I, Alison Gardner, hereby submit this original work as part of the requirements for the degree of Master of Science in Nutrition.

It is entitled:
Association of Maternal Adipokines with Infant Anthropometry in Obese, Pregnant Women

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Association of Maternal Adipokines with Infant Anthropometry in Obese, Pregnant Women

A thesis submitted to the
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By

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ABSTRACT

Objectives. To determine if, and to what extent maternal adipokines and glucose metabolism are associated with infant anthropometry at birth for obese and overweight pregnant mothers.

Design. Prospective cohort study

Subjects. Fifty-two healthy, non-smoking, overweight and obese pregnant women (pregravid BMI ≥ 25) between 18 and 40 years old were recruited from the greater Cincinnati metropolitan area.

Methods. Pregravid body mass index (BMI= kg/m²) was derived from self-reported anthropometric data. A fasting venous blood sample was collected at 26 weeks gestation to assess maternal adipokines and glucose metabolism. HOMA-IR was calculated from fasting glucose and insulin values. Infant anthropometry was self-reported by the mother following delivery. Fetal overgrowth was defined three ways: as macrosomia for infants ≥ 4000 grams at birth; as large-for-gestational-age (LGA) for birthweight ≥90th percentile for gestational age, and as ponderal index ≥90th percentile for weight-to-length ratio.

Results. Infant ponderal index was positively correlated with maternal insulin concentration ($r=+0.324$, $P<0.05$) and HOMA-IR ($r=+0.292$, $P<0.05$). Maternal insulin explained 16% of the variance in ponderal index ($R^2 = .16$, $F = 7.32$, $P < 0.01$).

Conclusion. Obese pregnant women have a metabolic profile similar to women diagnosed with gestational diabetes mellitus, and experience insulin resistance, inflammation, and the subsequent effects on fetal growth. Insulin resistance remains the most significant predictor of fetal overgrowth in this population. In this study, the maternal adipokines leptin, adiponectin, IL-6 and TNF-α were not associated with infant anthropometry at birth.
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Introduction

Significant changes in maternal metabolism occur during pregnancy to support fetal growth. The placenta and maternal adipose tissue are metabolically active, secreting adipokines that have paracrine and endocrine actions.\(^1\) One function of these adipokines is to mediate insulin resistance throughout pregnancy. Maternal insulin resistance is a normal physiologic response to pregnancy in order to provide nutrients for the growing fetus. In women with pregravid obesity, levels of adipokines are further elevated, contributing to poor outcomes for the mother (pre-eclampsia, gestational diabetes, thromboembolism) and the fetus (fetal overgrowth, late-fetal death, obesity, and cardiometabolic diseases later in life).\(^2\)

Although the mother’s weight and body mass index (BMI= kg/m\(^2\)),\(^3\) and degree of insulin resistance\(^4\) have been shown to be associated with infant anthropometry at birth, there is less evidence examining the relationship of the adipokines leptin, adiponectin, TNF-\(\alpha\) and IL-6 to birth size. Levels of these inflammatory markers tend to be elevated in obese individuals and are associated with insulin resistance.\(^5\) Adipokines may influence fetal growth indirectly through modulation of insulin resistance, or directly through regulation of placental nutrient transport.

The high prevalence of obesity creates concern regarding its consequences for pregnant women and their infants. Typically associated with gestational diabetes mellitus, fetal overgrowth has become more common in obese, pregnant women without diabetes. Infants born to obese mothers are at greater risk of fetal overgrowth than those born to lean women because of the increased insulin resistance associated with obesity.\(^6\) Because maternal obesity is more prevalent than maternal diabetes, the metabolic abnormalities associated with obesity may be a more significant determinant of fetal growth at the population level.
Obesity, Inflammation and Pregnancy

The prevalence of obesity has risen dramatically in the last 20 years. The World Health Organization (WHO) estimates that 500 million adults are obese (BMI \(\geq 30\text{ kg/m}^2\)).\(^7\) In the United States, one-third of women of reproductive age are obese.\(^8\) Though there is limited data detailing the prevalence of obesity in pregnancy, population data indicates that the prevalence of obesity in women of child-bearing age parallels the rise in the population as a whole.\(^9\) The Pregnancy Risk Assessment Monitoring System (PRAMS), a surveillance project conducted by the Centers for Disease Control, recorded a 70% rise in pregravid obesity from 1993-2003.\(^10\)

Obesity is characterized by excess stores of adipose tissue. Adipose tissue functions primarily as fat storage, but it is also a metabolically active tissue, secreting a range of cytokines, or adipokines.\(^11\) These factors affect inflammation and insulin resistance [tumor necrosis factor alpha (TNF-\(\alpha\)) and interleukin-6 (IL-6)], insulin sensitivity (adiponectin), and appetite and energy regulation (leptin).\(^12\) As a result, obesity is a state of low-grade inflammation and insulin resistance.\(^13\)

Pregnancy, a naturally inflammatory condition, shares a pattern of insulin resistance with obesity. Elevated secretion of inflammatory adipokines from maternal adipose tissue is supplemented by the placenta, promoting insulin resistance and enhancing the availability of glucose for fetal needs.\(^1\) As pregnancy progresses, insulin resistance increases by 40-60%. To compensate, the pancreas secretes additional insulin to maintain euglycemia.\(^14\) In normal pregnancies, the secretion of additional insulin is sufficient to maintain glucose homeostasis. The increased fat stores associated with obesity, however, lead to elevated levels of circulating free fatty acids, which contribute to further insulin resistance. Resulting from this combination of
pregnancy-induced and pre-existing insulin resistance, obese women are four times more likely to develop gestational diabetes mellitus, or pregnancy-induced diabetes, than lean women.\textsuperscript{15}

Defining Fetal Overgrowth

Infant birthweight is increasing in the United States and worldwide.\textsuperscript{16} Definitions of high birthweight and fetal overgrowth vary widely in the current literature based on the timing of measurements and the infant anthropometric data available. Macrosomia is frequently used to categorize fetal overgrowth. Several definitions have been used, including birthweight > 4000, 4100, 4500, or 4536 grams.\textsuperscript{4} Weight ≥ 4000 grams (8 pounds, 12 ounces) is the most commonly used classification for macrosomia worldwide. Normal birthweight ranges from 2820 to 3900 grams.\textsuperscript{17} In the United States, 8\% of newborns were born macrosomic in 2008,\textsuperscript{18} similar to the rate of 7.9\% in 2003.\textsuperscript{19} This definition does not account for the gestational age of the infant at birth, and can therefore be an inaccurate description of fetal growth.

Fetal size increases with length of time in utero, therefore classification of birthweight based on gestational age is a better indicator of optimal fetal growth. Plotted on standardized growth charts, birthweight can be categorized as small- (SGA), appropriate- (AGA), or large-for-gestational age (LGA). SGA corresponds to birthweight ≤ 10\textsuperscript{th} percentile for gestational age in weeks; AGA falls between 10\textsuperscript{th} - 90\textsuperscript{th} percentiles, and LGA refers to infant birthweight ≥90\textsuperscript{th} percentile for gestational age.\textsuperscript{20} Over 100,000 infants are born large-for-gestational-age (LGA) in the United States each year.\textsuperscript{21} In 2003, 12\% of term newborns were classified as LGA.\textsuperscript{20} This is a significant increase from 6.6\%, recorded from 1985 through 1998.\textsuperscript{22} The incidence of LGA births is notably higher than incidence of macrosomia, as LGA classification includes a wider range of birth weights based on gestational age.
These indices, however, are based only on birthweight and do not consider fetal weight-to-length ratio. Because infants differ in terms of body composition, the ponderal index was developed to assess fetal growth and adiposity.\textsuperscript{23} Calculated as

\[ \frac{\text{mass}}{\text{height}^3} \]

the ponderal index is plotted on percentile charts based on the gestational age of the infant at birth.\textsuperscript{24} These percentile charts are based on national birth data and account for gender variations, as male and female infants are plotted on separate graphs.\textsuperscript{25} Ponderal index \( \geq 90^{\text{th}} \) percentile is classified as large. By scaling mass with the third power of height, the ponderal index yields valid results in individuals with short-stature, namely infants and children. For this reason, the ponderal index is used instead of body mass index (kg/m\(^2\)), which is used as a surrogate indicator adiposity in adults.\textsuperscript{26}

Adverse Outcomes Associated with Fetal Overgrowth

There are short- and long-term implications for neonates born LGA or macrosomic. LGA infants have a two- to three-fold greater risk of intrauterine death.\textsuperscript{2} Fetal overgrowth also increases risk of delivery complications. Compared to normal birthweight infants, macrosomic infants are at greater risk for shoulder dystocia, brachial plexus injury, clavicle fractures, respiratory distress syndrome and asphyxia, all consequences of the challenge associated with vaginal delivery of a large fetus.\textsuperscript{4,27} To prevent these injuries, large infants are often delivered surgically by means of caesarean section. Though this procedure is common, it is not without risk. Aside from the immediate risk of operative complications, infants birthed by caesarean section have an increased risk of developing respiratory distress syndrome or asthma later in life.\textsuperscript{28}
The adverse outcomes linked to fetal overgrowth, however, extend beyond the perinatal period. Macrosomia is associated with metabolic abnormalities and chronic disease later in life. LGA infants are more likely to be overweight or obese as adolescents and adults than normal weight newborns,\textsuperscript{20, 29} and have a greater risk of developing type 2 diabetes and metabolic syndrome.\textsuperscript{30, 31}

Pathogenesis of Fetal Overgrowth

Fetal growth is influenced by non-modifiable and environmental factors. Non-modifiable factors include genetics, fetal gender, maternal age and height, and uteroplacental function. Early fetal growth is controlled by genes, especially genes coding for insulin, insulin-like growth factor, and their receptors.\textsuperscript{32} Genetic influence may account for anywhere between 25-80\% of birthweight variation.\textsuperscript{32} Some degree of variation is attributable to fetal gender, as the average full-term male birthweight is 150-200 grams (0.33-0.44 pounds) greater than the average female.\textsuperscript{33} Maternal age at first pregnancy, which is increasing in the United States and other countries, is also associated with higher birthweight.\textsuperscript{34} Women who have previously birthed a macrosomic infant, or were born at a high birthweight themselves, are more likely to give birth to a large infant than those who have birthed normal-size infants.\textsuperscript{4} Maternal height, but not paternal height, is also a positive predictor of infant birthweight.\textsuperscript{6}

Fetal growth slows at 30 weeks gestation and remains slow through the third trimester. Late growth restriction seems to be more pronounced, in adolescent and nulliparous pregnancies.\textsuperscript{35} In normal pregnancies, the uterus and placenta size begin to limit growth when fetal weight reaches approximately 3000-3200 grams (6.6-7.1 pounds).\textsuperscript{16} Growth beyond this
point is largely a product of the intrauterine environment; which, in turn, is primarily determined by maternal metabolic status and nutrition.

Fetal overgrowth is largely attributed to maternal hyperglycemia, as proposed by Jorgen Pedersen in 1954. Termed the maternal hyperglycemia-fetal hyperinsulinemia hypothesis, Pedersen theorized that maternal hyperglycemia, a result of insulin resistance initiated by maternal hormones (progesterone, prolactin and estradiol), placental lactogens and obesity, allows for increased glucose transport to the fetus. Maternal insulin, however, does not cross the placenta. In response to elevated maternal glucose levels, the fetal pancreas secretes insulin, which promotes glucose uptake by the fetus and accelerates the accumulation of fetal adipose tissue. As a result, macrosomia and LGA births are more prevalent in cases of maternal glucose intolerance, as observed in women with type 1, type 2 or gestational diabetes.

Historically associated with diabetic pregnancies, macrosomia has become more common in pregnancies complicated by obesity. Overweight or obese pregravid BMI (≥ 25 kg/m²) is an independent risk factor for delivering a macrosomic or LGA newborn. Pregravid BMI may be stronger predictor of macrosomia than glucose intolerance and diabetes. Because maternal obesity is more prevalent than maternal diabetes, at the population level, obesity may be a more significant contributor to fetal overgrowth.

Glucose Metabolism in Pregnancy

Obese pregnant women experience greater reductions in insulin sensitivity during gestation compared to lean pregnant women, and the associated elevations in blood glucose levels contribute to fetal overgrowth. Because fetal fat deposition and linear growth occurs
primarily in the third trimester, maternal glucose metabolism in the third trimester predicts infant size at birth.\textsuperscript{42}

Insulin and Glucose

In normal physiologic conditions, insulin stimulates glucose uptake by peripheral tissues, where skeletal muscle acts as the primary site of glucose disposal in the body. As blood glucose levels rise, pancreatic β-cells secrete insulin. Insulin binds to insulin receptors (IR) on cell membranes of myocytes and adipocytes. This binding activates phosphoinositide 3 (PI-3)-kinase, protein kinase B and protein kinase C isoforms that phosphorylate tyrosine residues on insulin receptor substrates (IRS)-1 and -6. This activation stimulates translocation of GLUT-4 transporters to the cell membrane, promoting facilitated diffusion of glucose into the cell. Intracellular glucose enters glycolysis, where it is converted to energy, or becomes substrate for protein and lipid synthesis.

In normal pregnancy, maternal hormones and adipokines promote insulin resistance in maternal peripheral tissues. Plasma levels of maternal hormones progesterone, prolactin, estradiol, and placental lactogens increase steadily over the course of pregnancy and are the primary origins of insulin resistance.\textsuperscript{43} Elevated insulin secretion, however, is able to maintain euglycemia during the first trimester.\textsuperscript{44} Insulin resistance increases 40-60\%, and insulin secretion 200-250\%, from pregravid levels to late gestation.\textsuperscript{45} Pregnancy-induced insulin resistance is necessary to elevate blood glucose levels, providing fuel for the growing fetus, but excessive insulin resistance and poor glucose control may lead to fetal overgrowth. Maternal serum insulin is positively correlated with neonatal ponderal index\textsuperscript{46} and is a predictor of high birthweight in overweight women with healthy pregnancies.\textsuperscript{47}
Placental glucose transport occurs by facilitated diffusion through GLUT transporters, such that net transfer of glucose to the fetus is strongly influenced by maternal blood glucose concentrations. Third trimester glucose is a stronger predictor of macrosomia than glucose levels in early pregnancy. Maternal fasting glucose between 26-32 weeks gestation was positively associated with birthweight and neonatal adiposity in non-diabetic pregnancies in the multi-center Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study. Similar studies confirm that third trimester fasting glucose is an independent predictor of macrosomia in non-diabetic and diabetic mothers.

Hemoglobin A1c

Glycosylated hemoglobin, or A1c, is a form of hemoglobin that conveys glucose control in the 8-12 weeks preceding measurement. A1c is formed in a non-enzymatic glycation pathway when hemoglobin interacts with blood glucose. A1c is expressed as a percent of hemoglobin that is glycosylated and increases with rising blood glucose values. Because the typical life-span of the erythrocyte is 120 days, A1c predicts average glucose control over a longer time period than fasting glucose and insulin values alone.

A1c values are typically higher in the third trimester of normal and diabetic pregnancies when compared to the first trimester. Third trimester A1c is positively associated with neonatal birthweight in diabetic and non-diabetic pregnancies. A1c values as low at 4.99% may predict LGA births in normal pregnancies, indicating that even mild glucose intolerance may contribute to fetal overgrowth.
Adipokines in Pregnancy

Adipokines have a multifunctional role during pregnancy. They are secreted by maternal adipose tissue and the placenta, but have been detected in fetal plasma, suggesting they may also be expressed in fetal tissues. There is an association between maternal adipokines and fetal growth, but this does not prove a direct or indirect effect on the fetus. The degree of influence of maternal adipokines on fetal growth is unclear.

Leptin

Leptin, a hormone produced by adipose tissue, functions in the regulation of appetite and metabolism. Circulating leptin is closely related to adiposity, so that a rise in fat stores confers an increase in leptin production. Transported across the blood brain barrier by soluble receptors, leptin acts on the hypothalamic arcuate nucleus (ARC), where it stimulates expression of proopiomelanocortin (POMC). POMC signals satiety, reducing food intake and increasing energy expenditure. Leptin stimulates another class of neurons within the ARC that produce NPY (neuropeptide Y) and AgRP (agouti-related protein) which signal hunger and stimulate food intake. This paradox of the conflicting actions of leptin has lead to the hypothesis that high circulating levels of leptin, as observed in obese individuals, leads to leptin resistance. This “leptin-induced leptin resistance” occurs when the transport of leptin to the central nervous system is impaired by chronically high leptin levels and decreased signaling of leptin receptor, leading to the absence of satiety signaling and appetite control.

During the first trimester of pregnancy, serum leptin increases 30% compared to pregravid levels. Leptin levels continue to rise through the second and third trimesters in normal and diabetic pregnancies. Although maternal fat stores increase during this time and may contribute to amplified leptin synthesis, the placenta is the primary source of leptin in
pregnancy.\textsuperscript{72} At least 95\% of placental leptin is released into the maternal blood supply, with the remaining 5\% available to the fetus.\textsuperscript{73} Unlike leptin derived from adipose tissue, which acts centrally on the hypothalamus, placental leptin acts peripherally on local tissue.\textsuperscript{74} Placental leptin functions to enhance mobilization of maternal fat stores, promoting transfer of free fatty acids across the placenta to the fetus.\textsuperscript{75} The presence of leptin receptors in the placenta suggests that leptin may be significantly involved in fetal growth.\textsuperscript{76} In the placenta, leptin also stimulates amino acid transporter system A, augmenting transfer of amino acids to the fetus.\textsuperscript{77} Leptin enhances the placental transport of substrate to the fetus, augmenting fetal growth in the third trimester.

Obese pregnant women experience elevated leptin concentrations in the third trimester compared to lean pregnant women.\textsuperscript{78,79} Hyperleptinemia is the product of inflammation and insulin resistance. Placental leptin mRNA synthesis is upregulated by the cytokines TNF-\(\alpha\) and IL-6\textsuperscript{80}, and to a greater extent, insulin.\textsuperscript{81} Increased leptin production may be a protective response to offset the effects of inflammatory cytokines.\textsuperscript{82}

The abundance of leptin receptors in fetal cartilage and bone tissue suggests that leptin is involved in the regulation of fetal growth.\textsuperscript{75} Low concentrations of leptin have been observed in SGA infants, while high levels have been detected in the blood of macrosomic newborns of diabetic pregnancies.\textsuperscript{82} Umbilical cord concentrations of leptin are positively correlated with infant weight, length, head circumference, and ponderal index at birth,\textsuperscript{83} but because infant size is also associated with other hormones known to influence intrauterine growth (insulin and insulin-like growth factor-I),\textsuperscript{84} it is difficult to determine the extent the influence of leptin on fetal growth.
This association, however, is not observed in cases of gestational diabetes mellitus. In these cases, studies have shown an inverse relationship between leptin and infant anthropometry.\textsuperscript{71, 85} Despite the hyperleptinemia observed in women with gestational diabetes,\textsuperscript{71, 85} the euglycemia achieved through insulin therapy may reduce glucose transport to the fetus, preventing fetal overgrowth. A result of tight glucose control, infant birthweight and length is sometimes lower in cases of gestational diabetes and type 2 diabetes compared to normal pregnancies.\textsuperscript{86}

Adiponectin

Adiponectin is an anti-inflammatory, insulin-sensitizing hormone that is secreted by adipose tissue. In contrast to leptin and inflammatory cytokines, adiponectin expression is inversely correlated with adiposity.\textsuperscript{87} While elevated leptin, TNF-\textgreek{a}, and IL-6 levels are observed in obese individuals, serum adiponectin is significantly lower when compared to non-obese individuals.\textsuperscript{87} Adiponectin is the only adipokine known to be negatively correlated with obesity.\textsuperscript{88} Hypoadiponectinaemia is also apparent in other inflammatory diseases, including type 2 diabetes,\textsuperscript{89} coronary artery disease,\textsuperscript{90} and metabolic syndrome.\textsuperscript{91}

Adiponectin enhances insulin sensitivity by suppressing gluconeogenesis and improving insulin activity in peripheral tissue. By inhibiting activity of gluconeogenic enzymes, adiponectin lowers hepatic glucose infusion and increases fatty acid oxidation in the liver.\textsuperscript{92} In addition, adiponectin influences the regulation of skeletal myocyte insulin receptor tyrosine phosphorylation, promoting glucose uptake in peripheral tissues.\textsuperscript{93} Adiponectin secretion is regulated by insulin, and by cytokines secreted by macrophages and adipocytes. Insulin,\textsuperscript{88} TNF-\textgreek{a} and IL-6 downregulate adiponectin expression.\textsuperscript{94}
There is conflicting data regarding longitudinal changes in adiponectin throughout pregnancy. The placenta secretes adiponectin and its receptors in late pregnancy, but does not appear to influence serum levels. A decrease in maternal serum adiponectin from pregravid levels has been shown in some studies, and non-significant decreases have been observed in others. Reductions in serum adiponectin are likely attributed to insulin resistance associated with pregnancy, and maintenance of levels detected in some pregnancies may be a protective mechanism against insulin resistance. The most conclusive evidence comes from pregnancies complicated by gestational diabetes, where hypoadiponectinaemia is observed and is negatively correlated with maternal BMI.

The conflicting results regarding maternal adiponectin concentrations may be attributed to the activity of oligomeric forms of adiponectin. In serum, adiponectin exists as trimers, hexamers, and high-molecular weight species. Depending on the form, adiponectin can have contrasting effects. Hexameric and high-molecular weight forms activate nuclear factor-κB (NF-κB), a transcription factor that regulates many genes involved in chronic inflammation. Alternately, the trimeric forms induce AMP-activated protein kinase (AMPK) in muscle, leading to increased glucose uptake, independent of insulin. Adiponectin trimers also suppress NF-κB signaling, reducing macrophage production of IL-6 and TNF-α. Because most research has focused on the effects of total adiponectin, it is difficult to distinguish the role of adiponectin isomers in pregnancy and fetal growth.

Given the role of adiponectin in insulin metabolism, and the significance of insulin to regulation of nutrient supply to fetal tissues, it is probable that adiponectin is involved in the regulation of fetal growth. High adiponectin concentrations are present in cord blood and fetal serum at birth, but contrary to adults, have been shown to be positively correlated or not.
related\textsuperscript{107} to the infant’s weight. In LGA neonates, however, cord blood adiponectin is lower, corresponding to increased fat mass at birth. \textsuperscript{108}

Maternal adiponectin is a high-molecular-weight protein (30 kDa), and consequently, does not cross the placenta,\textsuperscript{109} but may control nutrient transfer across the placenta to the fetus. Full-length adiponectin inhibits insulin-stimulated amino acid transport in placental cell cultures,\textsuperscript{110} suggesting that adiponectin may directly influence nutrient availability necessary for fetal growth.

Maternal adiponectin may also indirectly regulate fetal growth through regulation of insulin sensitivity. This has been observed in cases of gestational diabetes, where serum adiponectin at 11 weeks\textsuperscript{111} and 24 weeks gestation\textsuperscript{112} was negatively correlated with infant birthweight. This association has also been observed in normal pregnancies, where macrosomia is negatively correlated with maternal adiponectin at 37 weeks gestation.\textsuperscript{109} Lower levels of adiponectin in pregnancy may lead to insulin resistance, leading to dysregulation of fetal growth in utero. This effect may be more pronounced in obese, pregnant women.

IL-6

IL-6 is a pleiotropic cytokine involved in both immune regulation and non-immune events in a variety of cell types and tissues.\textsuperscript{113} Adipose tissue is a significant source of IL-6 production in obese individuals, where IL-6 can be released by adipocytes,\textsuperscript{114} or macrophages that have infiltrated adipose tissue.\textsuperscript{115} In fact, an estimated 10-35\% of circulating IL-6 is derived from adipose tissue.\textsuperscript{114} IL-6 induces cellular signaling by binding its membrane bound receptor (IL-6r) or soluble (sIL-6r) receptor,\textsuperscript{116} enabling it to associate with glycoprotein 130 to initiate intracellular signal transduction.\textsuperscript{117} At this stage, IL-6 can exert pro- or anti-inflammatory
effects. Activation of the Janus-activated protein kinases (JAK)/signal transducer and activator of transcription (STAT3) pathway induces transcription of suppressors of cytokine signaling (SOCS), which applies negative feedback on the pathway, resulting in anti-inflammatory effects. Alternately, the activation of the mitogen-activated protein kinase (MAPK) induces inflammation through activation of Extracellular signal-Regulated Kinases (ERK) and c-Jun NH(2)-terminal protein Kinases (JNK). The stimulation of a specific pathway may depend on the metabolic state of the cell or a combination of external factors such as the presence of other inflammatory cytokines or soluble receptors.

Transient increases in IL-6 concentration, as observed in acute illness or during exercise, may contribute to normal glucose homeostasis in healthy individuals by promoting glucose uptake in skeletal muscles through activation of AMPK. In states of chronic inflammation, such as obesity, sustained elevation of IL-6 levels may prompt insulin resistance. Through activation of the JNK pathway, chronic IL-6 exposure inhibits IRS-1 activation, thus reducing glucose uptake in peripheral tissues. There is strong evidence that IL-6 also contributes to hepatic insulin resistance. In hepatocytes, IL-6 inhibits tyrosine phosphorylation of IRS-1, stimulating glycogenolysis and gluconeogenesis, and further promoting glucose infusion into the blood.

IL-6 levels rise during pregnancy due to placental production and increased maternal fat stores. During pregnancy, IL-6 is secreted by maternal adipose tissue and the placenta, and is thought to contribute to maternal insulin resistance necessary for fetal growth. Serum IL-6 is significantly higher in the third trimester, when fetal linear growth is most significant, compared to early pregnancy. Obese pregnant women have elevated levels of IL-6 compared to pregnant
women with a healthy body weight. IL-6 is also elevated in cases of gestational diabetes, suggesting that IL-6 is related to hyperglycemia and insulin resistance in pregnancy.

Elevated maternal IL-6 may also directly influence fetal growth. Maternal IL-6 has been shown to be positively correlated with fetal adiposity in normal and diabetic pregnancies. IL-6 stimulates fatty acid accumulation and amino acid transporter system A activity in human placental cell cultures, suggesting that IL-6 may increase fatty acid and amino acid transfer to the fetus and contribute to fetal overgrowth.

**TNF-α**

TNF-α is an inflammatory cytokine produced primarily by macrophages and adipocytes. The principal physiologic functions of TNF-α are to induce insulin resistance, inflammation, and apoptotic cell death, and prevent viral replication. TNF-α negatively influences glucose metabolism and insulin sensitivity through the inhibition of insulin-stimulated tyrosine phosphorylation of insulin receptors in muscle and adipose tissue, which hinders translocation of glucose transporter 4 (GLUT 4) to the cell membrane. TNF-α further induces insulin resistance in skeletal muscle through suppression of AMPK activity, reducing fatty acid oxidation. This leads to accumulation of diacylglycerol in muscle tissue, causing resistance to insulin activity and reducing glucose uptake in peripheral tissues. TNF-α stimulates IL-6 production and inhibits adiponectin production further escalating inflammation and insulin resistance. TNF-α is elevated in the insulin resistant states of obesity and type 2 diabetes, where increases may be the result of oxidative stress caused by hyperglycemia. Elevated levels appear to be both a consequence and a mediator of insulin resistance.
TNF-α levels rise during pregnancy due to placental production and increased maternal fat stores.\textsuperscript{133} Moderate increases in TNF-α, similar to other adipokines, contributes to the insulin resistance necessary for glucose transport to the growing fetus. Pregravid BMI is the best predictor of TNF-α levels in normal and diabetic pregnancies, as elevated levels are associated with higher BMI.\textsuperscript{96} TNF-α is further elevated in the third trimester of pregnancy compared to pregravid levels.\textsuperscript{134} A significant amount of TNF-α is derived from the placenta, where infiltrated macrophages express high levels of proinflammatory cytokines.\textsuperscript{134,135}

Elevated maternal TNF-α may contribute to fetal overgrowth. At birth, the concentration of TNF-α is three times higher in the placentas of obese (>16% body fat) newborns compared to lean controls.\textsuperscript{136} TNF-α stimulates amino acid transporter system A activity in human placental cells, indicating that TNF-α may contribute to fetal overgrowth through excessive amino acid transfer to the fetus.\textsuperscript{16} Data from animal models suggest that exposure to elevated TNF-α in utero results in programming of obesity and fetal overgrowth.\textsuperscript{137} TNF-α, similar to leptin, adiponectin, and IL-6, may influence fetal growth directly by stimulating placental nutrient transport, and indirectly through modulation of insulin resistance.

**Purpose**

Although the relationship between pregravid maternal BMI and insulin resistance to fetal growth is well established, the role of adipokines is less clear. Current data regarding the association of adipokines to birthweight is limited. Much of this data is derived from women with pregnancies complicated by gestational diabetes, where macrosomia and LGA births are more common. Obese women may experience hyperglycemia below the diagnostic threshold for gestational diabetes and the subsequent effects on fetal growth.
The purpose of this sub-study is to determine if, and to what degree, a relationship exists between maternal adipokines (leptin, adiponectin, TNF-α, IL-6), indices of insulin resistance (insulin, glucose, A1c, HOMA-IR) and infant size at birth [birthweight, size for gestational age (SGA, AGA, LGA), macrosomia, ponderal index] in non-diabetic, obese pregnant women.

Hypotheses

Null (H₀)

1. There is no relationship between maternal leptin at 26 weeks gestation and infant birthweight, size for gestational age (SGA, AGA, LGA), macrosomia, or ponderal index.

2. There is no relationship between maternal adiponectin at 26 weeks gestation and infant birthweight, size for gestational age (SGA, AGA, LGA), macrosomia, or ponderal index.

3. There is no relationship between maternal IL-6 at 26 weeks gestation and infant birthweight, size for gestational age (SGA, AGA, LGA), macrosomia, or ponderal index.

4. There is no relationship between maternal TNF-α at 26 weeks gestation and infant birthweight, size for gestational age (SGA, AGA, LGA), macrosomia, or ponderal index.

5. There is no relationship between maternal fasting glucose at 26 weeks gestation and infant birthweight, size for gestational age (SGA, AGA, LGA), macrosomia, or ponderal index.

6. There is no relationship between maternal fasting insulin at 26 weeks gestation and infant birthweight, size for gestational age (SGA, AGA, LGA), macrosomia, or ponderal index.
7. There is no relationship between maternal HOMA-IR at 26 weeks gestation and infant birthweight, size for gestational age (SGA, AGA, LGA), macrosomia, or ponderal index.

8. There is no relationship between maternal A1c at 26 weeks gestation and infant birthweight, size for gestational age (SGA, AGA, LGA), macrosomia, or ponderal index.

Methods

This sub-study is part of the larger intervention study, “DHA, Inflammation, and Insulin Sensitivity in Obese Pregnant Women,” (5R21HL093532-02) granted to Dr. Debra Krummel, principal investigator. Approval of the study protocol was granted by the University of Cincinnati Institutional Review Board (IRB) and Cincinnati Children’s Hospital Medical Center (CCHMC) IRB.

Subjects

Pregnant women 18-40 years of age, with pregravid BMI ≥ 25, singleton pregnancy, and < 26 weeks gestation were recruited for participation in the study. Exclusion criteria included gestational diabetes mellitus controlled with insulin or medication (diet-controlled was acceptable for the study), hypertension, high unusual intake of docosahexaenoic acid (DHA) (more than 1 fish meal per week, use of DHA-fortified foods, or DHA supplement use), concurrent inflammatory, vascular or metabolic disease; current or previous use of tobacco, street drugs, or medications such as corticosteroids that affect inflammatory markers; and inability to travel to the Clinical Translational Research Center (CTRC) at CCHMC for study visits.
Participant incentives included a gift card ($150), a children’s story book and a plush baby toy upon completion of the study. Parking was free in the CCHMC garage and bus tokens were provided to subjects as needed.

Recruitment and Screening

Subjects were recruited from the greater Cincinnati area. Study staff distributed flyers to physicians offices, prenatal clinics, and participating hospitals (University of Cincinnati Hospital, the Christ Hospital, and Mercy Fairfield Hospital). Participants were also recruited through advertisements in direct mailings, including REACH magazine, the CCHMC booklet "Research Studies at Cincinnati Children's - A sampling of studies", and Cincinnati Family Magazine. Participants could also find information regarding the study on the study website: http://www.uc.edu/pregnancystudy.html or on Facebook.

Screening of potential candidates was conducted by telephone using a standardized script and screening form. Individuals meeting the screening criteria were invited to participate in the study, and scheduled for the study visit at 26 weeks gestation. Self-reported pregravid height and weight, to be used for body mass index (BMI) calculation, was collected at the time of screening.

Study Visit

The study visit was conducted at the Clinical Translational Research Center (CTRC) at Cincinnati Children’s Hospital Medical Center (CCHMC), at the beginning (26 weeks gestation) of the third trimester of pregnancy. Participants completed informed consent and Heath Insurance Portability and Accountability Act (HIPAA) forms prior to the start of the visit.
CTRC nursing staff obtained height and weight data, and 10 mL of blood via veinipuncture for analysis of glucose, insulin, A1c, leptin, adiponectin, IL-6 and TNF-α.

Sample Collection and Processing

Insulin, leptin, and adiponectin. A minimum of 2 mL of blood was collected in a gold top (SST) tube. After holding upright at room temperature for 30 minutes, tube was centrifuged at 3500 rpm for 10 minutes. Equal amounts of serum aliquoted into 3 tall plastic freezer tubes. Tubes were labeled with patient ID number, study visit number (1), date and time of specimen collection, and either “insulin,” “leptin,” or “adiponectin.” If the full 2 ml of blood could not be obtained, serum was aliquoted first into the adiponectin tube, second into the insulin tube, and third into the leptin tube. Samples were stored at -20 ºC, for later transfer to -80 ºC.

TNF-α and IL-6. A minimum of 2 mL of blood was collected in a EDTA tube. Inversion of the tube three to five times to allowed the anti-coagulant to thoroughly mix with the blood. After holding upright at room temperature for 10-15 minutes, the sample was centrifuged at 3500 rpm for 10 -15 minutes. Plasma was pipetted from the cell pellet, with 0.5 mL of plasma aliquoted into plastic freezer tubes. Tubes were labeled with patient ID number, study visit number (1), date and time of specimen collection, and “cytokines- lab”. If the full sample could not be obtained, plasma was aliquoted into as many tubes as possible. Samples were stored at -20 ºC, for later transfer to -80 ºC.
Radioimmunoassay

Plasma leptin and insulin levels were obtained via radioimmunoassay. In this process, a labeled tracer antigen incubated with a constant dilution of the antiserum. When added to the system, unlabeled antigen competes with the labeled tracer antigen for binding sites on the antibody. The amount of tracer that is bound to the antibody decreases as the concentration of unlabeled antigen increases. The amount of tracer antigen and bound antibody was calculated and a standard curve was set up to determine the amount of unlabeled antigen in the unknown samples. Both the leptin and insulin assays were obtained from Linco Research. The leptin assay uses $^{125}$I-labeled Human Leptin and a Human Leptin antiserum to determine the level of Leptin in the serum by using a double antibody/PEG technique. The insulin assay uses $^{125}$I-labeled Human Insulin and a Human Insulin antiserum to determine the level of Insulin in the serum by using a double antibody/PEG technique. This analysis was conducted by Theresa Kenney in the Clinical Laboratory at CCHMC.

ELISA

Adiponectin, IL-6 and TNF-α were measured from plasma samples using enzyme-linked immunosorbent assays (ELISA). Milliplex™ Multiplex kits (Millipore, Billerica, MA) were used according to the manufacturer protocol. Forty samples were run in duplicate on a 96-well plate using luminex technology on the Bio-Plex™ (Bio-Rad, Hercules, CA). The concentrations were calculated from standard curves using recombinant proteins. The analysis was conducted by the Cytokine and Mediator Measurement Core Laboratory run by Dr. Marsha Wills-Karp at CCHMC.
Blood Glucose

A drop of blood from venipuncture was analyzed using a bedside glucometer at the time of the blood draw by the nursing staff at the CTRC.

Hemoglobin A1c

A1c was measured through a modification of a high-performance-liquid-chromatography (HPLC). A minimum of 500 μl of EDTA whole blood was collected by venipuncture. An Alliance 2690/2695 HPLC (Waters Corporation) and a PolyCAT A (PolyLC, Inc.) column were used to separate the hemoglobin fractions by cation-exchange chromatography. The hemoglobin A1c was then quantified using a dual wavelength detector (model 2487, Waters Corp.) and Empower Software (Water Corp.). Results are reported as percent (normal range 3.5-6.3%). The coefficient of variation based on the normal control <3.2% and <1.3% for the elevated control. The HPLC method is sensitive to 0.05%. The analysis was conducted in the Hemoglobinopathy Lab at CCHMC.

Insulin Resistance

Homeostatic model assessment of insulin resistance (HOMA-IR) is an index of insulin resistance that is calculated from fasting glucose and insulin values. This index provides an accurate estimation of whole body insulin resistance, without the cumbersome methods involved in measurement by euglycemic clamp or oral glucose tolerance tests,\textsuperscript{138} and has been validated as an accurate alternative to the euglycemic clamp for the third trimester in obese, pregnant women.\textsuperscript{139} HOMA-IR index is calculated from the formula:\textsuperscript{140}
Infant Anthropometry

Each participant, after successful completion of all clinic study visits, was contacted by mail within six weeks following her estimated due date. The mailing included a note card expressing gratitude for participation in the study and a self-addressed, stamped postcard for collection of birth data (Appendix 1). The participant recorded the requested birth data (infant date of birth, gender, weight, length, head circumference, Apgar score, and delivery type) and returned the postcard to study staff via mail.

Gestational age of the infant was ascertained from the self-reported date of last menstrual period or ultrasound records and infant’s date of birth. Birthweight for gestational age was plotted on the chart developed from 1999-2000 U.S. Natality reference data (Appendix 2). Designation of LGA was based on a birthweight ≥ 90th percentile for gestational age and gender in the United States. Infants between the 10th and 90th percentile for gestational age were appropriate-for-gestational-age (AGA). Infants below the 10th percentile were considered small-for-gestational-age (SGA).

Macrosomia was defined as birthweight ≥4000 grams for infants born between 37 to 42 weeks gestation.

Ponderal index was calculated using the formula:

\[ \text{Large birthweight} \text{ was defined as ponderal index} \geq 90^{th} \text{ percentile, as plotted on a ponderal index for gestational age chart (Appendix 3).} \]
**Statistical Analysis**

All data is expressed as mean ± SD, with \( P < 0.05 \) the level of significance. The distribution of maternal metabolic variables (glucose, insulin, A1c, HOMA-IR, leptin, adiponectin, IL-6, TNF-\( \alpha \)) and infant anthropometry (birthweight, ponderal index) were checked for normality (Kolmogorov-Smirnov \( P > 0.05 \)).

The data set was assessed for outliers, and variables exceeding 3 SDs above the mean were excluded from analysis. The exclusion criteria restricted women with pre-existing inflammatory conditions from participation in the study, but a few women presented with other conditions that corresponded to abnormally high adipokine levels. The following data points were excluded from analyses: leptin data from participants with polycystic ovarian syndrome \((n=2)\), a condition shown to be associated with elevated circulating leptin,\(^{142}\) TNF-\( \alpha \) data from participants with food allergies \((n=2)\), where TNF-\( \alpha \) concentrations are abnormally elevated,\(^{143}\) IL-6 data from participants with diet-controlled gestational diabetes \((n=1)\) and abnormally high lipid profile \((n=1)\), both shown to be associated with elevated IL-6 concentrations.\(^{71}\)

Pearson’s correlation coefficient was used to characterize relationships between independent variables (maternal adipokines) and dependent variables [infant birth size (birthweight, ponderal index)]. Pearson’s correlation was also used to determine relationships between maternal glucose metabolism and infant birth size (birthweight, ponderal index). Pearson’s correlations were controlled for maternal pregravid body weight, as this was positively correlated with infant anthropometry. Linear regression analysis was conducted on variables that were significantly correlated.
Differences in maternal adipokines and markers of glucose metabolism among categories of infant size (SGA, AGA, LGA, ponderal index percentiles, and macrosomia) were calculated using analysis of variance (one-way ANOVA).

Data was analyzed using the software Statistical Package for the Social Sciences (version 18.0, 2010, SPSS, Inc, Chicago, IL).

Results

Maternal demographic and anthropometric data is reported in Table 1. Based on self-reported pre-pregnancy height and weight, all participants were either overweight, \( n=18 \) (BMI \( \geq 25 \text{ kg/m}^2 \)) or obese, \( n=34 \) (BMI \( \geq 30 \)) prior to pregnancy.
Table 1. Sample Characteristics of the Mother, \((n=52)\)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (± SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27.3 (5.0)</td>
<td>18–39</td>
</tr>
<tr>
<td>Education level</td>
<td>Highest Level Achieved %</td>
<td></td>
</tr>
<tr>
<td>High school diploma/GED or less</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Some College</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>College Degree</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Post-baccalaureate coursework</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, non-Hispanic</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Black, non-Hispanic</td>
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<tr>
<td>Hispanic</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Asian or Pacific Islander</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Native American/Alaskan</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Parity (# infants carried to term)</td>
<td>1.53 (1.52)</td>
<td>0–5</td>
</tr>
<tr>
<td>Height (inches)</td>
<td>64.3 (2.5)</td>
<td>59.0–69.0</td>
</tr>
<tr>
<td>Pregravid weight (lb)</td>
<td>203.5 (52.1)</td>
<td>139–347</td>
</tr>
<tr>
<td>Pregravid BMI (kg/m²)</td>
<td>34.6 (8.3)</td>
<td>25.0–57.7</td>
</tr>
<tr>
<td>Weight at 26 weeks gestation (lb)</td>
<td>218.7 (52.1)</td>
<td>147.0–371.1</td>
</tr>
<tr>
<td>Gestational weight gain (lb)</td>
<td>25.5 (13.9)</td>
<td>-11.7–59.5</td>
</tr>
</tbody>
</table>
### Table 2. Maternal Markers of Glucose Metabolism at 26 Weeks Gestation

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean (± SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (uU/mL)</td>
<td>47</td>
<td>23.7 (9.9)</td>
<td>9.4–52.2</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>52</td>
<td>84.6 (8.7)</td>
<td>58–102</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>47</td>
<td>5.0 (0.34)</td>
<td>1.7–10.7</td>
</tr>
<tr>
<td>A1c %</td>
<td>52</td>
<td>4.5 (0.5)</td>
<td>3.4–5.4</td>
</tr>
</tbody>
</table>

### Table 3. Maternal Adipokines at 26 Weeks Gestation

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean (± SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/mL)</td>
<td>45</td>
<td>26.5 (13.5)</td>
<td>11.5–81.6</td>
</tr>
<tr>
<td>Adiponectin (ng/mL)</td>
<td>50</td>
<td>4756 (1636)</td>
<td>1252–9040</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>50</td>
<td>7.8 (7.7)</td>
<td>1.3–38.6</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>50</td>
<td>5.8 (1.7)</td>
<td>1.1–7.8</td>
</tr>
</tbody>
</table>
Maternal Adipokines and Infant Anthropometry

Leptin

Table 4. Infant Characteristics at Birth, (n=52)

<table>
<thead>
<tr>
<th></th>
<th>Mean (± SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wks)</td>
<td>39.1 (1.6)</td>
<td>34.2–42.1</td>
</tr>
<tr>
<td>Birthweight (lb)</td>
<td>7.6 (1.1)</td>
<td>4.3–9.1</td>
</tr>
<tr>
<td>Length (in)</td>
<td>20.1 (1.1)</td>
<td>17.0–22.0</td>
</tr>
<tr>
<td>Male Sex %</td>
<td></td>
<td>56</td>
</tr>
<tr>
<td>Ponderal index [(g*100)/cm^3]</td>
<td>2.6 (0.4)</td>
<td>1.9–4.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percentile</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;90th</td>
<td>25</td>
</tr>
<tr>
<td>50th-90th</td>
<td>39</td>
</tr>
<tr>
<td>10th-50th</td>
<td>21</td>
</tr>
<tr>
<td>&lt;10th</td>
<td>15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Size for Gestational Age</th>
<th>Percentile</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGA (&gt;90th)</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>AGA (10-90th)</td>
<td></td>
<td>69</td>
</tr>
<tr>
<td>SGA (&lt;10th)</td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

| Macrosomia (>4000g) %    | 14         |

Maternal leptin and infant weight ($r=.040$, $P>0.05$) or ponderal index ($r=.085$, $P>0.05$) were not significantly correlated when controlled for pregravid body weight. There was no difference in mean leptin among categories of infant size at birth ($F (2, 42) = 1.61$, $P>0.05$), ponderal index ($F (1, 43) = .364$, $P>0.05$), or macrosomia ($F (1, 43) = .007$, $P>0.05$). This supports the null hypothesis, that there is no relationship between maternal leptin at 26 weeks
gestation and infant birthweight, size at birth (SGA, AGA, LGA), macrosomia, or ponderal index. However, there was a non-significant increase in maternal leptin across categories of size for gestational age (Figure 1).

![Graph showing maternal leptin across categories of size for gestational age](image)

**Figure 1. Maternal Leptin across categories of size for gestational age**

**Adiponectin**

Maternal adiponectin and infant weight ($r=0.093, P>0.05$) or ponderal index ($r=-0.017, P>0.05$) were not significantly correlated when controlled for pregravid body weight. There was no difference in mean adiponectin among categories of infant size at birth ($F(2, 47) = 0.440, P>0.05$), ponderal index ($F(1, 48) = 0.840, P>0.05$), or macrosomia ($F(1, 48) = 0.171, P>0.05$). This supports the null hypothesis, that there is no relationship between maternal adiponectin at 26 weeks gestation and infant birthweight, size at birth (SGA, AGA, LGA), macrosomia, or ponderal index.
Maternal IL-6 and infant weight ($r = -0.026, P > 0.05$) or ponderal index ($r = -0.132, P > 0.05$) were not significantly correlated when controlled for pregravid body weight. There was no difference in mean IL-6 among categories of infant size at birth ($F(2, 47) = 0.051, P > 0.05$), ponderal index ($F(1, 48) = 0.204, P > 0.05$), or macrosomia ($F(1, 48) = 0.197, P > 0.05$). This supports the null hypothesis, that there is no relationship between maternal IL-6 at 26 weeks gestation and infant birthweight, size at birth (SGA, AGA, LGA), macrosomia, or ponderal index. However, there was a non-significant increase in maternal IL-6 across categories of size for gestational age (Figure 2).

![Figure 2. Maternal IL-6 across categories size for gestational age](image-url)
TNF-α

Maternal TNF-α and infant weight ($r= -0.031, P > 0.05$) or ponderal index ($r= 0.080, P > 0.05$) were not significantly correlated when controlled for pregravid body weight. There was no difference in mean TNF-α among categories of infant size at birth ($F (2, 47) = 0.008, P > 0.05$), ponderal index ($F (1, 48) = 0.836, P > 0.05$), or macrosomia ($F (1, 48) = 0.996, P > 0.05$). This supports the null hypothesis, that there is no relationship between maternal TNF-α at 26 weeks gestation and infant birthweight, size at birth (SGA, AGA, LGA), macrosomia, or ponderal index. There was an non-significant increase in maternal TNF-α concentration with increasing ponderal index percentile (Figure 3).

Figure 3. Maternal TNF-α across categories of ponderal index percentile
Maternal Glucose Metabolism and Infant Anthropometry

Fasting glucose

Maternal fasting glucose and infant weight \( r = -0.005, \ P > 0.05 \) or ponderal index \( r = -0.041, \ P > 0.05 \) were not significantly correlated when controlled for pregravid body weight. There was no significant difference in mean fasting glucose among categories of infant size at birth \( F(2, 49) = .310, \ P > 0.05 \), ponderal index \( F(1, 50) = .059, \ P > 0.05 \), or macrosomia \( F(1, 50) = .032, \ P > 0.05 \). This supports the null hypothesis, that there is no relationship between maternal fasting glucose at 26 weeks gestation and infant birthweight, size at birth (SGA, AGA, LGA), macrosomia, or ponderal index. There was an non-significant increase in maternal fasting glucose with increasing size for gestational age (Figure 4).

![Figure 4. Maternal Glucose across categories of size for gestational age](image)

Figure 4. Maternal Glucose across categories of size for gestational age
Fasting Insulin

Maternal fasting insulin and ponderal index \( (r=+0.324, P<0.05) \) but not infant weight \( (r=-0.131, P>0.05) \) were significantly correlated when controlled for pregravid body weight. Maternal insulin explained 16% of the variance in infant ponderal index \( (R^2 = .16, F = 7.32, P < .01) \) (Figure 5). There was no difference in mean fasting insulin among categories of infant size at birth \( (F (2, 44) = .088, P>0.05) \), ponderal index \( (F (1, 45) = 1.497, P>0.05) \), or macrosomia \( (F (1, 46) = .863, P>0.05) \). However, there was a non-significant increase in maternal insulin across categories of size for gestational age (Figure 6) and ponderal index percentile groups (Figure 7). This supports rejection of the null hypothesis, which states there is no relationship between maternal fasting insulin at 26 weeks gestation and ponderal index.

Figure 5. Regression plot of maternal insulin and ponderal index
Figure 6. Maternal insulin across categories of size for gestational age

Figure 7. Maternal insulin across categories of ponderal index percentile
HOMA-IR

Maternal HOMA-IR and ponderal index ($r=+0.292$, $P<.050$), but not infant weight ($r=-.111$, $P>0.05$) were significantly correlated when controlled for pregravid body weight. There was no difference in mean HOMA-IR among categories of infant size at birth ($F(2, 44) = .074$, $P=.928$), ponderal index ($F(1, 45) = .973$, $P>0.05$), or macrosomia ($F(1, 45) = .795$, $P>0.05$). Maternal HOMA-IR also showed a non-significant increase across categories of size for gestational age (Figure 8) and ponderal index percentiles (Figure 9). This supports rejection of the null hypothesis that states there is no relationship between maternal HOMA-IR at 26 weeks gestation and infant ponderal index.

![Figure 8. Maternal HOMA-IR across categories of size for gestational age](image-url)
A1c

Maternal A1c and infant weight ($r=-.050$, $P>0.05$) or ponderal index ($r=.085$, $P>0.05$) were not significantly correlated when controlled for pregravid body weight. There was no difference in mean A1c among categories of infant size at birth ($F(2, 49) =1.00$, $P>0.05$), ponderal index ($F(1, 50) = .024$, $P>0.05$), or macrosomia ($F(1, 50) = 1.481$, $P>0.05$). This supports the null hypothesis that states there is no relationship between maternal A1c at 26 weeks gestation and infant birthweight, size at birth (SGA, AGA, LGA), macrosomia, or ponderal index.
Discussion

The levels of maternal adipokines observed in the present study were similar to those previously reported in pregnant women. Mean maternal leptin in our sample (26.5 ng/mL) were comparable to those reported in obese (24.0 ng/mL)\textsuperscript{144} and healthy weight (25.8 ng/mL)\textsuperscript{145} pregnant women in the third trimester. Mean TNF-\(\alpha\) levels in the current study (5.8 pg/mL) were also similar those measured in healthy, pregnant women (4.66 ng/mL).\textsuperscript{146} Mean maternal adiponectin concentrations (4756 ng/mL) were similar to those detected at 28 weeks gestation in pregnancies complicated by impaired glucose tolerance (4500 ng/mL).\textsuperscript{147}

Maternal markers of glucose metabolism in the present study, however, were similar to concentrations typically seen in pregnancies complicated by impaired glucose tolerance. Third trimester maternal fasting insulin in the present study matched concentrations observed in cases of gestational diabetes mellitus (26.1 uU/mL)\textsuperscript{148} more closely than typical in normal pregnancy (16.8 uU/mL).\textsuperscript{149} Likewise, fasting glucose in the study population (84.6 mg/dL) was more comparable to fasting values observed in women with gestational diabetes (85.2 mg/dL)\textsuperscript{148} versus normal pregnancies (80.9 mg/dL).\textsuperscript{51}

These data comparisons confirm that non-diabetic, obese pregnant women experience a considerable degree of insulin resistance and inflammation, despite falling below the diagnostic threshold for gestational diabetes. These women have some of the metabolic features associated with diabetes, and therefore have an increased risk of birthing a large infant.

The incidence of large infant births was higher in our sample of obese, pregnant women when compared to United States reference data. In this study, 7 of 52 infants (14%) were classified macrosomic, which is over 1.5 times the rate observed in the United States (8%) in
2008. However, this high rate of macrosomia corresponds to incidence data reported previously in obese, pregnant women.\textsuperscript{383}

In the United States, 12% of term newborns were classified LGA in 2003.\textsuperscript{39} In the present study, over 21% of infants were born LGA (11 of 52), almost double the national rate. This corroborates previous research that notes higher incidence of LGA births to obese, pregnant women.\textsuperscript{39}

In the present study, 25% of infants (13 of 52) were classified above the 90\textsuperscript{th} percentile for ponderal index. Infants with ponderal index $\geq$ 90\textsuperscript{th} percentile are considered to have high adiposity.\textsuperscript{24} Currently, no reference data exists for the ponderal index, as it is used more frequently in research than in clinical settings or by government agencies. Other indices of fetal growth do not consider the sex of the infant in the determination of fetal size. Males are an average of 150-200 grams larger than females at birth.\textsuperscript{33} Ponderal index percentile charts include separate ranges for male and female for accurate identification of fetal overgrowth. When compared to other indices of fetal growth, ponderal index provides the best estimate of infant adiposity.

There was no statistical relationship between maternal adipokines and infant size at birth in the present study. Previous research in humans and animals has shown that infant size at birth is related to maternal adipokines in the third trimester.\textsuperscript{63} The absence of such a relationship in this study could be the result of the low statistical power associated with a smaller sample size ($n=52$).

Maternal leptin was markedly higher in mothers birthing LGA infants ($M=28.9$ ng/mL) versus SGA infants ($M=17.1$ ng/mL), however, these differences were not significant. Low leptin concentrations have been detected in the blood of SGA neonates, while high levels have
been measured in macrosomic newborns of diabetic pregnancies. Previous studies have established the relationship between fetal leptin and size at birth, but few have been able to establish a connection between maternal leptin and fetal growth.

Leptin was not related to insulin ($r=.156, P=.301$) or HOMA-IR ($r=.193, P=.199$) in this study, suggesting that the relationship of leptin to infant size at birth may not be linked to insulin resistance. The mechanism of action of leptin on fetal growth is likely associated with placental function. Leptin receptors are abundant in the placenta, where leptin stimulates amino acid transporter system A, augmenting transfer of amino acids to the fetus. Placental leptin also enhances mobilization of maternal fat stores, promoting transfer of free fatty acids across the placenta to the fetus. This increased availability of substrate may allow for accelerated growth in utero and the subsequent birth of infants LGA in pregnant women with hyperleptinemia.

Maternal IL-6 concentration was higher across categories of size for gestational age; however, these differences were not significant. Levels of IL-6 were noticeably higher in mothers delivering LGA ($M=8.4$ ng/mL) versus AGA ($M=7.7$ ng/mL) and SGA ($M=6.9$ ng/mL) infants. A similar relationship was observed in a cohort of obese pregnant women, where maternal IL-6 concentrations increased by 1 pg/mL across tertiles of neonatal adiposity. This evidence in humans is further supported by data from animal studies, where pregnant rodents injected daily with human IL-6 rendered offspring with 30-40% greater adipose tissue weight than controls.

Evidence suggests that IL-6 is involved in fetal fat deposition, but the exact mechanism of action is unclear. The influence of IL-6 on fetal growth may be indirect through the modulation of maternal insulin resistance, or direct through regulation of placental nutrient transport. Studies of obese, non-pregnant populations show that IL-6 is elevated in cases of
insulin resistance and type 2 diabetes, and multiple animal models have confirmed the role of IL-6 in the pathogenesis of insulin resistance of skeletal muscle and hepatocytes. In this sample, however, IL-6 was not correlated with insulin ($r=-.003$, $P=.983$), or HOMA-IR ($r=.006$, $P=.971$), indicating that IL-6 influences fetal growth through means other than insulin resistance.

In addition to the effects on insulin resistance, IL-6 has been shown to directly stimulate placental nutrient transport. In placental cell cultures, IL-6 stimulates fatty acid accumulation and amino acid transporter system A activity, increasing the availability of substrates necessary for fetal growth. These activities of IL-6 may combine to augment fetal growth in obese women with elevated IL-6 concentrations during pregnancy.

Maternal TNF-α was visibly higher in mothers delivering infants with ponderal index $>50^{th}$ percentile (2.0 pg/mL) compared to infants with ponderal index $<50^{th}$ percentile (1.4 pg/mL), however this increase was not significant. Ponderal index is a measure of adiposity in infants, therefore the association with TNF-α suggests a possible role of the cytokine in the development of fetal adiposity. A similar relationship was observed in obese pregnant women, where TNF-α concentration was three times higher in the placentas of obese (>16% body fat) newborns compared to lean controls. Data from animal models suggest that exposure to elevated TNF-α in utero results in programming of obesity and fetal overgrowth. Infusion of TNF-α in pregnant rodents corresponds to an increase in maternal insulin resistance and a 30-40% increase in adipose tissue weight in offspring compared to controls.

The influence of TNF-α may influence fetal growth indirectly through to the induction of maternal insulin resistance, or directly through the activation of placental nutrient transport. High concentrations of TNF-α have been found in cases of insulin resistance in obese pregnant and obese, non-pregnant individuals. TNF-α has been shown to be correlated with insulin
resistance and fetal overgrowth in pregnancies complicated by gestational diabetes. In this sample, however, there was no correlation between TNF-α and insulin \((r=-.030, P=.839)\) or HOMA-IR \((r=-.042, P=.778)\), suggesting that the effect of TNF-α on fetal growth may be a function of influence on placental nutrient transport. In placental cell cultures, TNF-α stimulates amino acid transporter system A activity, indicating that TNF-α may contribute to excessive amino acid transfer to the fetus. High concentrations of TNF-α in the placenta also stimulate phospholipase A2, promoting fetal fat accretion in utero. Evidence from animal studies, and the present study, suggests that TNF-α may influence fetal adiposity at birth.

Mean maternal fasting glucose was slightly higher in cases of LGA (86.4 mg/dL) versus AGA (83.8 mg/dL); however, this was not significant. Placental glucose transport occurs by facilitated diffusion, such that net transfer of glucose to the fetus is strongly influenced by maternal blood glucose concentrations. Maternal fasting glucose in the third-trimester is a stronger predictor of macrosomia, and is positively associated with birthweight and neonatal adiposity in non-diabetic pregnancies. Glucose did not predict macrosomia in the present study, likely because numbers of macrosomic infants in the study were relatively low. The association of maternal insulin and HOMA-IR with infant birth size confirms the role of insulin resistance in the pathogenesis fetal overgrowth. Maternal insulin resistance creates elevations in placental glucose transport to the fetus. Because maternal insulin does not cross the placenta, the fetal pancreas secretes insulin, facilitating glucose uptake. In excess, glucose promotes fetal fat deposition.

Maternal insulin was positively correlated with infant ponderal index, validating the importance of insulin in the regulation of fetal adiposity. This association is apparent in other
studies, where maternal serum insulin was positively correlated with infant ponderal index\textsuperscript{46} and was a predictor of high birthweight\textsuperscript{47} in overweight women with normal pregnancies. Maternal insulin explained 16.1\% of variance in infant ponderal index in a linear regression model (Figure 5). This association is stronger than previously observed, where maternal insulin sensitivity, as measured by euglycemic clamp, explained 7\% of neonatal adiposity.\textsuperscript{154}

HOMA-IR was also positively correlated with ponderal index. Maternal insulin resistance, as measured by HOMA-IR, has been shown to be positively correlated with fetal adiposity in obese women in a previous study.\textsuperscript{155} Similarly, insulin resistance, determined by OGTT, is associated with high infant birthweight and incidence of LGA.\textsuperscript{156}

The connection of insulin resistance to fetal overgrowth, despite no correlation with maternal adipokines, suggests that insulin resistance can influence fetal growth in utero, independent of maternal inflammation.

\textbf{Conclusion}

Non-diabetic, obese pregnant women experience insulin resistance, inflammation and the subsequent effects on fetal overgrowth. An indicator of fetal adiposity, ponderal index gives the best estimation of fetal overgrowth. Insulin resistance remains the most significant predictor of fetal overgrowth in this population, but there is some evidence that high concentrations of maternal adipokines, particularly leptin, IL-6, and TNF-\(\alpha\) may contribute to increased infant size at birth. Though the mechanism for this cannot be ascertained from this study, the lack of correlation between adipokines and insulin resistance suggests that adipokines influence placental nutrient transport and ensuing fetal growth.
Training and Support

University of Cincinnati Academic Health Center HIPAA training and NIH Protection of Human Subjects certification was completed by all study personnel. Approval of the study protocol was granted by the University of Cincinnati Institutional Review Board (IRB) and Cincinnati Children’s Hospital Medical Center (CCHMC) IRB.

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Appendix 1. Postcard used for infant anthropometry data collection

![Postcard for Infant Anthropometry Data Collection](image1)

**A New Arrival!**

- Date of Birth: / 20__
- Gender: male female
- Weight: lbs oz
- Length: inches
- Head Circumference: cm
- Delivery Type (circle one): vaginal / c-section
- Apgar Score:

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**Congratulations!**

Now that your baby has arrived, we’d love to hear about it!
Please fill out this card with information about your baby and drop it in the mail. We extend our warm wishes to you and your new baby!

Omega-3 Pregnancy Study
3202 Eden Avenue #201
ML 0394
Cincinnati, OH 45267-0394

Subject ID# ___
Appendix 2. Distribution of Size for Gestational Age by percentile

Appendix 3. Distribution of Ponderal index by Percentile

References


