] | 120 (92–152)*, # [16.7 (12.8–21.2)]*, # |
| HOMA-R | 1.5 (1.2–2.2) | 1.9 (1.8–3.8) | 4.2 (3–5.1)*, # |
| 1st phase insulin secretion | 1202 (870–1454) | 1397 (1198–1595)* | 3685.4 (2735–4834)* |
| 2nd phase insulin secretion | 463 (343–595) | 628 (477.6–786)* | 989.4 (823.2–1012.5)* |

* Denotes statistically significant change ($p < 0.05$) from 1st trimester.
\# Denotes statistically significant change ($p < 0.05$) from 2nd trimester.

PYY and active GLP-1, after aprotinin were added to all samples in order to prevent proteolysis, were measured in plasma simultaneously with a multiplex assay (MILLIPLEX® Human Metabolic Hormone Panel, Millipore Corp., St. Charles, Missouri, USA) on the Luminex-100 Integrated System (Luminex Corporation, Austin, TX, USA) with intra- and inter-assay CVs at less than 11% and 19%, respectively, according to the manufacturer's instructions.

### 2.5. Indices of carbohydrate metabolism

Insulin resistance was estimated by the homeostasis model assessment (HOMA-R = [insulin at baseline, pmol/L × glucose at baseline, mmol/L]/135) [32,33]. Beta cell secretion of insulin was estimated by the following indices [34]: Predicted index of first phase of insulin secretion (1st PHIS) = 1283 + [1.289 × insulin at 30 min (pmol/L)] − [138.7× glucose at 30 min (mmol/L)] + [3.772 × insulin at baseline (pmol/L)], and predicted index of the second phase of insulin secretion (2nd PHIS) = 287 + [0.4164 × insulin at 30 min (pmol/L)] − [26.07× glucose at 30 min (mmol/L)] + [0.9226 × insulin at baseline (pmol/L)].

### 2.6. Statistical analysis

Data are described as mean ± SD or median and interquartile range (25th–75th percentile) for data not normally distributed. To test the change of each variable during pregnancy the one-way repeated measures ANOVA test was used in the case of normally distributed variables and the non-parametric Friedman ANOVA test in the case of non-normally distributed variables. To test for associations between different variables and to test correlations between differences the Spearman correlation analysis was performed. Stepwise multiple regression analysis was undertaken to define second and third trimester predictive variables for neonatal birth waist and cord blood insulin, respectively. A p-value of <0.05 was considered to be significant. The SPSS statistical software was used for statistical analysis (SPSS Statistics Inc., version no 22, Chicago, IL, USA).

**Table 2**
Neonatal anthropometric, hormone and metabolic variables at delivery (N = 80). Variables expressed as mean (±SD) or median (25th–75th interquartile range).

| Birth weight (g) | 3075 (2715–3444) |
|---|---|
| Neonatal waist circumference (cm) | 29.6 (3.5) |
| Neonatal %fat | 10.17 (1.25) |
| Cord glucose (mmol/L) | 5.4 (0.8) |
| [mg/dL] | [98.0 (15.0)] |
| Cord insulin (pmol/L) | 70.3 (50–104.7) |
| [(μIU/mL)] | [9.8 (7–14.6)] |
| Cord C-peptide (ng/mL) | 0.8 (0.4–1.1) |

## 3. Results

### 3.1. Maternal weight, hormone and metabolic variables during pregnancy

Maternal weight (Table 1) increased significantly from the 1st to the 2nd and 3rd trimesters ($p < 0.05$) with a median increase of 13.7 ± 1.5 kg. Neonatal birth weight and waist were within normal values. Maternal fasting plasma active ghrelin levels increased significantly from the first to the second ($p < 0.05$) and the third trimesters ($p < 0.05$). Active GLP-1 levels changed significantly from the second to the third trimester ($p < 0.05$) while total PYY levels did not change significantly during pregnancy. There was a trend towards increase of maternal leptin levels from the 1st to the 3rd trimester ($0.05 < p = 0.085 < 0.1$). Birth weight was within normal range. Table 2 shows all neonatal anthropometric, cord blood hormone and metabolic variables.

### 3.2. Correlations among maternal weight, hormone and metabolic variables during pregnancy, and neonatal anthropometric, cord blood metabolic variables

There was no correlation between maternal weight and maternal active ghrelin levels during pregnancy ($p > 0.05$). There was no correlation between maternal active ghrelin change and maternal weight change during pregnancy ($p > 0.05$).

In the first trimester maternal plasma active GLP-1 and active ghrelin levels correlated positively with each other ($r = 0.55$, $p = 0.001$) while active ghrelin levels correlated negatively with maternal HOMA-R ($r = -0.48$, $p = 0.003$), 1st PHIS ($r = -0.43$, $p = 0.004$) and 2nd PHIS ($r = -0.44$, $p = 0.004$). First trimester maternal weight correlated positively with percent total neonatal body fat ($r = 0.65$, $p = 0.001$).

In the second trimester maternal plasma active ghrelin levels correlated positively with neonatal waist circumference ($r = 0.75$, $p < 0.001$) (Fig. 1A) while in the second trimester maternal plasma active GLP-1 levels correlated negatively with birth weight ($r = -0.40$, $p = 0.03$). The change (delta, Δ) of active GLP-1 levels between the second and third trimesters correlated positively with the corresponding Δ active ghrelin levels ($r = 0.42$, $p = 0.02$). Furthermore, the second trimester maternal weight correlated positively with birth weight ($r = 0.45$, $p = 0.003$) and with percent total neonatal body fat ($p < 0.001$, $r = 0.76$). Maternal leptin correlated positively with maternal weight ($r = 0.52$, $p = 0.001$).

In the third trimester maternal plasma active GLP-1 and active ghrelin levels correlated positively with each other ($r = 0.49$, $p = 0.004$). Maternal plasma active ghrelin levels correlated positively

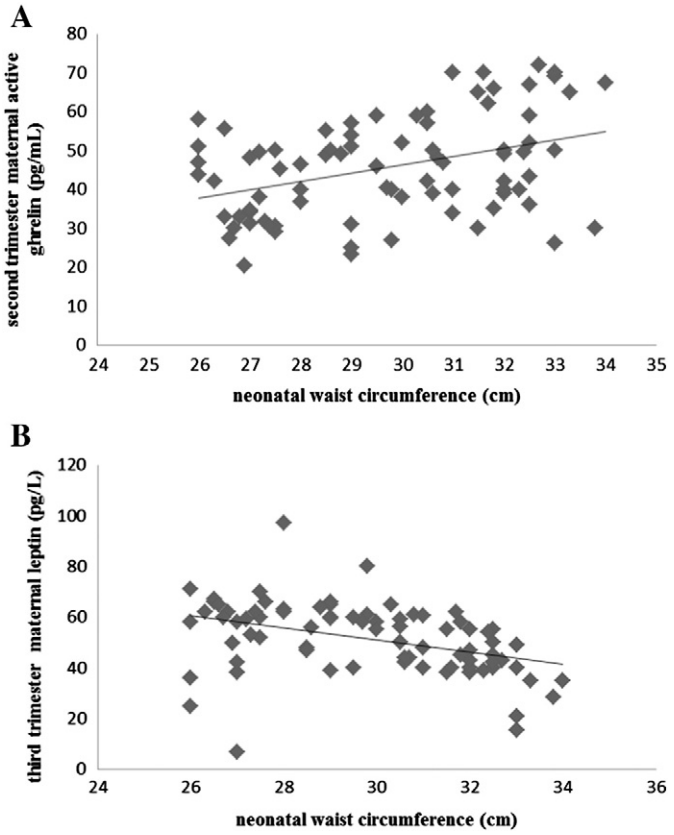


**Fig. 1.** A. Correlation of second trimester maternal active ghrelin levels with neonatal birth waist circumference ($p < 0.001$, $r = 0.75$). B. Correlation of third trimester maternal leptin levels with neonatal birth waist circumference ($p < 0.001$, $r = -0.81$).

with birth waist ($r = 0.70$, $p < 0.001$), and negatively with percent total neonatal body fat ($r = -0.94$, $p < 0.001$) and cord blood insulin levels ($r = -0.82$, $p < 0.001$). Maternal weight correlated positively with birth weight ($r = 0.50$, $p = 0.002$) and with percent neonatal body fat ($r = 0.97$, $p < 0.001$). Maternal leptin levels correlated negatively with neonatal birth waist ($r = -0.81$, $p < 0.001$) (Fig. 1B) and positively with maternal weight ($r = 0.60$, $p = 0.002$). Maternal HOMA-R correlated positively with birth weight ($r = 0.76$, $p < 0.001$).

### 3.3. Predictor of neonatal waist circumference

Stepwise multiple regression analysis revealed that the second trimester maternal circulating active ghrelin levels were the best positive predictor ($p = 0.03$, beta $= 0.84$) of neonatal waist circumference among the second trimester fasting plasma leptin, HOMA-R, active GLP1 and total PYY levels and maternal weight change (or maternal weight).

The first trimester variables did not show any statistical significance ($p > 0.05$) in the model regarding their predictive values.

### 3.4. Predictor of cord blood insulin

Stepwise multiple regression analysis revealed that the third trimester maternal circulating active ghrelin levels were the best negative predictor ($p = 0.02$, beta $= -0.99$) of cord blood insulin levels among third trimester fasting plasma leptin, active GLP1 and total PYY levels.

The first trimester variables did not show any statistical significance ($p > 0.05$) in the model regarding their predictive values.

## 4. Discussion

We investigated the role of circulating maternal fasting gut hormones (active ghrelin, active GLP-1, total PYY) and adipocytokine leptin, all part of the maternal appetite physiology, in non-diabetic pregnant women, upon neonatal total percent fat and central adipose tissue stores as expressed by waist circumference at birth. Furthermore, the role of these hormones upon fetal insulin secretion as expressed by cord blood insulin levels was explored. Circulating maternal fasting active ghrelin levels increased significantly from the first to the second trimester whereas fasting active GLP-1 levels increased significantly from the second to the third trimester and there was a trend of increase in circulating maternal leptin levels. Circulating maternal fasting total PYY did not change significantly during pregnancy.

The second trimester circulating maternal active ghrelin levels were the best positive predictors of neonatal waist circumference among all studied hormones and maternal weight or weight gain. Birth waist is a measure of liver volume and visceral adiposity which reflect the energy deposits of the neonate. It is considered a vital index for the beginning of neonatal life and predictive of its future growth and metabolic health [20–22]. Thus, it seems that neonatal waist circumference might depend on energy balance mechanisms which in their turn are influenced by circulating maternal active ghrelin levels. Recently it has been shown in mice that active ghrelin acting through its receptor inhibits the AMP-activated protein kinase (AMPK) activity in the liver and the visceral fat (mesenteric fat) [35]. AMPK is an intracellular energy sensor and energy regulator switching on ATP-producing pathways such as glucose uptake and fatty acid oxidation [15]. Thus, the positive correlation in the second and third trimesters of maternal circulating active ghrelin levels with neonatal waist circumference might reflect a switching-off of ATP producing pathways in fetal liver and visceral fat resulting to enhanced visceral fat deposition. Maternal ghrelin, a key regulator of human appetite [36], plays an important role in rat fetal development during pregnancy [18]. Of note, ghrelin is also found in the placenta and circulating maternal levels in addition reflect placental excretion [37]. Furthermore, in this study the third trimester maternal circulating leptin, known to suppress hypothalamic AMPK activity and contribute to restriction of food intake [15], correlated negatively with neonatal birth waist circumference whereas maternal weight was only associated positively with neonatal percent total fat and birth weight.

Interestingly, there was no association during pregnancy between maternal active ghrelin levels and maternal weight while the first trimester maternal active ghrelin levels correlated negatively with maternal insulin secretion and resistance indices. In addition, the third trimester maternal active ghrelin levels correlated negatively with neonatal percent total body fat and cord blood insulin and predicted negatively cord blood insulin levels. To explain the finding of positive and negative association of maternal active ghrelin with neonatal waist circumference and percent total fat, respectively, one should take into account that the percent total fat of the neonate as estimated by skinfold measurements represents subcutaneous-peripheral fat and not intra-abdominal visceral fat [38]. The latter is estimated by waist circumference. In neonates it is known that the amount of deep body fat at the abdominal site is small compared to subcutaneous fat [31, 39]. Measurement of subcutaneous fat thickness may be used as a non-invasive method to give a first rough estimate of body fat and body composition in infants. Indeed total body fat estimation using skinfold measurements is only an indication of fat patterning. Studies in humans show that ghrelin concentrations decrease with hyperglycemia and hyperinsulinism [40] and that in pregnancy, with or without GDM, they are markedly decreased [41]. Furthermore, in women with PCOS, an insulin resistance-associated state, serum ghrelin levels correlate negatively with HOMA-R [42]. Thus, maternal active ghrelin levels seem to counteract maternal and fetal hyperinsulinemia probably via inhibition of pancreatic insulin release [16,17]. Consequently, the positive correlation of maternal circulating active ghrelin levels with

neonatal waist circumference seems to be the result of energy regulation mechanisms rather than of insulin-resistance development during pregnancy. On the other hand, maternal weight during all three trimesters of pregnancy was associated positively with neonatal percent total fat and with birth weight (in second and third trimesters) confirming previous observations associating maternal weight with neonatal birth weight [43,44]. Indeed a large Danish population-based study (366,886 singletons) showed that pre-gestational maternal body mass index, although it affects neonatal waist circumference, correlates more closely to birth weight than to waist circumference itself [24].

During the first and third trimesters maternal fasting active GLP-1 levels correlated positively with maternal plasma active ghrelin levels. In addition, the change of active GLP-1 and ghrelin levels between the second and third trimesters correlated positively with each other. This positive correlation between the anorexigenic GLP-1 and the orexigenic active ghrelin has not been reported in non-pregnant states and it might reflect a homeostatic mechanism during normal pregnancy. No other significant findings regarding the role of the other maternal fasting peptides involved in the appetite physiology (leptin, total PYY, active GLP1) were noted. Not measuring the maternal postprandial levels of active GLP-1 could be considered a weakness of this study because the insulinotropic action of this hormone is clearly proven. However, studies using clamps in healthy subjects have demonstrated a similar effect at basal glucose levels as well [45]. In fact, the chronic basal long-term effects of these circulating maternal hormones on the maternal central nervous system and appetite and thus on neonatal central energy stores and metabolism can be investigated by measuring their fasting levels. Furthermore although absolute levels of appetite-related hormones were measured in the present study it should be acknowledged that sensitivity to these hormones might also change, thereby influencing the observed relationships.

In summary, maternal appetite-related hormones during pregnancy, as measured by active ghrelin and leptin levels, were shown to be associated with neonatal visceral energy storage, expressed by neonatal waist circumference at birth, while maternal weight was only associated positively with neonatal percent total fat and birth weight.

During pregnancy both maternal- and placental-derived active ghrelin, by increasing maternal appetite, might increase nutrient supplies to the fetus resulting in its positive energy balance. There is a need for further studies to investigate the role of maternal active ghrelin in fetal anthropometry and metabolism in gestational diabetes and in obese pregnant women. Future studies will need to monitor perceived appetite and actual nutritional intake.

## Conflict of interest

No author has any conflict of interest by any author that could be perceived as prejudicing the impartiality of the research reported.

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