Early Human Development 90 (2014) 487–492

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Best practice guidelines

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

Georgios Valsamakis [a,c], Dimitrios C. Papatheodorou [a], Aikaterini Naoum [a], Alexandra Margeli [b], Ioannis Papassotiriou [b], Efthymios Kapantais [d], George Creatsas [a], Sudhesh Kumar [c], George Mastorakos [a,*]

[a] Endocrine Unit, 2nd Department of Obstetrics and Gynecology and Pathology Department, Aretaieion University Hospital, Athens Medical School, Athens, Greece
[b] Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece
[c] Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism (WISDEM), University Hospital Coventry and Warwick Medical School, Coventry, UK
[d] Department of Obesity and Diabetes, Metropolitan Hospital, Athens, Greece

ARTICLE INFO

Article history:
Received 4 March 2014
Received in revised form 11 June 2014
Accepted 1 July 2014

Keywords:
Pregnancy
Maternal active ghrelin
Appetite
Birth waist
Cord blood insulin

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

* Corresponding author at: 3 Neofytou Vamva Str, 10674 Athens, Greece.
E-mail address: mastorakg@ath.forthnet.gr (G. Mastorakos).

http://dx.doi.org/10.1016/j.earlhumdev.2014.07.001
0378-3782/

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