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Adipokine-myokine-hepatokine compartment-system in mothers and children: An explorative study

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ABSTRACT

Objective: Maternal lifestyle during pregnancy has an effect of gestational development and neonatal outcome. Overweight gravidas and gravidas with excessive weight gain have an increased risk of gestational complications and neonatal metabolic disorder. The underlying mechanisms are still under discussion, but the hormonally active fat mass and its biomarkers, adipocytokines, may play a key role by potentially having a direct impact on the metabolic homeostasis of the system in concert with other biomarkers like hepatokines and myokines. Up to now little is known in terms of lifestyle habits and their effect on this complex model on maternal and fetal outcome. Therefore, we aim to investigate the influence of maternal lifestyle clusters during pregnancy on the maternal and fetal biomarkers of compartments, specifically those implying maternal fat and muscle mass, maternal liver and the placenta and who are associated with maternal body composition and birth weight.

Methods: In this exploratory pilot study at least 100 singleton pregnancies and their newborns will be included. The women will undergo assessments of anthropometric measurements, venous blood samples will be drawn and physical activity and nutritional status will be collected through questionnaires. Newborns will undergo assessments of anthropometric measurements, umbilical cord samples will be drawn and birth outcomes will be evaluated. We will measure adipokines, myokines and hepatokines and relate them to maternal lifestyle clusters and fetal outcome.

Conclusion: Our study will be the first to examine the relationship between maternal body composition, birth weight and potential biomarkers based on an innovative compartment model.

1. Introduction

Incidences of overweight and metabolic syndrome in children increase worldwide, thereby increasing the risk for developing chronic diseases in later life. This is negatively influenced by prematurity, fetopathia diabetic, maternal obesity and gestational diabetes (GDM) [1]. Maternal obesity is an important factor of excessive birth weight and increasing risk for later obesity and metabolic disorder [2]. The additional cause next to genetic predisposition is assumed to be perinatal programming. This process describes the reaction of the developing embryonic organs, such as the hypothalamus, to the intrauterine environment influenced by the mother. One of the influences that is prominently known to affect the embryonic organs is the mother's nutrition. The perinatal programming may have lifelong consequences to metabolism as a result from faulty materno-placental nutrient supply [3]. Therefore, children's long–term reaction to carbohydrates and amino acids are programmed during sensitive fetal phases by the maternal "offer" [4]. Our compartment model describes the individual metabolically active organs and their known endocrinological functions. The effect of those organ systems upon each other and onto the mother's entire metabolism and that impact on the fetus is the basis of our examinations. Particular endocrinological factors such as insulin and leptin, but also other biomarkers such as adiponectin, resistin and TNF-alpha (TNF-α) play a key role in the development of GDM [5,6]. Examples show that these cytokines originate from different compartments like maternal liver, muscle,

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and adipose tissue, but also in the placenta [7–12] (Table 1), corresponding with each other and more or less with the fetus (Fig. 1). Of the biomarkers to be researched, the following is known about their function and mechanisms of action: Leptin is secreted in adipose tissue with serum levels proportional to the adipose mass. It has been demonstrated that obese subjects have higher levels than subjects with healthy body weight [11–13]. Another operant regulator of glucose homeostasis is resistin from adipose tissue, muscle cells, endothelial cells, placental villi and throphoblasts [11,14,15]. Some studies found a positive correlation between resistin levels and body fat mass, others do not confirm this link [14]. Serum levels first increase during the third trimester and then at term. The effect of the maternal metabolism is an increase in insulin resistance. Higher umbilical resistin levels at term increase neonatal hepatic glucose production and may protect neonatal hypoglycemia [15]. Adiponectin is expressed contrary to leptin, in that it enhances insulin sensitivity by increasing insulin activity and reducing glucose production. In obese mothers adiponectin levels are reduced. Presumably adiponectin is produced in the human placenta [16,17]. The present adipokines may affect pregnancy outcome and fetal growth. Leptin and resistin levels in GDM are controversial, while data relating to adiponectin levels show a decrease in GDM.

The increase of fat mass in pregnancy is associated with an exacerbated inflammatory state. Higher circulation concentrations of inflammatory cytokines like C-reactive protein (CRP), Interleukin-6 (IL-6) and TNF-α are detected [18–20]. Also identified are myokines, a subspecies of cytokines which have a role in exercise associated metabolic adaption [21]. Myokines probably have a beneficial effect regarding chronic disease. Little is known about their impact during pregnancy. Brain-derived neurotrophic factor (BDNF) is identified as a regulator of controlling body fat mass and energy balance [21,22]. Low serum levels are associated with obesity and diabetes mellitus type 2 [23]. In contrast, physical activity may increase serum levels [22,24]. Irisin levels are inversely correlated with obesity, diabetes mellitus and GDM. Irisin precursors are expressed by the placenta. Mothers with GDM have significantly lower irisin levels than those without GDM [25]. FetuinA is a hepatokine associated with insulin resistance during pregnancy and is detected as an acute phase protein [26].

As shown in Table 1, the relevance of individual biomarkers has been established. However, the intricate interactions amongst and between biomarkers and compartments remain largely unknown. This pilot study intends to detect these interactions between the different lifestyle factors (nutrition and physical exercise) and relevant biomarkers. In order to do so, blood from the mother and the baby at the time of birth will be sampled from a vein and the umbilical cord respectively. Also, both their anthropometric data will be registered as well as the mother's lifestyle factors determined by means of retrospective questionnaire. The aim of the study is to examine the influence of maternal lifestyle clusters on maternal weight gain, birth weight and the underlying biomarkers.

2. Material and methods

2.1. Ethical consideration

We will conduct a pilot study in a cross-sectional cohort over a 5 month period at the German Sport University Cologne and the University Bonn Medical School, Germany. The protocol was submitted to the University Bonn Medical School Ethics Committee and approved under the number 269/13. Also, the following procedures will be followed: Participation in the study will only occur after reading the consent form and giving written consent. All women will be guaranteed the right to not participate in the study. The study will be carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). We will ensure confidentially of the collected data and document numbers (identifications).

2.2. Subjects and sample size

This study will include women who are admitted for delivery at the labor ward, Department of Obstetrics and Gynecology, University Bonn, with a singleton pregnancy and gestational age between 36 and 42 weeks who have given written consent. Women with multiple pregnancy, gestational age <36 weeks, the inability to speak German, mental illness and a high-risk pregnancy will be excluded from the study. The target sample size is 100 mothers and their newborns. Nearly all quantitative studies can be subjected to a sample size calculation. However, they may be of little value in early exploratory studies where scarce data are available on which to base the calculations (though this may be addressed by performing a pilot study first and using the data from that) [27].

2.3. Anthropometric and clinical data

Clinical data such as height or pregnancy related diseases are retrieved from the patients' antenatal and inpatient files. We will also consider aspects during pregnancy and relating to birth which may be important variables including: maternal age, parity, ethnicity, level of education, smoking and GDM during pregnancy,

Table 1
Selected cytokines during pregnancy (modified to D'Ippolito et al., 2012 [28]).

Cytokines	Leptin	Adiponectin	Resistin	IL-6	TNF-α	Irisin	BDNF	FetuinA
Maternal								
Circulating levels	↑	↓	↑ (3.Trim)	↑	↑ (3.Trim)	↑/↓	↑/↓	↑ (2.Trim)
Metaboliceffects	growth of adipose tissue	increases insulin activity and sensitivity; reduces glucose production	increases insulin resistance	?	reduces insulin sensitivity	negatively correlated with GDM	positively correlated with exercises	increases insulin resistance; acute phase protein; fat accumulation in liver
Fetal								
Umbilical level at term	↑	↑	↑	?	?	?	?	?
Function	increases: throphoblast-proliferation; IL-expression; VEGF-secretion; placental lipolysis (?)	decreases of transplacental insulin-mediated amino acid transport; enhancement fetal insulin sensitivity	increases hepatic glucose production; protect of neonatal hypoglycemia	Regulates hepatic immune response	increases placenta inflammation	?	?	negative regulation of neonatal bone development (?)

↑ = upregulation, ↓ = downregulation, ↑/↓ = upregulation as wall as downregulation possible, ? = currently no data available.

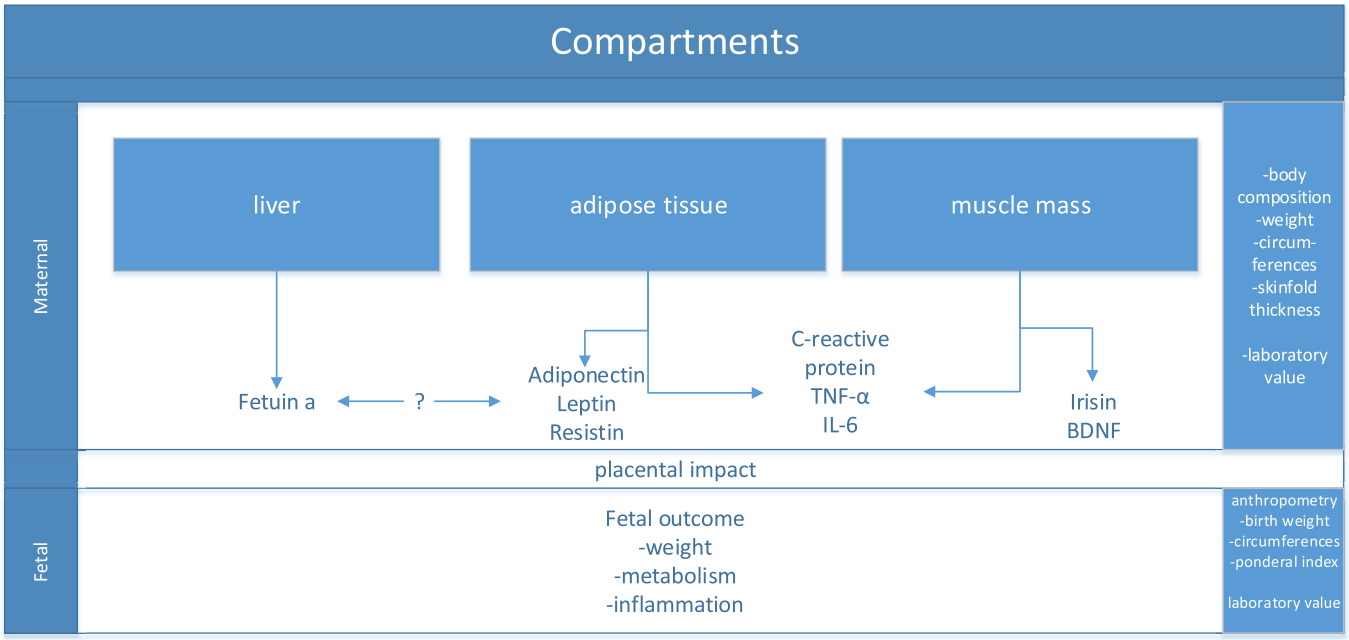

Fig. 1. Adipokine-myokine-hepatokine compartment model.

caesarean delivery or vaginal delivery and other complications during childbirth. Furthermore, maternal body weight before delivery will be measured by the gynecologist at the preceding appointment and will be retrieved from the antenatal chart. Women's Body Mass Index (BMI = kg/m^2) will be calculated by body weight in relation to body size. To estimate maternal weight gain we will use the self-reported pre-pregnancy weight. To classify women's weight gain during pregnancy we will use the Institute of Medicine (IOM) guidelines based on BMI (Table 2).

Anthropometric measurements will be performed during admission for delivery. Maternal mid-arm circumferences will be measured on the right side to the nearest 0.1 cm with a non-extensible, flexible tape. Skinfold thickness will be measured using a Harpenden skinfold caliper (John Bull British Indicators Ltd., Harpenden UK) with a constant pressure of 10 g/mm^2. The procedure will be carefully standardized and the measurement will be made in triplicate; the results will be averaged. Three points are measured to the nearest 0.2 mm: triceps, thigh, and suprailiac [29].

In addition, we will estimate neonatal Ponderal Index (PI = 100x(kg/m^3)). The birth weight will be measured using an electronic scale to the nearest 1 g and height will be measured from crown-heel in the recumbent position to the nearest 1 mm by midwives within 2 h after delivery.

We will examine the following neonatal outcomes: birth weight, length, head circumference, and Apgar score; additionally, the pH in the umbilical cord artery will be analyzed within 10 min after delivery. Gestational age at birth will be calculated from the last menstrual period and verified by first-trimester ultrasound measurements.

2.4. Laboratory value generate from maternal venous blood and umbilical cord

Venous blood samples will be collected into 7.5 mL serum tube (S-Monovette; Sarstedt/Germany). The maternal blood sample will be collected on admission to the labor ward; the umbilical cord sample will be collected from the placental part of the umbilical cord immediately after clamping. The samples will be stored at +4° Celsius for a maximum of 48 h. The samples will be centrifuged (4000 rpm for 10 min at 4 °C), and serum will be pipetted into aliquots and stored at −20° Celsius until assaying. Samples will be thawed only once and all reagents will be run in duplicate. Adiponectin and leptin will be measured in a TECAN reader (Nano Quant infinite M200 Pro, Switzerland) by a direct sandwich ELISA kit from MERCK/Millipore, Germany, according to the manufacturer's instructions. A seven point standard curve will be generated and samples will be interrogated with a lower level of detection of 1.28 ng/ml and 0.78 ng/ml for adiponectin and leptin, respectively. Serum concentrations of TNF-α, IL-6, resistin and BDNF will be investigated by a multiplex immunoassay from eBioscience conducted according to the manufacturer's instructions. This sandwich ELISA-like bead-based suspension array allows measurement of multiple analytes in one well. Measurement of serum duplicates will be conducted in a Luminex 200 reader (Luminex, Austin, TX, USA). A seven point standard curve was generated on each plate for each analyte and samples will be interrogated with a lower level of detection of 9.1 pg/ml, 9.1 pg/ml, 6.01 pg/ml and 1.88 pg/ml for TNF-α, IL-6, resistin and BDNF, respectively (calculated with Bio-Plex Manager 6.1, Bio-Rad, Hercules, CA, USA). In addition, CRP, Irisin and Fetuin-A will be measured by a single ELISA kit, according to manufacturer's instructions. Insulin will be measured by radioimmunoassay (RIA Kit; Roche Diagnostics Germany).

Table 2
Weight gain recommendations during pregnancy according to the Institute of Medicine [36] 2009; kg = kilogram; lbs = pounds.

Pre-pregnancy BMI	Total weight gain	
	Range in kg	Range in lbs
Underweight (BMI < 18.5 kg/m^2)	12.5–18	28–40
normal-weight (BMI 18.5–24.9 kg/m^2)	11.5–16	25–35
Overweight (BMI 25.0–29.9 kg/m^2)	7–11.5	15–25
Obese (BMI > 30 kg/m^2)	5–9	11–20

2.5. Questionnaire

Several questionnaires will be used to measure physical activity, nutrition and socio-demographic factors. At the beginning, demographics and other covariates such as pre-pregnancy weight, socioeconomic status, level of education, ethnicity or smoking habits will be asked by closed questions. To measure physical activity before and during pregnancy, we will use the Pregnancy Physical Activity Questionnaire (PPAQ) as described by Chasan-Taber et al. [30]. It is a reliable, validated instrument and a reasonably accurate measure of a broad range of physical activity during pregnancy. Women are asked to select the category that best approximates the amount of time spent in 32 activities including household/caregiving, occupational, sports/exercise, and inactivity during the current trimester. Women will be classified according to self-reported physical activity. Time spent in each activity will be multiplied by its intensity to calculate the average "daily total energy expenditure in Metabolic Equivalent of Task (MET) – hours per day. In addition, activity will be classified in the following intensity groups: sedentary (<1.5 METs), light (1.5 $\leq$ 3.0 METs), moderate (3.0–6.0 METs) or vigorous (>6.0 METs) [30]. To assess nutrition during pregnancy, we will use a semi-quantitative food frequency questionnaire with pregnancy-specific adaptations as described elsewhere. The nutritional part of the questionnaire records the dieting behavior of the women and collects data about the consumed amounts of the separate food groups: grains, meat, fish, dairy, fruit and vegetables. Additional categories are beverages and supplements. The questionnaire supplies data about general dietary decisions and behavior and their changes since the beginning of the pregnancy [31].

2.6. Data analysis

Nutrient calculations will be performed using Prodi ® 6.2 Software (Science publishing society Stuttgart 2013, Germany).

We will use linear regression analyses to explore the bivariate and multivariate effects of physical activity and nutrition (lifestyle clusters) on maternal weight gain and birth weight. The aforementioned are analyzed in relation to the different biomarkers of the four compartments (fat mass, muscle mass, liver, placenta) with regard to the covariates like parity, ethnicity, smoking and GDM. We will divide physical activity levels during pregnancy into quartiles and compare the birth weights among the four groups using analysis of variance. Descriptive comparisons will be calculated by the standard procedures such as t-test, Analysis of Covariance (ANCOVA) and chi-square test. All statistical analyses will be performed using the SPSS statistical package (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

3. Discussion

Maternal health and physical activity are important for a healthy infant outcome [32–35]. Several studies support these findings, but little is known about the underlying biological mechanisms [3,6,15]. Leslie Myatt describes a cascade of fetal programming beginning at the placenta. Dysfunctions in the placenta effect changes in trophoblasts and the vascular system of the placenta, leading to hypoxic conditions that ultimately impair the growth of the fetus. This oxidative stress may cause retardation in fetal growth or pregnancy complications such as preeclampsia. It is being speculated that aberrant hormones are leading to this development. However, the causes have not been able to be specified further to date [3]. The study at hand aims to analyze those correlations and potential key factors in the context of adipokines. Boa et al. examined 2015 adipokine levels in gravidas with and without GDM. They were able to verify that adipokines play a key role in the development of GDM [6]. Cortelazzi et al. conducted similar research with resistin and adiponectin. The influence of the mother's energy balance onto the fetal growth has been verified in a similar fashion [15]. Still the underlying mechanism has not been established yet nor the influence of the maternal lifestyle or body composition. The goal of this study is, therefore, to examine the correlation between maternal and infantile biomarkers taking into consideration the individual lifestyle during pregnancy and the body composition. Details about the complex interactions between cytokine pathways and changes in terms of different body composition and maternal lifestyle may thus be detected. Equally, more knowledge may be gained about the interaction between adipokines, myokines and hepatokines, the effect of maternal exercise and nutrition and how this relates to the fetal programming and outcome.

To our knowledge, this is the first study examining the relationship between maternal body composition, birth weight and potential biomarkers based on this compartment model. Therefore, we developed this pilot study project with the intention to incorporate a statewide study in numerous centers.

Results of the presented study might offer the relationship between weight gain in pregnancy, maternal lifestyle (physical activity and nutrition) with maternal and fetal biomarkers and potential birth complications. The authors envision that attained knowledge will serve as a guide to optimal gestational advice and for improving maternal and fetal health.

Sources of study

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Conflict of interest

The authors declare that they have no competing interest.

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