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CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE
DA CRIANÇA E DO ADOLESCENTE

O AMBIENTE INTRAUTERINO COMO MODULADOR DOS PROCESSOS METABÓLICOS DO RECÉM-NASCIDO

SUZANA MARIA RAMOS COSTA

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TESE APRESENTADA AO PROGAMA DE PÓS-GRADUAÇÃO EM SAÚDE DA CRIANÇA E DO ADOLESCENTE DA UNIVERSIDADE FEDERAL DE PERNAMBUCO, PARA OBTENÇÃO DO TÍTULO DE DOUTOR EM SAÚDE DA CRIANÇA E DO ADOLESCENTE.

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"FAÇA O QUE PUDER,
COM O QUE TIVER,
ONDE ESTIVER."

THEODORE ROOSEVELT

DEDICATÓRIA

ÀS FAMÍLIAS, MÃES E BEBÊS, QUE PARTICIPARAM DESTE TRABALHO

À MINHA FAMILIA, QUE SEMPRE ME DEU O SUPORTE NECESSÁRIO PARA CHEGAR AO FIM DESTA CAMINHADA, ESPECIALMENTE AOS MEUS FILHOS, EMANUELE E PEDRO HENRIQUE, QUE ME DERAM O AMOR E A FORÇA PARA SUPERAR AS DIFICULDADES

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SUMÁRIO

LISTA DE SIGLAS

ADA: American Diabetes Association

ANOVA: Analysis of Variance

BCA: Bicinchoninic Acid

BMI: Body Mass Index

CDC: Centro de Controle e Prevenção de Doenças

cDNA: DNA complementar

CE: Cholesterol Esters

CEP IMIP: Comitê de Ética do IMIP

CNPq: Conselho Nacional de Pesquisa

CONEP: Comissão Nacional de Ética em Pesquisa

DAG: Diacylglycerol

DNA: Ácido Desoxirribonucleico

EUA: Estados Unidos da América

FFA: Free Fat Acids

Fulbright/CAPES: Colaboração Fulbright/CAPES

GC-MS: Gas Chromatography-Mass Spectrometry

GDM: Gestational Diabetes Mellitus

HAPO: Hyperglycemia and Adverse Pregnancy Outcome

HbA1c: Glycated Hemoglobin

HDL: High Density Lipoprotein

HGF: Hepatocyte growth factor

HIV: Human Immunodeficiency Virus

HOMA-IR: Homeostasis Model Assessment of Insulin Resistance

HUVEC: Human Umbilical Vein Endothelial Cells

IGF-I: Fator de Crescimento Insulina - símile Tipo I

IGF-II: Fator de Crescimento Insulina - símile Tipo II

IL-1β: Interleucina 1 subunidade β

IL-6: Interleucina 6

IL-8: Interleucina 8

IMC: Índice de Massa Corpórea

IMIP: Instituto de Medicina Integral Prof. Fernando Figueira

IR/IGR-IR: Hybrid Receptor

LDL: Low Density Lipoprotein

LIKA/UFPE: Laboratório de Imunologia Keizo Asami /Universidade Federal de Pernambuco

LMP: Last Menstrual Period

MCP-1: Monocyte Chemoattractant Protein-1

miRNA: microRNA

OGTT: Oral Glucose Tolerance Test

OMS: Organização Mundial da Saúde

OPAS: Organização Pan-Americana de Saúde

PAI-1: Plasminogen Activator Inhibitor type-1

PBS: Phosphate Buffered Saline

PKC: Protein Kinase C

PPIE: Ciclofilina E

qRT-PCR: Quantitative Reverse Transcriptase-Polymerase Chain Reaction

RNA: Ácido Ribonucleico

SDS: Sodium Dodecyl Sulfate

TG: Triglycerides

TNF-α: Tumor Necrosis Factor-α

VLDL: Very Low Density Lipoprotein

WHO: World Health Organization

RESUMO

A obesidade é reconhecida como um dos principais problemas de saúde pública mundial, e já se caracteriza como uma *pandemia*. As pesquisas apontam para a interação entre o genótipo e os fatores ambientais como a origem dos processos subjacentes à obesidade, sendo o período intra-útero o mais importante nesta gênese. Os receptores híbridos de insulina e IGF-I (fator de crescimento insulina-símile tipo 1) surgem como uma potencial etiologia molecular para explicar a base fisiopatológica da obesidade e das doenças associadas, que é a resistência à insulina. Então, revisamos sua importância no desenvolvimento da placenta e no crescimento fetal. Esta tese é composta por mais dois artigos originais, no primeiro avaliamos dados antropométricos maternos e do recémnascido, marcadores inflamatórios e bioquímicos, na busca de um biomarcador que pudesse identificar os recém-nascidos de maior risco para desenvolver obesidade.
Encontramos que o **í**ndice de **m**assa **c**orpórea (IMC) materno, o HOMA-IR (**Ho**meostasis **M**odel **A**ssessment of **I**nsulin **R**esistance) e a glicemia materna estão associados aos valores de insulina no cordão umbilical e o HOMA-IR à leptina do feto. Estes dados sugerem que mães obesas e/ou com resistência à insulina transferem mais nutrientes aos fetos, os quais respondem com maior produção pancreática de insulina e deposição de gordura, sendo o incremento da massa gorda responsável pelo aumento da leptina encontrada no cordão umbilical. No outro artigo original, procuramos a razão mais provável de resistência à insulina em nível intracelular. Verificamos se havia a formação de mais receptores híbridos de insulina e IGF-I. Porém, isso não foi comprovado. Alguns estudos mostram que a hiperglicemia e o excesso de lipídeos podem sobrecarregar as vias
oxidativas da glicose e dos ácidos graxos na mitocôndria originando a resistência à insulina. Em nosso trabalho, o soro das mães obesas apresentava hiperglicemia e excesso de lipídeos, que poderiam proporcionar esta alteração celular. Nossos resultados sugerem que o estado nutricional e a composição corporal maternos modulam o metabolismo energético durante o período intra-útero.
Considerando a complexidade da gestação, que envolve a mãe, o feto e a placenta, somada à plasticidade deste período de tão rápido desenvolvimento, tornam-se necessários mais estudos relacionados especialmente à influência da nutrição e de outros fatores ambientais maternos na regulação dos processos metabólicos do concepto.

PALAVRAS-CHAVES: RECÉM- NASCIDOS; OBESIDADE; METABÓLICO; AMBIENTE; INTRAUTERINO

ABSTRACT

Obesity is recognized as major public health problems worldwide, and already Characterized as a pandemic. Some studies indicate that the interaction between genotype
and environmental factors as the beginning of the underlying processes of the obesity, being the period intrauterine the most important in this genesis. The hybrid receptors for insulin and **i**nsulin-simile **g**rowth **f**actor type 1 (IGF-I) appear as a potential molecular etiology explaining the pathophysiological basis of the obesity and related diseases, which is insulin resistance. Then, we review its importance in placental development and fetal growth. This thesis consists of two original articles, the first article evaluated maternal and newborn anthropometry, biochemical and inflammatory markers to look for a biomarker that could identify newborns at higher risk for developing obesity. We found that maternal **b**ody **m**ass **i**ndex (BMI), **ho**meostasis **m**odel **a**ssessment of **i**nsulin **r**esistance (HOMAIR) and maternal glucose p-values were associated with cord blood insulin but only HOMA-IR was correlated with leptin. These data suggest that obese mothers and/or with insulin resistance transfer more nutrients to the fetus, whose respond with increased pancreatic production of insulin and fat deposition, and consequently the improved fat mass is responsible for the enhance of leptin found in the umbilical cord. In other article original, we was looking for the most likely reason for insulin resistance in intracellular level. We verified if there was more formation of hybrids receptors for insulin and IGF-I. However, this was not proven. Some studies have shown that hyperglycemia and excess lipids can overload the oxidative pathways of glucose and fatty acids in mitochondria leading to insulin resistance. In our study, the serum of obese mothers showed higher glycemia and lipids, which could provide this cellular alteration. Our results suggest that nutritional status and maternal body composition modulate energy metabolism during intrauterine life.
Considering the complexity of pregnancy, which involves the mother, the fetus and placenta, in addition to the plasticity of this period of very rapid fetal development, more studies concerning to the influence of nutrition and other maternal environmental factors in the regulation of metabolic processes of fetus are necessary.

KEY-WORDS: NEWBORN; OBESITY; METABOLIC; ENVIRONMENT; INTRAUTERINE

APRESENTAÇÃO

Nos últimos anos, em todo o mundo, tem se observado um aumento na prevalência da obesidade em países desenvolvidos como em desenvolvimento, em indivíduos de diferentes classes sócio-econômicas e em todas as faixas etárias. A Organização Mundial de Saúde (OMS)[1], a Organização Pan-Americana de Saúde (OPAS)[2] e o Centro de Controle e Prevenção de Doenças (CDC)[3] reconhecem a obesidade como um dos principais problemas de saúde pública, caracterizando-a como uma *pandemia.* A obesidade é um fenômeno complexo apenas podendo ser explicado por um modelo multicausal.

Meu interesse em estudar obesidade adveio da minha prática clínica, primeiro como pediatra e, posteriormente, como endocrinologista pediátrica, quando me deparei inúmeras vezes com crianças e adolescentes com sobrepeso e obesidade com problemas de integração na escola e com baixa autoestima. Esta situação era agravada na adolescência, quando os jovens têm mais necessidade de aceitação pelo grupo. Como o dever do pediatra é proporcionar o desenvolvimento biopsicossocial da criança e do adolescente, me senti motivada a fazer uma contribuição pontual na compreensão desta doença tão complexa. Assim, para um melhor esclarecimento da gênese das alterações observadas no metabolismo energético encontrado na obesidade, retornei aos conhecimentos de genética e biologia molecular adquiridos no mestrado.

Deste modo, descobri que atenção especial tem sido dada pelos pesquisadores à intrincada interação entre o genótipo do concepto e os diferentes fatores ambientais intra-uterinos. Em relação à obesidade materna, tem sido observado que há um maior risco de filhos de mães obesas desenvolverem síndrome metabólica na vida adulta, já sendo observado um maior risco para o aparecimento da obesidade nos primeiros anos de vida[4,5]. Neste contexto, a instalação da resistência periférica à insulina parece ser a chave para um melhor entendimento de uma série de problemas crônicos associados à obesidade, que já podem se manifestar clinicamente na faixa etária pediátrica. Uma possível explicação para a resistência à insulina é a observação de um defeito na sinalização da insulina e do IGF-I ao nível do receptor de insulina[6,7]. Então, percebi a necessidade de realizar uma revisão da literatura para me apropriar do conhecimento existente sobre a presença dos receptores de

insulina, IGF-I e seus híbridos na gestante focando na importância dos mesmos no desenvolvimento da placenta e crescimento fetal.

Em decorrência do artigo de revisão intitulado "O ambiente intra-uterino como fator de risco para a síndrome metabólica" publicado na Revista Brasileira de Saúde Materno Infantil[8] surgiram duas perguntas: 1) quais os parâmetros clínicos e laboratoriais maternos que podem identificar precocemente o recém-nascido propenso a desenvolver alterações metabólicas como resistência à insulina? e 2) a alteração da sinalização da insulina difere em nível dos receptores ou da cascata intracelular nos recém-nascidos de mães obesas e de mães com peso adequado? Procuramos respondê-las com os artigos apresentados a seguir.

No primeiro artigo original intitulado "Fetal cord blood insulin and leptin are correlated in offspring of obese mothers", que será enviado à *Pediatrics Diabetes*, observamos, pela avaliação do HOMA-IR, que as mães obesas têm mais resistência à insulina, favorecendo a transferência de mais glicose para o feto, que por sua vez, produz uma maior quantidade de insulina. Também nos chamou a atenção a correlação positiva do IMC materno com sua fração plasmática de LDL-colesterol, que dá indícios de uma maior disponibilidade de lipídeos para o feto. Além disso, a interleucina-6, um modulador do sistema A de transporte de aminoácidos da placenta, mostrou-se diretamente correlacionada com o IMC materno. Contrariamente, a leptina, que estimula a transferência de nutrientes da placenta para o feto, não mostrou associação com o IMC, mas forte correlação positiva com o HOMA-IR e a insulina do cordão umbilical. Estes dados dão força à idéia de um maior fluxo de nutrientes da placenta para o feto em mães obesas e/ou com resistência a insulina, que respondem com uma maior produção pancreática de insulina e deposição de gordura. Isso é responsável pelo aumento respectivo da insulina e leptina no cordão umbilical, os quais poderiam indicar uma adaptação do metabolismo fetal desde o período intra-uterino.

No segundo artigo original "Fetal cord blood lipids in offspring of obese mothers" será submetido à seção de comunicações breves do *The Journal of Pediatrics* em virtude da novidade da publicação. A pergunta condutora deste artigo foi: a alteração da sinalização da insulina difere em nível dos receptores ou da cascata intracelular nos recém-nascidos de mães obesas e de mães com peso adequado? Para respondê-la avaliamos a expressão gênica dos receptores de insulina e de IGF-I em busca da formação de mais receptores

híbridos, porém, isso não foi comprovado. Então, estudamos a via de sinalização intracelular. Encontramos maiores níveis glicêmicos e de lipídeos plasmáticos em mães obesas, que poderiam sobrecarregar as vias oxidativas na mitocôndria causando a interrupção da cascata de eventos dentro das células.

Para compreender os mecanismos pelos quais o ambiente intra-uterino desorganizado poderia alterar o metabolismo fetal era necessário um laboratório com técnicas avançadas já bem estabelecidas. A bolsa da Fulbright permitiu a utilização destas no laboratório da Dra. Mary Elizabeth Patti, situado no Joslin Diabetes Center da Harvard University, uma pesquisadora reconhecida no campo de pesquisa de diabetes melllitus tipo 2, tornando possível a elaboração dos dois artigos originais acima citados e gerando um banco de dados ainda a ser analisado, que permitirá novas contribuições ao problema em estudo.

CAPITULO I

ARTIGO DE REVISÃO DA LITERATURA

REVISÃO / REVIEW

The maternal intrauterine environment as a generator of children at risk of metabolic syndrome: a review

Suzana Maria Ramos Costa [1]
Giselia Alves Pontes da Silva [2]

O ambiente intrauterino como fator de risco para a síndrome metabólica: uma revisão

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Abstract

Nowadays, scientists are paying special attention to the increasing prevalence of obesity and associated co-morbidities, especially metabolic syndrome. This is due to observation of the spread of this syndrome from one generation to another and the growing number of obese pregnant women, which seems to exacerbate this situation. It is not yet well established whether the pathophysiological process underlying metabolic syndrome, namely insulin resistance, is due to changes in the receptor or in the cascade of intracellular processes. This narrative review aims to report on physiological and pathological changes occurring in pregnancy and the presence of Insulin receptor, Insulin Growth Factor-I receptor and the hybrid receptor, focusing on the presence of hyperinsulinemia in the growth and development of fetuses susceptible to metabolic syndrome.

Key words *Obesity, Metabolic syndrome, Insulin, Fetal development*

Resumo

O mundo científico está dando atenção especial ao crescimento da prevalência da obesidade e de suas co-morbidades, de modo particular da síndrome metabólica. Esse fato deve-se à observação da propagação dessa síndrome através de gerações e ao crescimento do número de gestantes obesas que parece agravar esta situação. Ainda não está bem estabelecido se o processo fisiopatológico subjacente à síndrome metabólica, a resistência à insulina, é por alteração no seu receptor ou na cascata de processos intracelulares. Esta revisão visa relacionar as alterações fisiológicas e patológicas da gestação e a presença dos receptores de insulina, Insulin Growth Factor-I e seus híbridos, focando na presença da hiperinsulinemia no crescimento e desenvolvimento do feto, a predisposição à síndrome metabólica.

Palavras-chave *Obesidade, Síndrome metabólica, Insulina, Desenvolvimento fetal*

Introduction

Given the increasing prevalence of obesity and its co-morbidities around the world, this issue has been the subject of much study. The presence of obesity is usually associated with other factors related to metabolic syndrome - characterized by the association between obesity, hypertension, cardiovascular disease, glucose intolerance, type 2 diabetes mellitus and dyslipidemia[1,2] - a major concern being the relation between this syndrome and the increasing prevalence of diabetes and consequent mortality related to cardiovascular disease. Metabolic syndrome or syndrome X is also called insulin resistance syndrome, owing to its pathophysiological basis.

Another reason for attention is the spread of this syndrome across generations. The growing number of obese pregnant women has contributed to this, as both maternal obesity prior to conception and excessive weight gain during pregnancy increase the risk of gestational diabetes mellitus and hypertensive disorders related to pregnancy.[3,4] Pregnancy would appear to be a catalyst of the pathophysiological process of metabolic syndrome, namely insulin resistance, which brings about a change in the receptor and other in a cascade of intracellular processes.[5] During pregnancy, the physiological reduction of insulin response in the tissues stimulates synthesis, thereby resulting in hyperinsulinemia.[5] This condition overloads the already compromised metabolism of obese individuals that makes them prone to such diseases, and changes subsequently occur in order to maintain the equilibrium of the intrauterine environment during gestation.[3,5]

This review aims to examine changes in the insulin receptor, the insulin growth factor-I receptor and its hybrid receptor and the physiological and pathological processes of pregnancy, focusing on the presence of these receptors in the placenta and their importance for growth of the fetus and predisposition to metabolic syndrome.

The hybrids of insulin receptors and IGF-I

In 1989, the hybrid receptors were described. They are composed of one α subunit and one β insulin receptor subunit and one α and one β IGF-I growth-factor receptor.[6] These receptors have subsequently been associated with insulin resistance syndrome,[7] because greater expression of hybrid receptors has been observed in the skeletal muscle[8] and adipose[9] tissues of patients with type 2 diabetes mellitus, in addition to the presence in greater quantities of these receptors in the skeletal muscle of patients with obesity[10] and a higher prevalence of chronic primary hyperinsulinemia.[11] Subsequently, hybrid receptors have been found in the endothelial cells of coronary arteries[12] and vascular smooth muscle cells[13] of healthy individuals. Studies have shown that hybrid receptors may represent the molecular defect of insulin resistance and its location provides the connection between changes in glucose metabolism and cardiovascular diseases associated with metabolic syndrome. The question was whether increased expression of the receptor hybrid is capable of giving rise to insulin resistance.

Li *et al*.[14] have shown that insulin at physiological concentrations does not activate hybrid receptors, although it can do so in large concentrations. Finally, in 2008, a study showed that human microvascular endothelial cells are insulin resistant as a result of the isolation of insulin receptors by hybrids, suggesting that these receptors are involved in the same molecular etiology of insulin resistance, as it has been reported that the affinity of insulin for this receptor is low and does not activate the cascade of intracellular events when insulin is in physiological quantities.[15]

Currently, the liveliest discussion with regard to hybrid receptors addresses the question of whether they precede hyperinsulinemia or are secondary to it. The work of Valensise *et al*.[16] was a milestone in this regard, because it showed that there were more hybrid receptors in the placentas of women with hyperinsulinemia than in those of women with normal levels of insulin. This study thus showed that excess insulin in the mother can induce the emergence of these receptors in the placenta. Another fetal tissue that presents hybrid receptors is the endothelium of the umbilical cord,[17] although it is not known whether there are changes in the quantities of the receptor in children of mothers with hyperinsulinemia.

Interestingly, the metabolic response and cell proliferation and differentiation during embryonic and fetal development depends on the binding of insulin and IGF receptors for insulin and IGF-I. Since there can be no changes in the amounts of these receptors by the expression of hybrid receptors in women with hyperinsulinemia, the whole process may be compromised. This may partly explain the insulin resistance, the restriction of growth and transmission of this trait to offspring.

Nevertheless, additional studies are required to ascertain whether the expression of hybrid receptors is increased in other fetal tissues, as an excess of

these receptors modifies the regulation of the passage of nutrients between mother and fetus and the mechanisms involved in activation and intracellular signal transduction in various tissues. This is a promising field of research because it will provide understanding of the molecular processes underlying a clinical disease that is extremely common in contemporary society.

From conception to fetus

Conception is a complex process that involves the mother, the placenta and the fetus itself. We will therefore present an explanation of how some of these factors interact to form an organism capable of survival.

Fetal "Programming"

The ultimate goal of the mother is to produce healthy offspring capable of transmitting their genes to a whole line of descendants. The term "mother system" would include all aspects of the physiology of maternal behavior that contribute to the production and growth of offspring. Through evolution, the "mother system" would have seized upon mechanisms that can lead to greater survival of offspring in both the intra- and the extra-uterine environment, thereby favoring the perpetuation of the species.[18] In adverse conditions, however, all the maternal stress involved in keeping the fetus alive may trigger adjustments to the fetus. In some extra-uterine environments, these adaptations may lead to a disposition to unfavorable diseases.[2]

Nowadays, two additional hypotheses are accepted to explain these mechanisms. In 1962, Neel[19] proposed the hypothesis of the "thrifty genotype" to explain the transmission of diabetes mellitus. According to this hypothesis the "thrifty" genes were selected by evolution, when food sources were scarce and there was need for an immediate response to insulin as a way of promoting a rapid increase in fat reserves. Fetal nutrition and growth responses in the prenatal period seem to vary as a result of this genotype.[19] Studies show that the fetal response to prenatal environment has no single outcome, but rather that there are a variety of responses that are potentially modulated by the selected genes.

In 1989, the hypothesis of the "thrifty phenotype",[2] also called the "hypothesis of fetal origin of adult disease" proposed by Barker and colleagues, based on epidemiological studies that linked low fetal weight, catch-up growth during the first years of life and subsequent development of diabetes and metabolic syndrome as a result of fetal malnutrition. This hypothesis suggests that fetal growth restriction is an adaptive response to an inadequate supply of nutrients to the fetus resulting in the diversion of these to some organs at the expense of others. An altered availability of nutrients to rapidly growing organs may lead to changes in size, structure and metabolic activity as a whole or in part, and other possible consequences. While these fetal responses help to increase the chances of survival in the intrauterine environment with limited supplies, they also seem to be associated with a long-term cost for the health of the individual. In the intrauterine period, the individual thus "schedules" its metabolism to be suitable for the estimated supply of extra-uterine environmental nutrients. If this environment is not restrictive as expected during the intrauterine period, this may facilitate the onset of diseases later in life.

In the postnatal period, catch-up growth occurs in most infants with intrauterine growth restriction as a result of adjustments of metabolic regulation for the provision of energy. There is thus rapid growth and increased weight gain when the body is exposed to an adequate diet or a diet rich in carbohydrates associated with little physical activity. This acceleration of the growth rate is particularly likely to occur in the first six months of life, but can be seen up to about two years of age.[20,21]

It has been shown that children born small for gestational age who underwent catch-up growth also had insulin resistance during this period, while those who did not undergo catch-up had normal insulin sensitivity. During the catch-up growth after birth, the accumulation of fat mass is faster than muscle mass. This rapid weight gain predisposes the child to obesity, type 2 diabetes mellitus and cardiovascular diseases.[20-22]

These assumptions can be viewed as complementary because the "thrifty" genes selected during evolution would change the metabolism and growth structures in the fetus, in response to intrauterine environmental stimuli, thereby providing it with protection. The “mother system” can thus serve to regulate the interaction between genes and the environment.

It is possible that an environment with scarce food has selected individuals with a gene expression mechanism that favors greater production of hybrid receptors when exposed to an adverse intrauterine environment. A larger quantity of these receptors would allow adaptation to the intrauterine environment and subsequently make the individual more susceptible to metabolic

syndrome.

Gametogenesis

Environmental effects on the genome are already seen in the early stages of development. During gametogenesis some genes in the egg are subject to the maternal imprinting system. This alters gene expression during development and can result in patterns of non-Mendelian inheritance. The mother can also vary the supply of nutrients and hormones to the gametes, changing the probability of survival of the embryo. These homeostatic processes can occur in many different ways and begin before conception. Ovulation is very sensitive to maternal energy reserves. It limits the beginning of new gestation periods in which nutritional status is more capable of supporting a pregnancy.[23]

The gametes and the preimplantation embryo

The maternal organism tries to optimize production of gametes and survival of the preimplantation embryo by giving them the ability to adapt their metabolic needs, the number of cells and the degree of differentiation prior to implantation *in utero*, thereby resulting in changes while still within the uterine environment.[23,24] Beside the nutritional support provided by the granulosa cells, there is bidirectional communication between the oocyte and granulosa in its normal maturation and development. The IGF-I receptor is present in both granulosa and in human oocytes. Insulin and IGF-I act through this receptor to regulate the expression and translocation of glucose transporters in both the embryo and in oocytes, where glucose uptake has been found to be mediated by such transporters.[24]

The preimplantation embryo uses pyruvate and lactate as its main sources of energy, although it expresses several glucose transporters. The rate of use of pyruvate decreases progressively from the eight-cell stage to the morula, coinciding with increased uptake of glucose into cells. During this period of compaction glycolytic metabolism begins, while the oxidation of lactate and pyruvate continues. Uptake of glucose is present in all stages of development. Before compaction, this is necessary for the development of the fetus, for the prevention of apoptosis and the transcription of some genes. However, it remains essential even after compaction.[24]

In addition to the glucose transporters, the preimplantation embryo expresses insulin and IGF-I receptors at the same time as starting glycolytic metabolism. The IGF-I receptor increases glucose use in response to insulin and IGF-I. Activation of insulin and IGF-I receptors, to allow the entry of glucose, trigger anti-apoptosis signals that are important for normal metabolism of the embryo. High concentrations of insulin or IGF-I lead to a drop in quantities of the IGF-I receptor. This would reduce the uptake of glucose-induced insulin and stimulate apoptosis in the blastocyst and the trophoblast cells.[24]

Fetus

During pregnancy, the "mother system" provides an environment for full fetal development and growth. It is thus possible to observe the physiological changes and pathological conditions that can alter this course.

It is well established that pregnancy is a normal period of physiological resistance to insulin. Throughout pregnancy, there is a progressive increase in resistance to provide an adequate provision of substrates for rapid development and a growth of the fetus. Maternal insulin resistance is important during normal gestation, as it plays an important role in the release of metabolites for fetal growth.[3,5]

Catalano *et al*.[25] showed that there is an increase of 120% in response to the first phase of insulin and 50% in the second phase response in pregnant women between 12 and 14 weeks, showing the early onset of insulin resistance in normal pregnancies. It was also observed that there is a one-third reduction in insulin sensitivity in pregnant women and a three-times higher response to insulin normal pregnant women compared with non-pregnant women. The levels of fasting plasma insulin increase throughout gestation, but these changes do not occur at the same time as reductions in concentrations of glucose.[5] This suggests that concentrations of glucose and insulin are not directly connected unless insulin sensitivity is altered or "glucostatic" pancreatic beta cells altered.

Obese women have a reduced tolerance to carbohydrates, which is aggravated by pregnancy. In obese women, therefore, fasting and post-prandial plasma insulin concentrations are higher than in non-obese women. In obese women, the excess insulin resistance induced by pregnancy or excess weight associated with an insufficient amount of insulin can result in the development of gestational diabetes.[3,5]

Insulin resistance is present in normal pregnancy and obesity is also involved in the pathogenesis of various complications of pregnancy such as gestational diabetes and hypertension.[5] As mentioned above, obese women have a higher concentration of insulin and lower insulin sensitivity when compared

to non-obese women. Despite these changes in insulin, there are cases where blood glucose levels rise. However, there are studies showing that women with a pre-conception body mass index greater than 30 kg/m² have 3.6 times greater risk of developing gestational diabetes than those with normal weight. Women with gestational diabetes have a high prevalence of hypertension (35-40%) compared to normoinsulinemic patients (5-10%).[3]

Obesity in pregnancy appears to be a risk factor for hypertension even in the absence of diabetes.[5] Pregnant women with hyperinsulinemia present systolic and diastolic blood pressure greater than those with normal levels of insulin, even when they cannot be classified as hypertensive women.[26] A positive association has been demonstrated between hyperinsulinemic women in the second quarter and the development of hypertension and pre-eclampsia.[27] Martinez *et al*.[28] showed an insulin concentration four times higher in women with pre-eclampsia in the oral glucose tolerance test when compared to the control group. The prevalence of pregnancy-induced hypertension in obese women is double that among those of normal weight, with an increased incidence of 4.8% in non-obese women compared to 10.2% in those who are obese.

It can thus be seen that obesity, diabetes and hypertension during pregnancy are interconnected and also that there is a fine line between the insulin resistance of normal pregnancy and high-risk pregnancies complicated by these diseases.

These changes during pregnancy have been implicated in the spread of the phenotype of insulin resistance across generations. Obesity is more common in the children of obese mothers, especially if the latter had gestational diabetes or if the children were macrosomic Obese mothers thus contribute to the geometric growth of the pandemic of obesity and its comorbidities.[5,26,29]

As mentioned above, the children of mothers with pregnancy problems such as obesity, diabetes and hypertension are more likely to develop insulin resistance. Wang *et al*.[30] have shown that this is seen very early in the postnatal period. It is not known, however, whether the early parameters of metabolic syndrome depend on individual genetic predisposition and/or exposure to environmental factors during life.

Subjection of the fetus to a favorable intrauterine environment triggers predictive adaptation to the extra-uterine environment, and failure to predict this correctly may lead the emergence of diseases. This phenomenon is called "fetal programming".[2]

The follow sections outline the more interesting aspects of the insulin receptor and IGF-I located in the placenta that may be involved in this "programming".

The placenta as a sensor of nutritional ecology

Nutrients and other factors establish the local nutritional ecology of the intrauterine fetus. The sensitivity of fetal growth to received nutrition means that the nutrients released by the placenta stimulate the production of fetal insulin and IGFs, which are essential for maintaining the fetal growth rate.[31] A sophisticated repertory of maternal metabolic responses helps maintain a stable intrauterine nutritional environment. During pregnancy, the woman should have the ability to maintain fetal nutrition even when intake is compromised. It is notable that maternal nutrition plays a role in both ways: meeting the energy demands of the mother and having a positive effect on the growth and weight of the fetus.[23]

The placenta supplies the fetus with nutrients and oxygen. The functional capacity of the placenta to meet this demand is controlled by maternal and fetal signals. Fetal-placental signaling regulates the demand for nutrients by the fetus. The nutrients transferred by the placenta are used for energy production and growth. Fetal demand is the main determining factor in the supply of nutrients through the placenta and is adjusted according to the needs of fetal irrespective of the size of the placenta. The placenta seems to be able to change its structure and number of carriers in response to the demand for nutrients for growth of the fetus. Placental efficiency and growth are part of a closely controlled dynamic system that is able to respond to changes in the intrauterine environment.[32,33]

The insulin – IGF axis

The placenta expresses large quantities of insulin receptors compared to other body tissues. The location of these receptors changes with development. At the beginning of pregnancy, they are located in the microvilli of the syncytiotrophoblast, while, at the end, they are found predominantly in the endothelium. This finding strongly suggests a change in control of insulin-dependent processes from the mother in early pregnancy to the fetus towards the end. Insulin stimulates mitogenesis by acting on the trophoblast and the metabolic process when it stimulates the endothelium. This explains the biphasic growth of the placenta and fetus.[34]

The location of the IGF-I receptors in the placenta differs from the location of insulin receptors. In early gestation, the IGF-I receptors are in

the cytotrophoblast, which is consistent with its proliferative effect. At term gestation, the IGF-I receptors are in the syncytiotrophoblast and villous cytotrophoblast, which can bind to the IGF-I and IGF-II in the fetus' circulatory system.[34,35]

Insulin and the IGFs do not cross the placenta. The main function of insulin is to regulate metabolic processes, including the placenta. In the trophoblast, insulin stimulates vasculogenesis and near the end of gestation, the deposition of lipids. In the endothelium, it stimulates the synthesis of glycogen. The fetal insulin hypothesis suggests that fetal insulin regulates fetal IGF-I production. This could explain the effect of insulin on fetal growth.[35]

IGF-I and IGF-II are important growth factors synthesized by the placenta and the fetus. IGF-I is present in the syncytiotrophoblast and in the cytotrophoblast throughout gestation. The IGF-II is only expressed in villous and extravillous cytotrophoblast during early gestation. It is no longer detected when pregnancy reaches term. In the early stages of pregnancy, IGF-II is a major modulator of embryonic growth and placental development, stimulating angio- and vasculogenesis.[34]

During the first half of pregnancy, the maternal IGF-I alters the division of nutrients between mother and fetus and enables adaptation to pregnancy. It increases the uptake of substrate and suppresses fetal catabolism, thereby contributing to the success of pregnancy.[31] In the placenta, it induces the uptake of glucose and aminoacids.[33] Coincidentally IGF-I receptors are present in the same membranes as the amino acid carriers.[33,34] Both IGFs stimulate the passage of placental aminoacids.[31] In humans, fetal IGF-I decreases when malnutrition or hypoxia is present in the mothers.[36,37]

The transport of glucose

Insulin indirectly controls the entry of glucose through the exposure of glucose transporters in the plasma membrane that act by way of facilitated diffusion. The passage of glucose from the placenta to the fetus is regulated by glucose transporters and by changes in acute and chronic concentrations of glucose.[32]

Glucose is the main energy substrate for the placenta and fetus and is essential for normal metabolism and growth of the fetus. During pregnancy, maintenance of the concentration of maternal glucose is the result of increased maternal glucose production and the development of maternal glucose intolerance associated with a physiological insulin resistance. Any glucose that goes to the fetus comes from the mother. The glucose that reaches the fetus is directly related to the concentration of maternal glucose. The passage of maternal glucose is regulated by the concentration of fetal glucose. If this concentration is low, the fetus provides a steeper concentration gradient of maternal-fetal glucose and there subsequently increased transfer of glucose to the fetus. The consumption of glucose by the placenta is directly related to the concentration of glucose in the fetal blood.[32,36,38]

During the second half of pregnancy the fetus grows sevenfold. The passage of glucose through the placenta also increases to meet the requirements of fetal metabolism. The increased capacity to transport glucose stems from the greater number of glucose transporters in the membranes caused by stimulation of uterine IGF-I. The energy needs of the trophoblast then increase considerably to maintain the amino acid transport system and cluster ions, which are active and thus only serve to expend energy.[32,38]

Hypoglycemia is the hallmark of pregnancies in which intrauterine growth is restricted. This phenomenon produces a steeper maternal-fetal glucose concentration gradient which helps to offset the reduced capacity of placental glucose transport and flow of glucose from mother to fetal circulation occurs because of the small size of the placenta. The rate of fetal glucose metabolism depends directly on the simultaneous interaction of plasma glucose concentrations and fetus insulin.[32,34,36,37]

The fetal insulin hypothesis proposes that the insulin secreted by the fetal pancreas in response to the maternal glucose concentration is the key to fetal growth.[39] Fetal insulin secretion is one of the determinants of fetal growth, mainly in the latter stages of gestation when the weight of the fetus increases greatly. Pederson[39] has proposed that fetal macrosomia is not the result of increased direct passage of nutrients, but is mediated indirectly by increased secretion of insulin by the fetus in response to maternal hyperglycemia. Insulin-related fetal growth thus reflects not only the fetal blood, but also genetic factors of the fetus that regulate insulin secretion by the fetal pancreas and the sensitivity of fetal tissue to the effects of insulin.

In humans, fetal insulin secretion is altered by changes in glucose concentration, depending on the pattern, magnitude and duration. In fetuses subjected to sustained chronic hyperglycemia, there is a decrease in glucose tolerance, in basal and glucose-induced insulin secretion. However, when hyperglycemia is intermittent, there is increased secretion of insulin.[32,38]

Interestingly, human fetuses submitted to chronic hypoglycemia also have reduced secretion of basal

and insulin-induced glucose, which are consistent with the findings of a reduction in development of the pancreas and its ability to secrete insulin in fetuses with intrauterine growth restriction.[30,32,36,37]

In sheep submitted to restricted intrauterine conditions it has been shown that there is a reduction in concentrations of insulin and IGF-I abnormalities, similar to those that occur in humans, an increase in the number of insulin receptors and a suppression of glycogen synthase kinase-3-β. Low levels of this enzyme favor the activity of glycogen synthase and hence increase the formation of glycogen.[32] With the increase in numbers of insulin receptors, there is also further stimulation of proximal signaling. This is not effective, however, because there is a decrease in the proteins that regulate protein synthesis, which are part of the cascade of intracellular events. In cases of intrauterine growth restriction, there is, therefore, a tendency to increase the energy producing substrates such as glucose, and decrease the ability of protein synthesis for growth. The glucose that reaches the fetus is then divided into a store of fat and glycogen and oxidation (energy production).[37] The behavior of insulin receptors in sheep with intrauterine growth restriction is similar to that of the hybrid receptors.

Transport of amino acids

The transport of amino acids by the placenta is essential for fetal growth, because his absence is associated with a deficiency of this transport. After going through the microvilli and basal membranes of the trophoblast, amino acids, such as glucose, can spread freely in the fetal connective tissue and cross the endothelium reaching the circulation of the fetus. Amino acids are important both for protein synthesis and as an energy source for some metabolic processes, such as the synthesis of nucleotides.[33] The transport of amino acids is a complex process because it involves multiple systems of active transport. In the placenta of human fetuses with growth restriction there is a decrease in the activity of system A transport of amino acids in the membrane of the microvilli.[36,37]

The study of women with pregnancies complicated by fetal growth restriction receiving supplementation of amino acids by direct infusion of amino acids in the maternal circulation showed increased concentrations of some amino acids in the umbilical vein.[40]

In pregnant women with controlled gestational diabetes mellitus, it was also observed that there was a higher concentration of amino acids in umbilical blood than in normal pregnant women. This finding is the opposite of that found for pregnancies with fetal growth restriction.[38]

Amino acids, like glucose, also stimulate the secretion of insulin from the pancreas of the fetus. Fetal growth is thus stimulated by insulin by a direct association between plasma concentrations of nutrients and growth.[34,37-39]

Final considerations

The condition of insulin resistance thus progresses throughout pregnancy, and may be exacerbated by the presence of hybrid receptors in the placenta of women with hyperinsulinemia. The change in the placental response to the action of the maternal hyperinsulinaemia could represent a stimulus to the fetus that results in higher levels of expression of hybrid receptors in the fetus. This change would cause an intrauterine predisposition to disease in adulthood. This is a complex process that is still not fully understood.

Unanswered questions include: whether the endothelial cells from the umbilical cord of babies born to obese women with hyperinsulinemia have a higher expression of hybrid receptors, whether this is related to the birth weight of newborns, whether the expression of hybrid receptors is increased in other fetal tissues, whether obese women with gestational diabetes and/or hypertensive pregnancy disorders have a different number of hybrid receptors expressed, whether there are changes in the transport of the nutrients and oxygen of these placentas, nutritional changes that lead to a predisposition to metabolic syndrome, and which factors in life outside of the womb trigger disease in adults.

In view of the foregoing, it can be concluded that maternal hyperinsulinemia is involved in fetal growth that is more or less in accordance with its nutritional status, as well as the supply of nutrients to the fetus during pregnancy.

Pregnant women with normal levels of insulin, proper weight and a healthy and adequate diet for normal development of the conceptus have newborns with appropriate weight for gestational age. If these same women had a higher insulin levels than expected for this gestation the fetus would probably have grown more and gained more weight. This increase in maternal insulin impacts the insulin resistance of maternal tissues that results in maternal hyperglycemia passing more glucose to the fetus. Fetal hyperglycemia, when intermittent, stimulates insulin production by the fetal pancreas, resulting in fetal hyperinsulinemia. This condition is associated with nutrients appropriate to the need for increased

secretion of IGF-I to stimulate fetal growth.

Women with adequate weight prior to conception but with inadequate nutrient intake during pregnancy or underweight before pregnancy will not have adequate passage of glucose through the placenta, even though it has hyperinsulinemia, because the small amount of maternal glucose must be shared between the woman and the fetus. Moreover, there is a deficiency of active transport of amino acids stemming from decreased availability and glucose being used as an energy source for this type of transport. In this case, there is no fetal hyperinsulinemia or increased IGF-I and, consequently, fetal growth will suffer.

Women who were obese prior to conception or who became obese during pregnancy pass more glucose to the fetus, and transfer more amino acids, as this is dependent on glucose. These fetuses grow more if maternal hyperinsulinemia is present during pregnancy, because this stimulates secretion of IGF-I by insulin and both are stimulated by the large quantity of available nutrients.

Whereas the presence of hybrid receptors brings a peripheral insulin resistance which could be beneficial, when there is an intrauterine environment with a shortage of or excess glucose, helping to maintain a glucose level suitable for the survival of the fetus during this period of life and a greater quantity of these receptors may be deleterious for the individual during extra-uterine life when he or she comes into contact with environmental factors that overwhelm the body metabolically. It can be argued that the greater quantity of these receptors, the types of tissues affected and the intensity of exposure to external environmental factors make possible the emergence of a broad spectrum of clinical manifestations of metabolic syndrome.

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CAPITULO II

METODO

MÉTODO

Nesta seção é apresentado o método da pesquisa que gerou os dados empíricos desta tese.

1. LOCAL E PERÍODO DO ESTUDO

O estudo foi colaborativo, realizado no período de abril de 2010 a maio de 2012. Os centros envolvidos foram o Centro de Assistência à Mulher (CAM) do Instituto de Medicina Integral Professor Fernando Figueira (IMIP), onde as participantes foram selecionadas e os cordões umbilicais foram coletados; o Laboratório do IMIP, onde foram realizadas as dosagens hematológicas e bioquímicas; o Laboratório de Imunopatologia Keiso Asami (LIKA/UFPE) onde foram isoladas as HUVECs e o Laboratório de Dra. Patti (Joslin Diabetes Center/Harvard University) onde foram processadas as análises por técnicas de biologia molecular.

2. DESENHO DO ESTUDO

Foi realizado um estudo tipo exploratório descritivo com componente analítico após dividir as gestantes em três grupos: gestantes eutróficas, gestantes com sobrepeso e gestantes obesas.

3. AMOSTRA

Das oitenta e uma gestantes brasileiras selecionadas, apenas 38 chegaram ao final das 3 etapas: acompanhamento do período pré-natal, o parto e as primeiras 48 horas após o parto. As perdas serão discutidas em detalhes, posteriormente, no item "Problemas Metodológicos".

3.1. CRITÉRIOS DE INCLUSÃO

As gestantes selecionadas tinham idade maior que 18 anos, eram eutróficas, com sobrepeso ou obesas segundo os critérios do Ministério da Saúde do Brasil[9] e apresentavam gestação única.

3.2. CRITÉRIOS DE EXCLUSÃO

Nenhuma das gestantes podia ter outras doenças associadas ou fazer uso de medicações que alterassem o metabolismo da glicose ou os níveis de insulina. O binômio mãe/recém-nascido foi excluído se o feto fosse prematuro, apresentasse malformações ou evidências de doenças genéticas, ou o parto tivesse ocorrido em outra maternidade.

4. VARIÁVEIS DO ESTUDO

Em relação à gestante: idade, condições sócio-econômicas (nível de instrução, estado civil), antecedentes mórbidos familiares (diabetes, hipertensão, obesidade) e pessoais (diabetes, hipertensão, infertilidade, cirurgia pélvica e infecção urinária), antecedentes obstétricos, estado nutricional pré-gestacional (Índice de Massa Corpórea Precoce), estado nutricional no final da gestação (Índice de Massa Corpórea Final), ganho ponderal na gestação, patologias na atual gestação, perfis glicídico e lipídico.

Em relação ao recém-nascido: sexo, peso, comprimento, perímetros cefálico, torácico e abdominal ao nascimento, idade gestacional, intercorrências nas primerias 48 horas de vida, hemoglogina glicada, dosagens de insulina, lipídeos e marcadores inflamatórios (IL-1β, IL-6, IL-8, TNF-α, MCP-1, HGF, Leptina, Adiponectina, Resistina, PAI-1) do sangue do cordão umbilical, e em relação às HUVECs, a expressão gênica dos receptores de insulina e de IGF-I.

5. OPERACIONALIZAÇÃO E COLETA DOS DADOS

Gestantes eutróficas ou portadoras de sobrepeso ou obesidade pré-gestacionais, segundo os critérios preconizados pelo Ministério da Saúde do Brasil[9] foram recrutadas no ambulatório de pré-natal do Serviço de Obstetrícia do CAM/IMIP. Consentimento livre e esclarecido (APÊNDICE B) foi obtido de todas as gestantes após a explicação da natureza e dos riscos do estudo. Após a assinatura do termo de consentimento foi feita a primeira entrevista para coleta dos dados. Posteriormente, os dados antropométricos e clínicos foram colhidos no cartão da gestante (APÊNDICE D) após as consultas de pré-natal. Os exames laboratoriais foram realizados como parte do atendimento rotineiro destas gestantes, exceto a insulinemia.

Após o parto, foi colhido sangue do cordão umbilical para dosagens de insulina e marcadores inflamatórios, e os cordões umbilicais eram usados para isolamento das células endoteliais para estudo da expressão gênica dos receptores. Também foram obtidos os dados antropométricos do neonato a partir do seu prontuário.

Ver organograma com a operacionalização do estudo no APÊNDICE E.

5.1. ISOLAMENTO DE CÉLULAS ENDOTELIAIS DA VEIA DO CORDÃO UMBILICAL

As células endoteliais da veia do cordão umbilical foram isoladas para verificarmos se havia diferença na expressão dos receptores de insulina, IGF-I e seus híbridos entre os recém-nascidos de mães obesas, com sobrepeso e eutróficas.

O cordão umbilical coletado na sala de parto era acondicionado em pote plástico com PBS a 1% gelado para transporte até o laboratório do LIKA/UFPE. As células endoteliais foram isoladas da veia do cordão umbilical, segundo o Protocolo da *Nature Protocols*[10] e eram armazenadas em *RNA later* a -20º C para posterior extração de RNA, DNA e proteínas.

5.2. EXTRAÇÃO DE RNA, DNA E PROTEÍNAS

O sedimento de células endoteliais armazenadas em *RNA later* a -20ºC, era descongelado e rehidratado com água destilada *RNAse Free*. Então, procedia-se à extração de RNA, DNA e proteínas com o Protocolo usando TRIzol ® (Invitrogen, USA) descrito a seguir:

Extração de RNA

1. As amostras eram homogeneizadas com TRIzol e incubadas durante 5 minutos à temperatura ambiente para permitir a dissociação completa dos complexos de nucleoproteína;
2.Adicionava-se 0,2ml de clorofórmio por 1mL de TRIzol utilizado para homogeneização. Fechava-se o tubo de forma segura;
3. Balançava-se o tubo vigorosamente com a mão por 15 segundos;
4. Incubavam-se as amostras eram durante 2-3 minutos à temperatura ambiente;
5. Centrifugavam-se as amostras a 12.000 x *g* durante 15 minutos a 4°C;
6. Removia-se a fase aquosa da amostra inclinando-se o tubo a 45° e, então a fase aquosa era retirada;
7. Colocava-se a fase aquosa em um tubo novo e procedia-se ao isolamento do RNA;
8. Adicionava-se 50ul de acetato de sódio 3M;
9. Adicionava-se 0,5ml de isopropanol a 100%;
10. Incubava-se à temperatura ambiente durante 10 minutos;
11. Centrifugava-se a 12.000 x *g* durante 10 minutos a 4°C.
12. Removia-se o sobrenadante do tubo, deixando apenas o sedimento de RNA;
13. Lavava-se o sedimento, com 1mL de etanol a 75%;
14. Misturava-se a amostra de forma breve no Vórtex, em seguida, centrifugava-se o tubo em 7500 x *g* durante 5 minutos a 4°C;
15. Descartava-se o sobrenadante;
16. Secava-se o sedimento de RNA ao ar durante 5-10 minutos;
17. O sedimento de RNA era ressuspenso em 30uL de água *RNase Free;*
18. Armazenava-se a -80°C.

Extração de DNA

O DNA era isolado a partir da camada de interfase de fenol-clorofórmio salvo do passo 6 da separação de fases.

1. Removia-se a fase aquosa restante e adicionava-se 0,3mL de etanol a 100%;
2. Fechava-se o tubo e invertia-se a amostra várias vezes para misturar;
3. Incubavam-se as amostras durante 2-3 minutos à temperatura ambiente;
4. Centrifugava-se o tubo a 2000 x *g* durante 5 minutos a 4°C;
5. Guardava-se o sobrenadante para extração das proteínas;
6. O sedimento de DNA era ressuspenso em 30uL de água destilada*;*
7. Armazenava-se a -80°C.

O controle da qualidade e a quantificação do RNA e do DNA eram realizados no aparelho chamado NanoDrop com programa ND-1000.

Extração das proteínas

As proteínas eram isoladas a partir da fase de fenol-etanol salvo do passo 5 de centrifugação do DNA.

1. Adicionava-se 0,75ml de isopropanol na fase de fenol-etanol;
2. Incubavam-se as amostras durante 10 minutos à temperatura ambiente;
3. Centrifugava-se a 12.000 x *g* durante 10 minutos a 4°C para sedimentar a proteína. Removia-se e descartava-se o sobrenadante;
4. Preparava-se uma solução de lavagem consistindo de cloridrato de guanidina 0,3M em etanol a 95%;
5. Lavava-se o sedimento de proteína com 1,5mL da solução de lavagem;
6. Incubava-se durante 20 minutos à temperatura ambiente;
7. Centrifugava-se a 7500 x *g* durante 5 minutos a 4°C;
8. Removia-se e descartava-se a solução de lavagem;
9. Repetiam-se os passos 5-8, mais duas vezes;

10. Adicionava-se 1,5mL de etanol a 100% ao sedimento de proteína após a terceira lavagem e misturava-se a amostra no Vórtex;
11. Incubava-se durante 20 minutos à temperatura ambiente;
12. Centrifugava-se a 7500 x *g* durante 5 minutos a 4°C. Removia-se e descartava-se o etanol da lavagem;
13. Secava-se o sedimento de proteína ao ar durante 5-10 minutos;
14. Ressuspendia-se o sedimento de proteína com 200uL de SDS a 1%;
15. Centrifugava-se a 10.000 x *g* durante 10 minutos a 4°C;
16. Transferia-se o sobrenadante contendo a proteína para um novo tubo e armazenava-se a amostra a -20°C.

A quantificação das proteínas foi feita pela técnica *BCA Protein Assay*

1. Preparavam-se os poços-controle:
Adicionava-se 5uL das soluções padrão nos poços-controle (em duplicata)
Adicionava-se 2uL do tampão de lise em cada poço-controle
Adicionava-se 3uL de água em cada poço-controle
2. Preparavam-se as amostras:
Adicionava-se 2uL da amostra em cada poço (em duplicata)
Adicionava-se 8uL de água em cada poço
3. Preparava-se a mistura dos reagentes do kit de BCA:
Usava-se a proporcao 1:50 do reagente B:A
Adicionava-se 200uL da mistura em cada poço
Misturavam-se as soluções na placa de 96 poços por 30 segundos e incubava-se a 37°C por 30 minutos.
Lia-se a placa com o comprimento de onda de 562nm.
Para analisar colocavam-se os dados obtidos num gráfico de dispersão, onde o eixo do X correspondia à absorbância e o do Y, à concentração. Ao gráfico, adicionava-se a equação e a linha de tendência, então se usava a equação para calcular a concentração de proteína por poço. Depois, dividia-se o resultado pela quantidade de amostra colocada em cada poço.

A separação do miRNA das frações dos RNAs maiores (> 200 nucleotídeos) foi feita com o Kit *Clean-up RNeasy MinElute* ® (Qiagen, USA)

O procedimento era o seguinte:

1. Media-se a quantidade exata de cada amostra com uma pipeta. Adicionava-se água *RNAse Free* para completar 50ul. Adicionava-se 175ul de tampão RLT, e misturava-se bem. Depois, adicionava-se 125ul de etanol a 100%, e misturava-se bem por pipetagem.
2. Pipetava-se a amostra, incluindo qualquer precipitado formado em uma coluna de centrifugação *RNeasy Mini* colocado num tubo de 2ml. Fechava-se a tampa suavemente e centrifugava-se a 8000 x *g* durante 15s à temperatura ambiente (15-25°C). O filtrado contém o miRNA.

- Purificava-se a fração de miRNA seguindo os passos A4-A10 e os RNAs maiores (> 200 nucleotídeos), os passos A11-A16.

A4. Adicionava-se 225ul de etanol a 100% ao miRNA filtrado através da etapa 2 e homogeneizava-se por agitação em vórtex. Não centrifugava.
A5. Pipetava-se toda a amostra em uma coluna de centrifugação *RNeasy MinElute* colocado num tubo de coleta de 2ml. Fechava-se a tampa suavemente e centrifugava-se durante 15s, a 8000 x *g* à temperatura ambiente (15-25°C). Descartava-se o filtrado.
A6. Adicionava-se 350uL de tampão RWT à coluna *RNeasy MinElute*. Fechava-se a tampa suavemente e centrifugava-se durante 15s, a 8000 x *g*. Descartava-se o filtrado.
A7. Pipetava-se 250uL de tampão RPE à coluna *RNeasy MinElute*. Fechava-se a tampa suavemente e centrifugava-se durante 15s, a 8000 x *g*. Descartava-se o filtrado.
A8. Adicionava-se 250ul de etanol a 80% à coluna de *RNeasy MinElute* de rotação. Fechava-se a tampa suavemente e centrifugava-se durante 2 minutos, a 8000 x *g* (10.000 rpm). Descartava-se o filtrado.
A9. Colocava-se a coluna de centrifugação *RNeasy MinElute* em um novo tubo de coleta de 2ml. Abria-se a tampa e centrifugava-se durante 5min a 8000 x *g*.

A10. Colocava-se a coluna de centrifugação *RNeasy MinElute* em um tubo de coleta de 1,5ml e pipetava-se 14uL de água *RNAse Free* sobre a membrana da coluna. Fechava-se a tampa suavemente e centrifugava-se durante 5min a 8000 x *g* para coletar a fração miRNA.

- Purificação dos RNAs grandes (> 200 nucleotídeos) usando a coluna de centrifugação *RNeasy Mini* (passos A11-A14).

A11. Pipetava-se 350uL de tampão RWT na coluna de centrifugação *RNeasy Mini*. Fechava-se a tampa suavemente e centrifugava-se durante 15s, a 8000 x *g*. Descartava-se o filtrado.
A12. Adicionava-se 250uL de tampão RPE à coluna *RNeasy Mini*. Fechava-se a tampa suavemente e centrifugava-se durante 15s, a 8000 x *g*. Descartava-se o filtrado. Repetia-se o procedimento 1 vez mais.
A13. Colocava-se a coluna *RNeasy Mini* em um novo tubo de coleta de 2ml. Abria-se a tampa e centrifugava-se a 12000 x *g*, durante 1 minuto.
A14. Colocava-se a coluna *RNeasy Mini* para um novo tubo de coleta de 1,5ml. Pipetava-se 30ul de água *RNAse Free* diretamente sobre a membrana da coluna. Fechava-se a tampa suavemente e centrifugava-se durante 5min a 8000 x *g* para recuperar o RNA total.

O RNA (> 200 nt) era utilizado para produzir cDNA, que posteriormente era usado no Real time quantitative RT-PCR (qRT-PCR).

5.3. ANÁLISE DA EXPRESSÃO DE RNA MENSAGEIRO DOS RECEPTORES DE INSULINA E DO FATOR DE CRESCIMENTO IGF-I COM REAL TIME QRT-PCR

A quantificação do RNA mensageiro das isoformas do receptor de insulina e do receptor do fator de crescimento IGF-I pelo Real time qRT-PCR foi feita para verificar a expressão gênica in vivo.

A síntese de cDNA era realizada usando 1 ug de RNA (> 200nt) de cada amostra em 20µL de reação com Taq DNA Polymerase® (Invitrogen, USA). O protocolo da

reação era o seguinte: 5min 25°C, 30min 42°C, e 5min 85°C. A cada produto desta reação era adicionava-se 200uL de água *RNAse Free*. Cada 20µL da reação de qRT-PCR continha 10µl of cDNA diluído e 10µl de iQ™ SYBR® Green Supermix (Bio-Rad:100mM KCl, 6mM $MgCl_2$, 40mM Tris-HCl, pH 8.4, 0.4mM of dATP, dCTP, dGTP and dTTP, iTaq DNA Polymerase 50U/mL, SYBR Green I, 20mM Fluoresceína), com forward e reverse primers a 100nM. Antes da amplificação por PCR, a *iTaq DNA polymerase* era ativada a 95°C por 10 minutos. Isto era seguido por 40 ciclos de amplificação composta por 30s a 95°C, 15s a 60°C, e 90s a 72°C. A fluorescência era detectada ao final de cada fase de extensão.

Os *primers* eram "desenhados" para os genes específicos e eram verificados usando o programa *NCBI nucleotide BLAST* (APÊNDICE F).

As quantidades relativas dos mRNAs dos receptores de IGF-I e das isoformas do receptor de insulina eram padronizadas fazendo-se uma relação com o gene housekeeping que codifica ciclofilina E. As reações de todas as amostras eram realizadas em duplicata e no mesmo termociclador. A quantidade relativa de mRNA de cada receptor foi calculada usando o método comparativo C_T.

Os marcadores inflamatórios (IL-1β, IL-6, IL-8, TNF-α, HGF, MCP-1, PAI-1, Leptin, Adiponectina e Resistina) eram quantificados por multiplex ELISA (Millipore, Billerica, MA).

As frações de lipídeos do soro do cordão umbilical eram mensuradas por cromatografia gasosa de alta resolução e espectrometria de massa (GC-MS)[11].

6. PROCESSAMENTO E ANÁLISE DOS DADOS

Os questionários foram checados regularmente quanto ao preenchimento dos dados, que foram posteriormente digitados para o banco de dados do programa Excel 2003.

A análise estatística foi realizada através do programa StatView e Excel 2003. A média e o desvio padrão foram calculados para as variáveis contínuas e usados para as variáveis categóricas. Para analisar as associações entre o índice de massa corpórea materna e os desfechos, as variáveis foram estudadas como contínuas. A correlação de

Pearson foi usada para avaliar as associações entre índice de massa corpórea materna e as variáveis paramétricas, e a correlação de Spearman foi utilizada para analisar as associações com as variáveis não-paramétricas. Todos os cálculos consideraram os valores de $p<0.05$ bicaudal como significativos.

7. ASPECTOS ÉTICOS

O protocolo deste estudo foi previamente analisado e aprovado pelo Comitê de Ética em Pesquisa em Seres Humanos do Instituto de Medicina Integral Professor Fernando Figueira (CEP IMIP/PE) e pela Comissão Nacional de Ética em Pesquisa (CONEP - Protocolo # 387/2011), Brasília, Brasil. Todas as participantes assinaram o Termo de Consentimento Livre e Esclarecido após a leitura do documento e explicação dos procedimentos. O estudo foi realizado em acordo com os princípios da Declaração de Helsinki.

8. PROBLEMAS METODOLÓGICOS

Alguns problemas encontrados durante a execução do trabalho de pesquisa resultaram em 54,3% das perdas da amostra (TABELAS 1 e 2), tais como, exclusão de pacientes por fetos malformados, natimorto e abortos (8,6%) ou partos em outras maternidades (17,2%), abandono por parte das pacientes (23,4%) e dificuldades com o isolamento das HUVECs e técnicas moleculares. Dentre estes, a superlotação da maternidade do IMIP, o grande número de natimortos e malformados e a viagem realizada ao laboratório da Dra. Mary Elizabeth Patti (algumas pacientes não pariram até a data da viagem) não estavam planejados no começo da coleta.

Em virtude da implementação da técnica de isolamento das HUVEC foram observados os seguintes problemas:

1. A distância entre o IMIP e o LIKA/UFPE dificultou o isolamento das HUVEC nas primeiras três horas após o parto como preconizado pelo protocolo da *Nature Protocols*[10];

2. Não havia caixa de congelamento para evitar o choque térmico das células colocadas a -20^{o}C após o isolamento do cordão umbilical;

3. Não foram conservadas a -80^{o}C por falta de acesso ao freezer adequado; e

4. O uso de *RNA Later* não deve ser usado em sedimento de células, mas apenas em tecidos. Isso inviabilizou a extração adequada de proteínas e DNA, resultando na perda dos mesmos.

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Tabela 1. Causas de perdas das amostras

Nasceram em nossa maternidade							Nasceram em outra maternidade		
Classificação Materna	VDRL +	Malformados/ Natimortos/ Abortos	Não pariram	Desapareceram	Abandonaram	Não avisaram	Laqueadura	Superlotação	Total
Eutrófica		4	1		1	2		2	10
Sobrepeso		1		1	2	5	1	6	16
Obesa	1	2	2	2	3	3	2	3	18
Total	1	7	3	3	6	10	3	11	44
Tipos das Causas	Não Preveníveis = 8		Possivelmente Preveníveis = 22				Preveníveis = 14		

Tabela 2. Causas de exclusão dos recém-nascidos

Classificação materna	Problema
Eutrófica	Anencefalia (aborto)
Eutrófica	Malformações de membros superiores e inferiores, tórax curto e provável cardiopatia → morte do recém-nascido na sala de parto
Eutrófica	Natimorto
Eutrófica	Polidactilia
Sobrepeso	Eclâmpsia → recém-nascido prematuro extremo → morte do recém-nascido
Obesa	Aborto espontâneo
Obesa	Transposição dos grandes vasos → morte do recém-nascido

CAPITULO III

ARTIGO 1

Title: "Fetal cord blood insulin and leptin can be a marker to predisposition of offspring obesity of obese mothers"

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Introduction

Obesity is considered a pandemic public health problem by World Health Organization (WHO). In 2010, the prevalence of obese women in USA was 40.6% of the total population[1], in United Kingdom and Canada were 26%, in Egypt was 48%, in Turkey was 32% and in Brazil was 25%.[2]. The amount of obese women is growing in developed and in developing countries.

Nowadays, this situation is worrisome, and the WHO estimates that 29% of non pregnant women ages 20 to 39 are obese[3]. A major concern being the growing number of

obese pregnant, who are transmitting the obese phenotype through future generations maintaining the obesity cycle. It is important the precocious detection of predisposing factors and interruption of this cycle. In this study, we assessed whether maternal obesity could transferring information to the fetus triggers insulin resistance during the intrauterine life. The aim of the present work is to identify maternal obesity markers associate with neonatal anthropometrics and metabolic parameters in the offspring which could contribute for future obesity.

Research Design and Methods

The protocol was approved by the Hospital Institutional Review Board (CEP IMIP/PE) and the National Committee for Ethics in Research at National Health Council (CONEP-Statement # 387/2011), Brasilia, Brazil. All participants gave written informed consent after the nature of the procedure was explained. The study was performed in accordance with the principles of the Declaration of Helsinki.

Subjects

All pregnant women were eligible to participate unless they had one or more exclusion criteria: age <18 years, date of last menstrual period not certain and no ultrasound estimation between 5 and 16 weeks of gestational age available, unable to complete the oral glucose tolerance test (OGTT) by 35 weeks gestation, diabetes antedating pregnancy or diagnosis of diabetes during the current pregnancy, or other systemic disease known before the present pregnancy or requiring treatment with medication that may interfere with insulin or glucose metabolism, multiple pregnancy, conception using gonadotropin ovulation induction or by in vitro fertilization, infection with human immunodeficiency virus or syphilis. The pairs mother-newborn were excluded if the newborns were premature (<36 weeks) or with malformations or genetic syndromes or stillbirth or the delivery was at another hospital.

Thirty one pregnant women were selected to participate in the study. They were grouped by the early body mass index (mean 15.0 ± 4.2 weeks of gestation): 14 normal

body mass index pregnant women, 5 overweight pregnant women and 12 obese women[4]. Data concerning smoking use, familiar history among first-degree members, anterior personal gestational history, previous diseases, demographic characteristics and clinical data (height, weight and blood pressure, LMP) were collected to fill the prenatal card of Ministry of Health of Brazil during the first visit. At the same moment, the fasting glucose, hematocrit, hemoglobin measurement, urine type 1 test, and HIV and syphilis sorological tests were requested. The maternal glycemia (1-hour 75g OGTT) and fasting insulin were performed in the second trimester (mean 25.0 ± 3.0 weeks of gestation), and the complete 75 g OGTT (fasting, 1-hour and 2-hour glycemia and insulin levels) and fasting lipids profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, and triglycerides) were assessed at 30.3 ± 4.0 weeks and analyzed using ADA criteria[5]; women with GDM were excluded from analysis, as well they had essential hypertension and/or evidence of other systemic disease. The final BMI was calculated at 36-40 weeks (last prenatal visit before the delivery). Neonatal anthropometrics were measured in the first 24 hours of life and blood cord was collected at birth for further analysis of insulin levels, inflammatory markers, hematocrit, hemoglobin, and hemoglobin glycated (HbA1c) measurements.

Oral Glucose Tolerance Test (OGTT) and HOmeostasis Model Assessment of IR (HOMA-IR)

Pregnant underwent a 1-hour glycemia and fasting insulin, with the use of a 75-g dose of glucose, between 22 and 28 weeks and a standard oral glucose-tolerance test and fasting, 1-hour and 2 hour insulin, with the use of a 75-g dose of glucose, between 26 and 34 weeks. And the insulin resistance (IR) was assessed by homeostasis model assessment of IR (HOMA-IR).

Lipids Profile Analysis

The blood for lipids profile analysis was collected in the fasting time of the complete OGTT.

Cord-blood Samples

Cord-blood samples were collected at delivery for the measurement of serum insulin levels, hematocrit, hemoglobin, hemoglobin glycated (HbA1c), inflammatory markers (Interleukin-1 beta (IL-1β), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Tumor Necrosis Factor-alpha (TNF-α), Hepatocyte Growth Factor (HGF), Monocyte Chemoattractant Protein-1 (MCP-1), Leptin, Adiponectin, Resistin, and Plasminogen Activator Inhibitor type-1 (PAI-1 total)).

Biochemical and Sorological Analysis

Plasma glucose, serum insulin and lipids levels, hematocrit and hemoglobin measurements, urine type 1 test, HIV and syphilis sorological tests were analysed at the clinical laboratory of Instituto de Medicina Integral Prof. Fernando Figueira (hospital name). Plasma glucose, hemoglobin glycated and lipids samples were analysed with the use of a colorimetric method (ARCHITECT c8000, Abbott). LDL-cholesterol levels were calculated by Friedwald formula. Serum insulin samples were analysed with the use of an electrochemiluminescence immunoassays. Urine type 1 test were analysed with the use of an automatic analyser (URiSCAN Pro II, YD) and optical microscope (Nikon). Hematocrit and hemoglobin were measured with the use of an automatic analyser (Sysmex XT-1800, Roche). HIV tests were performed with the use of AxSYM System, Abbott, and the syphilis sorological tests were performed with the use of immunoassay/ELISA (Elisys Quattro, HUMAN).

Cord IL-1β, IL-6, IL-8, Leptin, Adiponectin, Resistin, PAI-1 total MCP-1, HGF and TNF-α levels were determined by multiplex suspension array (5P30 DK 36836 Specialized Assay Core) at Joslin DERC Service.

Prenatal care, delivery, and neonatal care

Prenatal care, timing of delivery, and neonatal care were determined by means of the standard practice of the hospital staffs. Medical records were abstracted to obtain data regarding the prenatal course, labor and delivery, the postpartum course, and the newborn

course. Neonatal anthropometrics were measured in the first 24 hours by the hospital staffs.

Outcomes

Primary maternal groups were defined by early pregnancy body mass index: lean (<25kg/m^2), overweight (25-30kg/m^2), and obese (>30kg/m^2). Primary maternal variables beyond BMI included maternal insulin resistance (BMI) and glycemia. Primary neonatal outcomes were birth weight, abdominal circumference, and cord insulin level. Additioanl neonatal outcome variables included length, ponderal index, head and thorax circumference, and cord adipokines and proinflammatory markers.

Statistical Analysis

Mean and standard deviation are reported for continuous variables, which were also reported as categorical variables. For associations of maternal body mass index with primary and secondary outcomes, each measurement was analyzed as a continuous variable. Pearson or Spearman product-moment correlations were used to assess associations among maternal body mass index, maternal glycaemia, cord insulin levels and the other parameters. All analysis was conducted in StatView and Excel. All reported P-values are two-sided.

Results

The thirty one pregnants were Brazilian. Nobody used to smoke before or during the pregnancy. There were not significant differences in the groups about educational level, marital status, familiar history of mellitus diabetes or hypertension in first degree parents, obstetrician history of previous abortion and parity, presence of preeclampsia or haemorrhage in the present pregnancy, and type of delivery. None newborn presented hypoglycaemia symptoms. There was not significant difference in the presence of jaundice. Clinical and biochemical data of the three groups of the mothers and newborns

are summarized in Table 1, the inflammatory markers in the cord blood are in Table 2, and the correlations are in the Figure 1.

Early and final gestational maternal BMI were strongly associated ($r=0.88$, $p<0.0001$). Both maternal BMI were associated with maternal glycemia (2-hour OGTT) ($r=0.59$, $r=0.62$, both $p<0.001$) but only early gestational maternal BMI was correlated with LDL-cholesterol ($r=0.41$, $p\leq0.05$). Maternal weight gain during pregnancy was inversely related to initial BMI ($r=-0.44$, $p=0.01$), as expected, but was not associated with final BMI ($r=0.11$, $p=0.55$) or offspring cord insulin ($r=0.09$, $p=0.60$). We next assessed relationships between maternal metabolism and fetal physical and biochemical parameters. Both maternal BMI were associated with cord insulin (early: $r=0.35$, $p=0.04$; final: $r=0.36$, $p=0.02$), and cord insulin was strongly correlated with ponderal index ($r=0.50$, $p=0.002$) and tended to be correlated to birth weight ($r=0.30$, $p=0.06$). Beside, maternal glucose (2-hour OGTT) was associated with birth weight ($r=0.35$, $p=0.04$). In the other hand, maternal glycemia (2-hour OGTT) was not correlated with cord insulin($r=0.10$, $p=0.54$), neither maternal insulin resistance (HOMA-IR) ($r=0.13$, $p=0.47$), nor with birth weight ($r=0.09$, $p=0.63$) or length ($r=0.19$, $p=0.31$). However, final maternal BMI also was associated with abdominal circumference (final: $r=0.41$, $p=0.02$). The maternal weight gain was only inversely related with HGF ($r=-0.33$, $p=0.04$).

Newborn abdominal circumference, an early marker of adiposity risk, was strongly correlated with birth weight ($r=0.82$, $p<0.0001$), length ($r=0.54$, $p<0.005$), and cord insulin ($r=0.56$, $p<0.005$).

We next examinated levels of adipokines and inflammatory markers in the cord blood and correlations with maternal and fetal parameters. Leptin levels were significantly higher in neonates of overweight and obese groups, and were associated with final BMI ($r=0.39$, $p=0.01$) and maternal glucose (1-hour OGTT: $r=0.40$, $p=0.03$). Leptin was not associated with maternal HOMA-IR ($r=0.27$, $p=0.12$) or maternal weight gain ($r=0.06$, $p=0.71$), but tended to correlate with early maternal BMI ($r=0.28$, $p=0.0.08$) and maternal glycemia (2-hour OGTT: $r=0.32$, $p=0.06$). Leptin correlated with several newborn outcome measures, including cord insulin ($r=0.60$, $p<0.0001$), ponderal index ($r=0.52$, $p<0.005$) and abdominal circumference ($r=0.43$, $p=0.02$).

As well, HGF showed direct association with fetal resistin (r=0.56, p=0.001), and inverse with cord insulin (r=-0.39, p=0.03) and abdominal circumference (r=-0.39, p=0.03), beyond to tended to be correlated with PAI-1 (r=0.31, p=0.08) and inversely with ponderal index (r=-0.27, p=0.09), but not with birth weight (r=-0.07, p=0.68).

Discussion

Children of obese mothers have bigger risk for becoming obese. There is a consensus that maternal early or pre-pregnancy BMI associates with offspring BMI in later life. In general, the obese mother newborns are large-for-gestational-age and/or have macrossomia due to higher glucose transport from placenta to fetus (Pedersen Hypothesis)[6]. Moreover, the weight gain during gestation is important to mother stock energy that will be used in the end of gestation and neonatal period but it also affects the fetus in developing[7]. We found that the maternal weight gain during pregnancy was inversely related to initial BMI reflects the necessity of mothers with less weight to store more nutrients as energy source. Then, to determine correlated biomarkers to maternal obesity and offspring adiposity and obesity, we prospectively studied 31 pregnant women categorized by the maternal early BMI calculated at the first prenatal visit. Surprisingly, the newborn parameters was not different among the groups; however, both maternal BMI, maternal weight gain, maternal glycaemia were statistically significant by ANOVA. Although, the presence of metabolic syndrome symptoms is more common in small-for-gestational-age and large-for-gestational-age newborns, we studied the predisposing markers of adequate-for-gestational-age newborns of obese mothers to develop obesity. Besides, both maternal BMI were associated with maternal glucose level at 30 weeks and cord insulin. These data suggest that the newborns have more deposit in the adipose tissue resulting in different body composition and predisposition to develop obesity.

The HAPO study found strong association between maternal BMI and fetal adiposity[8] and between cord C-peptide and individual newborn skin folds after the adjustment for the confounders[6] beyond the maternal glucose levels were associated with higher birth weight and cord-blood serum C-peptide levels[9]. We found the association

among maternal BMI, maternal glucose levels, and cord insulin but no with birth weight due probably the small amount of participants.

Moreover, obesity is a state of chronic inflammation worsening by placental production of inflammatory mediators during the pregnancy[10]. Leptin is a modulator of placental development and fetal growth, which allow a cross-talking between fetus and placenta, because fetal growth stimulate the placental production of leptin, then the placenta increases the transfer of nutrients and the insulin production direct by leptin and indirect by higher nutrient supply[11]. Cord leptin levels correlated with placental size[11], maternal glycemic control and conceptus growth[12], as birth length and ponderal index[13]. In our study, inflammatory markers were in the normal range, except the IL-1β, which was below the normal reference probably as consequence of the short half-life and degradation, and leptin showed a tendency ($p=0.05$ by ANOVA) among the three groups. We found a strongly association between leptin and final maternal BMI and maternal glycemia, cord insulin, newborn abdominal circumference, and ponderal index. However, leptin was not associated with early maternal BMI or maternal weight gain. The maternal insulin resistance provides a higher nutritional support which favors the increase of insulin secretion and the development of adipose tissue by the fetus, therefore, the expansion of fat mass contribute for more production of leptin explaining the correlation with the ponderal index and abdominal circumference.

HGF promotes fetal liver development during the first and second trimester of gestation and hepatic maturation during late fetal to neonatal period[14]. And resistin induces hepatic insulin resistance by preventing the suppression of glucose production[15]. Our work showed association between cord HGF and resistin, and inversely correlation with maternal weight gain. It also suggests alterations of hepatic metabolism early in the fetal life as a result of the exposure to excess of nutrients.

We propone the maternal hyperglycemia triggers the process of fetal programming, which proceeds the high fetal insulin followed by larger accretion of fat mass. The development of fetal adipose tissue raises the leptin production, that stimulates the transference of nutrients from placenta to the fetus generating a vicious cycle. The excess of glucose and free fat acids become the pancreatic beta cells irresponsive. The

insulin resistance can orchestrate a complex phenomenon from which results in the modification of the metabolic processes, as suggested the alteration of fetal liver in early life in this paper.

Conclusion

These results demonstrate a positive association between maternal BMI, maternal glycemia, offspring cord insulin, leptin, HGF and resistin levels, newborn ponderal index and abdominal circumference even in mothers without GDM.

More studies are necessary to clarify the mechanisms mediating the relationships between maternal obesity and fetal programming. It will be an important research goal to optimizing pre-pregnancy BMI and metabolism aim at interrupt the obesity cycle.

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Table 1. Clinical and biochemical data of mothers and newborns.

	Category by Early BMI			
	BMI<25 kg/m^2	**BMI=25-30 kg/m^2**	**BMI>30 kg/m^2**	
	Lean (n=14)	Overweight (n=5)	Obese (n=12)	ANOVA P-value
Maternal data				
Early BMI	22.2±1.9^A (n=14)	27.3±1.8^B (n=5)	34.4±3.5^C (n=12)	<0.0001
Final BMI	28.4±2.9^A (n=14)	30.9±3.5 (n=5)	37.4±4.2^D (n=12)	<0.0001
Weight Gain (kg)	11.1±4.6^E (n=14)	9.8±3.3 (n=5)	6.4±3.7 (n=12)	0.02
Height (meters)	1.60±0.05 (n=14)	1.64±0.09 (n=5)	1.58±0.05 (n=12)	0.18
Age (years)	24.1±5.5^E (n=14)	29.0±5.0 (n=5)	30.2±4.6 (n=12)	0.01
Hematocrit (%)	35.7±3.2 (n=14)	34.6±1.5 (n=5)	36.9±2.7 (n=12)	0.30
Haemoglobin (g/dl)	11.5±1.0 (n=14)	11.4±0.4 (n=5)	12.1±1.0 (n=12)	0.25
Fasting Glucose 1st trimester (mg/dl)	78.3±8.4 (n=14)	77.7±9.6 (n=5)	78.5±6.5 (n=12)	0.97
Glucose 1h GTT 24 weeks (mg/dl)	105.0±24.5^E (n=14)	118.3±13.5 (n=5)	143.5±35.1 (n=12)	0.01
Fasting Glucose GTT 30 weeks (mg/dl)	75.4±5.0 (n=14)	69.3±4.9 (n=5)	78.4±8.4^F (n=12)	0.04
Glucose 1h GTT 30 weeks (mg/dl)	133.5±19.7 (n=14)	118.6±13.9 (n=5)	141.3±22.5^F (n=12)	0.12
Glucose 2h GTT 30 weeks (mg/dl)	116.2±14.1^E (n=14)	114.3±12.7 (n=5)	135.9±22.1^F (n=12)	0.01
Fasting Insulin GTT 30 weeks (uU/ml)	11.1±4.4 (n=13)	6.2±2.6 (n=5)	13.1±7.8^F (n=12)	0.10
Insulin 1h GTT 30 weeks (uU/ml)	111.1±51.1 (n=13)	80.3±14.0 (n=5)	128.2±92.2 (n=11)	0.42
Insulin 2h GTT 30 weeks (uU/ml)	103.7±59.4 (n=13)	69.7±25.2 (n=5)	144.9±100.4 (n=12)	0.16
FGIR (fasting glc/insulin ratio)	7.7±2.7 (n=13)	12.8±5.1^G (n=5)	8.1±5.4 (n=12)	0.09
HOMA-IR	2.0±0.8 (n=13)	1.0±0.4 (n=5)	2.6±1.7^F (n=12)	0.08
HOMA-beta %	497.9±588.2 (n=13)	596.3±603.4 (n=5)	393.6±243.6 (n=12)	0.71
Total Cholesterol (mg/dl)	249.8±30.6 (n=13)	232.0±34.0 (n=5)	226.3±49.0 (n=12)	0.32
HDL-Cholesterol (mg/dl)	64.9±10.9 (n=13)	61.8±15.8 (n=5)	71.5±10.9 (n=12)	0.39
LDL-Cholesterol (mg/dl)	144.3±27.5^H (n=13)	124.8±35.7 (n=5)	111.9±45.0 (n=12)	0.10
VLDL-Cholesterol (mg/dl)	40.7±10.9 (n=13)	36.0±2.9 (n=5)	50.1±2.7 (n=12)	0.24
Triglycerides (mg/dl)	203.7±53.6 (n=13)	179.0±13.8 (n=5)	213.1±57.6 (n=12)	0.47
C-section (%) inside each group	28.5 (n=4)	40.0 (n=2)	50.0 (n=6)	0.62*
Newborn data				
Gestational Age (weeks)	273.1±10.7 (n=14)	276.2±6.7 (n=5)	274.1±6.9 (n=12)	0.80
Birth Weight (grams)	3198.2±680.2 (n=14)	3432.2±216.1 (n=5)	3268.7±430.1 (n=12)	
Head circumference (cm)	33.7±1.6 (n=14)	35.0±0.7 (n=5)	34.2±1.6 (n=12)	0.28
Thorax circumference (cm)	32.6±2.2 (n=14)	33.8±0.4 ((n=5)	33.0±1.6 (n=12)	0.52
Abdominal circumference (cm)	31.3±2.9 (n=14)	32.0±1.7 (n=5)	31.5±1.9 (n=12)	0.87
Length (cm)	48.7±3.1 (n=14)	50.2±1.9 (n=5)	48.8±2.3 (n=12)	0.57
Ponderal Index (kg/m^3)	27.3±2.7 (n=14)	27.2±2.5 (n=5)	28.0±2.3 (n=12)	0.73
Cord Hematocrit (%)	41.7±3.7 (n=10)	36.8±5.0 (n=2)	42.4±3.9 (n=10)	0.20
Cord Haemoglobin (g/dl)	13.4±1.0 (n=10)	11.8±2.1 (n=2)	13.6±1.3 (n=10)	0.20
Cord Haemoglobin A1c (%)	1.4±0.14 (n=10)	1.3±0.06 (n=3)	1.3±0.10 (n=7)	0.26
Cord Insulin (uU/ml)	4.4±3.7 (n=13)	8.3±5.6 (n=5)	7.9±8.7 (n=12)	0.33

* Date calculated by Fisher

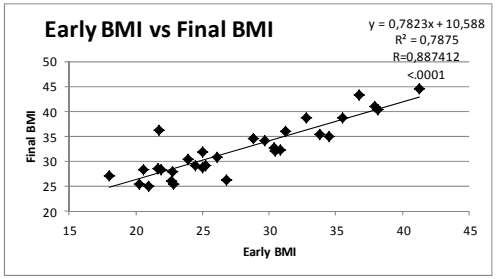
Letters indicate Fisher P<0.0001 - A: Lean vs. Obese; P<0.005 - B: Lean vs. Overweight; P<0.0001 C: Overweight vs. Obese; P<0.005 – D: Overweight vs. Obese; P<0.01 - E: Lean vs. Obese; P<0.05 – F: Overweight vs. Obese; G: Lean vs. Overweight; H: Lean vs. Obese

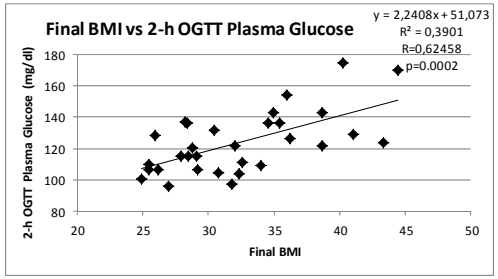
Table 2. Inflammatory Markers in the cord blood.

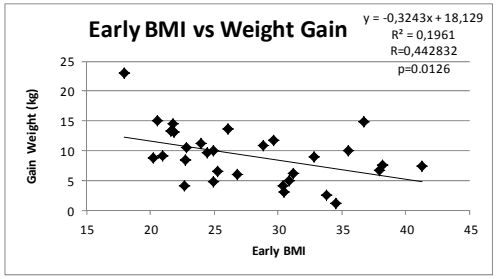
	Category by Early BMI			
	BMI<25 kg/m^2	**BMI=25-30 kg/m^2**	**BMI>30 kg/m^2**	
	Lean (n=14)	Overweight (n=5)	Obese (n=11)	ANOVA
Inflammatory Markers				P-value
Leptin (pg/ml)	22321.0±19183.0	58614.4±40473.1	40225.8±32296.5	0.05[A,B]
IL-8 (pg/ml)	87.6±159.3	33.0±41.3	113.3±289.6	0.77
HGF (pg/ml)	3303.9±2485.8	2674.8±938.4	3620.0±784.3	0.63
MCP-1 (pg/ml)	490.4±441.1	414.4±277.3	296.0±202.3	0.39
TNF-alpha (pg/ml)	9.4±2.4	11.1±4.0	12.2±4.0	0.13
IL-1b (pg/ml)	0.12±0.06	0.13±0.09	3.3±10.6	0.44
IL-6 (pg/ml)	14.5±12.5	10.8±4.6	47.5±72.5	0.15
Adiponectin (ug/ml)	467.9±374.4	264.7±100.4	310.3±205.5	0.27
Resistin (pg/ml)	201.9±182.7	126.4±75.5	236.0±129.9	0.42
Total PAI-1 (pg/ml)	184.9±59.4	134.8±27.9	160.2±26.1	0.10

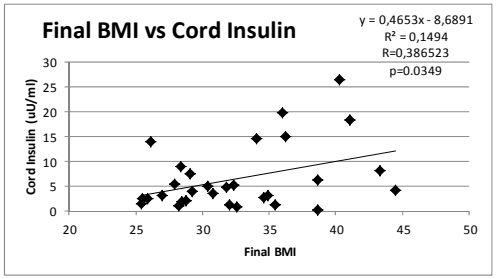
Letters indicate Fisher P=0.001 - A: Lean vs. Overweight; P<0.05 - B: Lean vs. Obese.

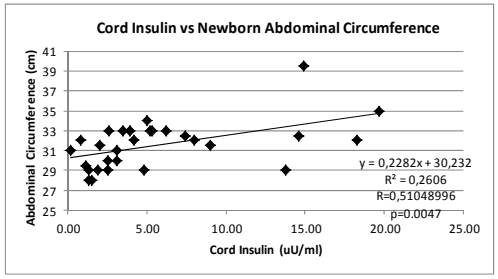
Figures 1. Significant Correlations

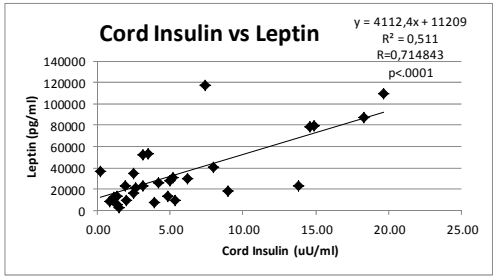


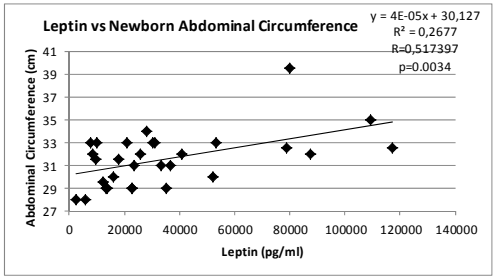


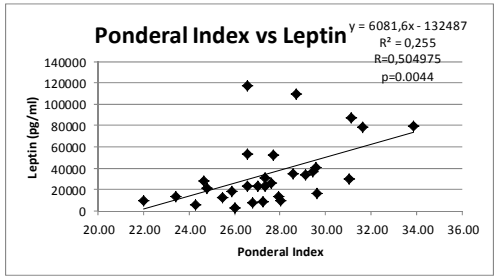


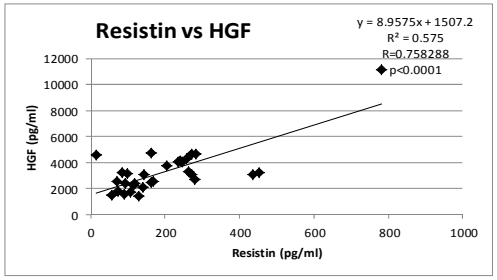












ARTIGO 2

Title: "Fetal cord blood lipids in offspring of obese mothers"

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Abstract

By virtue of the obesity being more common in the children of obese mothers, it is of vital public health importance to understand the mechanisms responsible for effects of obesity-linked maternal insulin resistance on offspring health[1]. We assess the hybrid receptor IR/IGF-IR formation and the lipidomic profile to identify whether is alterated in the fetus of obese mothers.

Introduction

Obesity is considered a pandemic public health problem by World Health Organization. The projected total numbers of obese adults in 2030 will be 573 million individuals[2]. Beside, the prevalence of childhood and adolescent obesity (2003-2006) between 2 and 19 years-old varies from 12% to 18%, and they are affected by the same

co-morbitidies than the adults[3,4]. It is well established that obesity being more common in the children of obese mothers[1]. Some studies have showed that the interaction between the genotype and the intrauterine environment can modify the fetus sensibility to insulin[5]. The insulin receptor/insulin-like growth factor type I hybrid receptor raise as a possible molecular etiology of insulin resistance[6]. Beside, the insulin resistance can be a result of changes in the intracellular metabolic pathways. In this study, we assessed the gene expression of insulin and IGF-I receptors to verify whether there is more formation of hybrids receptors in offspring of obese mothers, and if the lipids could trigger changes in the signaling of intracellular processes.

Methods

The thirty one selected pregnant women were grouped by the body mass index (mean 15.0 ± 4.2 weeks of gestation), calculated at the first prenatal visiting, in: 14 normal body mass index pregnant women, 5 overweight pregnant women and 12 obese women. The maternal glycemia (complete 75 g OGTT: fasting, 1-hour and 2-hour glycemia and insulin levels) and fasting lipids profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, and triglycerides) were assessed at 30.3 ± 4.0 weeks and analyzed using ADA criteria[7]; women with gestational diabetes mellitus were excluded from analysis. Cord blood samples were collected at delivery for the analysis of lipomic profile and, then, the **h**uman **u**mbilical **v**ein **e**ndothelial **c**ells (HUVECs) were isolated from umbilical cord[8]. RNA was extracted from HUVEC and performed Real Time **q**uantitative **R**everse **T**ranscription **P**olymerase **C**hain **R**eaction (Real Time qRT-PCR)[9]. The protocol was approved by the Hospital Institutional Review Board (CEP IMIP/PE) and the National Committee for Ethics in Research at National Health Council (CONEP-Statement # 387/2011), Brasilia, Brazil. ANOVA (Statview) was used to compare differences for continuous variables among the 3 categories for the maternal body mass index (lean<25kg/m^2, overweight from 25 to 30kg/m^2, obese>30kg/m^2).

Results

All women were Brazilian and had no essential hypertension neither evidence of other systemic disease. None pregnant smoked before or during the pregnant. There were not significant differences in the groups about education, marital status, familiar history of mellitus diabetes or hypertension in first degree parents, obstetrician history of previous abortion and parity, presence of preeclampsia or bleeding in the present pregnancy, and type of delivery.

Fasting and 2-h OGTT in the third trimester (both: $p<0.05$) were statistically significant by ANOVA among the groups, but LDL-cholesterol was not ($p=0.1$). Maternal BMI were correlated with maternal glycemia (2-hour OGTT) ($r=0.59$, $p<0.001$) and with LDL-cholesterol ($r=0.41$, $p<0.05$).

The gene expression of two insulin receptor isoforms and insulin-simile growth factor type I did not show difference among the 3 groups (IR short: $p=0.16$, IR long: $p=0.32$, and IGF-IR: $p=0.22$, respectively).

The analysis of lipidomic profile (phospholipids (PL), triglycerides (TG), free fat acids (FFA), cholesterol esters (CE), and diacylglicerols (DAG)) showed significantly difference of the total DAG ($p=0.04$) and the **18:0** of DAG fraction ($p=0.01$) when we analyzed the absolute concentration (Table 1). In addition to, the proportions of the **22:4w6 TG** ($p=0.03$) and **18:2 TG** fractions ($p=0.05$) among the groups were significant different among the 3 groups, and **20:4 TG** ($p=0.09$), **16:0 FFA** ($p=0.07$), **18:1w9 FFA** ($p=0.09$), **18:1w7 FFA** ($p=0.09$) tended to be different (Table 2).

Discussion

Obese mother has high glycemic levels than lean mother even when they are not diabetic because the physiological resistance to insulin during pregnancy is exacerbated by obesity[10]. In this condition the fetus is overloaded with glucose and lipids transported by the placenta[11]. In order to maintain the equilibrium, the fetus triggers adaptative responses, as changes in the insulin receptor and/or in the cascade of intracellular

processes. One potential mediator would be alterations in insulin receptor signaling due to differences in formation of hybrid receptors IR/IGF-IR which occurs when the IGF-IR mRNA/IR mRNA ratio is 7.1 +/- 1.5[9]. It is known the excess of glucose increases the DAG content and these lipids oversupply overloads the glucose-fat acid cycle[12] that results in insulin resistance due to changes in the intracellular processes. We did not find any difference in the gene expression among the groups. Thus, the other situation what could explain the attempt to restore the balance would be the larger amount transfer of LDL that could be converted over to DAG and its fractions beyond TG and FFA fraction. In this study the maternal BMI was associated with the maternal glycemia and LDL-cholesterol which are transfered by diffusion.

This work showed indications that the insulin resistance already starts in the intrauterine period. However it is necessary studies with a bigger amount of patients to confirm these data, to determine what is the altered intracellular pathway and what lipid fraction is more important.

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Table 1. Lipid Profile in the cord blood.

	Category by Early BMI			
	BMI<25 kg/m^2	**BMI=25-30 kg/m^2**	**BMI>30 kg/m^2**	
Absolute Values	Lean (n=14)	Overweight (n=8)	Obese (n=15)	ANOVA
Lipid Profile				P-value
Total PL	758.3±190.2	858.4±149.6	744.1±201.8	0.36
14:0 PL	2.2±0.6	2.5±0.6	2.2±0.7	0.71
16:0 PL	248.4±57.5	285.1±64.2	244.7±64.7	0.30
16:1 PL	6.1±1.9	7.4±1.0	6.2±2.9	0.44
18:0 PL	116.4±35.4	136.3±23.0	113.4±31.6	0.24
18:1w9 PL	54.1±9.7	64.4±13.3	52.2±18.4	0.15
18:1w7 PL	18.4±3.2	22.2±3.1	19.9±7.0	0.26
18:2 PL	63.6±22.0	66.6±14.4	64.11±21.7	0.94
20:3w6 PL	40.2±9.6	39.9±7.2	33.6±13.7	0.23
20:4 PL	141.4±41.5	163.2±31.2	133.1±51.4	0.30
20:5 PL	1.3±1.6	0.7±1.1	12.9±46.1	0.49
22:4w6 PL	5.6±2.1	6.2±3.0	5.2±1.9	0.56
22:5w6 PL	8.0±4.0	9.0±3.5	6.7±3.2	0.36
22:5w3 PL	3.7±2.4	3.6±1.9	3.0±2.3	0.69
22:6 PL	48.2±23.2	50.6±13.9	46.0±16.0	0.85
Total TG	288.0±136.2	242.4±76.3	382.4±354.7	0.37
14:0 TG	5.1±2.6	4.4±1.2	6.8±5.8	0.35
16:0 TG	95.3±41.7	81.9±28.4	125.6±125.5	0.45
16:1 TG	15.4±6.6	14.0±4.2	23.3±28.0	0.40
18:0 TG	26.9±11.8	18.5±6.9	23.5±13.2	0.28
18:1w9 TG	77.5±40.0	65.6±23.9	100.9±87.6	0.38
18:1w7 TG	9.2±3.6	8.3±3.3	11.6±9.4	0.46
18:2 TG	35.5±26.5	28.2±10.0	56.3±58.9	0.23
18:3w6 TG	08±1.1	0.8±0.7	1.6±1.8	0.26
18:3w3 TG	1.0±1.2	0.5±0.6	1.8±1.8	0.14
20:3w6 TG	2.9±2.8	1.8±0.8	3.6±4.5	0.49
20:4 TG	8.3±5.8	9.8±4.3	12.8±11.3	0.35
20:5 TG	1.3±1.6	0.7±1.1	12.9±46.1	0.49
22:4w6 TG	1.3±1.2	1.8±1.0	2.4±2.8	0.34
22:5w6 TG	2.4±1.3	2.4±1.2	3.4±2.3	0.30
22:5w3 TG	0.4±0.8	0.0±0.0	0.9±1,5	0.13
22:6 TG	5.1±3.5	3.6±2.1	6.8±5.2	0.20
Total FFA	97.9±36.7	110.3±37.2	131.0±51.1	0.13
14:0 FFA	0.9±0.5	1.1±0.2	1.2±0.6	0.23
16:0 FFA	35.1±12.5	43.0±12.2	48.1±21.7	0.13
16:1 FFA	15.4±6.6	14.0±4.2	23.3±28.0	0.40
18:0 FFA	116.4±35.4	136.3±23.0	113.4±31.6	0.24
18:1w9 FFA	54.1±9.7	64.4±13.3	52.2±18.4	0.15
18:1w7 FFA	1.6±0.9	1.3±0.5	2.0±0.8	0.14
18:2 FFA	12.6±7.2	11.9±8.5	15.9±7.3	0.37
18:3w6 FFA	40.2±9.6	39.9±7.2	33.6±13.7	0.23

18:3w3 FFA	0.4±0.4	0.4±0.4	0.6±0.4	0.47
20:4 FFA	2.7±1.3	2.3±1.3	2.9±1.2	0.54
22:6 FFA	1.1±0.7	1.0±0.8	1.4±0.6	0.41
Total CE	673.9±178.4	729.2±272.9	605.2±219.9	0.41
14:0 CE	5.8±2.0	7.1±3.9	5.1±1.9	0.21
16:0 CE	145.0±38.9	168.1±88.2	128.0±45.2	0.26
16:1 CE	49.4±12.7	60.7±34.7	44.3±22.4	0.27
18:0 CE	26.7±9.9	27.6±10.6	22.0±9.6	0.33
18:1w9 CE	172.4±36.6	187.1±63.7	165.6±78.7	0.73
18:1w7 CE	20.8±3.9	24.1±8.4	20.3±7.4	0.40
18:2 CE	12.6±7.2	11.9±8.5	15.9±7.3	0.37
18:3w6 CE	5.0±1.2	5.9±3.5	4.2±2.4	0.27
18:3w3 CE	0.5±1.0	0.1±0.3	0.5±0.9	0.48
20:3w6 CE	9.3±2.9	9.0±2.2	7.4±2.3	0.12
20:4 CE	88.6±37.6	99.9±32.9	79.4±25.4	0.35
20:5 CE	1.1±1.2	0.7±1.1	0.7±0.7	0.56
22:5w6 CE	0.3±0.6	0.1±0.3	0.4±0.6	0.54
22:6 CE	7.7±3.2	7.6±3.4	7.1±3.5	0.86
Total DG	10.7±6.2	16.4±10.4	8.8±4.5	0.04^{A}
14:0 DG	0.04±0.09	0.12±0.17	0.09±0.17	0.49
16:0 DG	3.1±1.4	4.2±2.7	2.6±1.4	0.15
16:1 DG	0.09±0.18	0.89±0.17	0.02±0.10	0.45
18:0 DG	4.6±4.0	9.0±6.2	3.8±2.3	0.01
18:1w9 DG	2.0±1.4	2.3±2.1	1.6±1.0	0.47
18:1w7 DG	0.04±0.11	0.0±0.0	0.01±0.05	0.40
18:2 DG	0.5±0.7	0.4±0.2	0.4±0.3	0.59
22:6 DG	0.02±0.10	0.0±0.0	0.0±0.0	0.45

Letters indicate Fisher $P<0.05$ - A: Overweight vs. Obese.

Table 2. Proportions of lipids in the cord blood.

	Category by Early BMI			
	BMI<25 kg/m^2	**BMI=25-30 kg/m^2**	**BMI>30 kg/m^2**	
Lipid Fraction (%)	Lean (n=14)	Overweight (n=8)	Obese (n=15)	ANOVA P-value
14:0 PL	0.30±0.07	0.29±0.06	0.31±0.12	0.82
16:0 PL	32.9±1.7	33.0±2.3	32.9±2.1	0.99
16:1 PL	0.87±0.37	0.87±0.11	0.84±0.35	0.98
18:0 PL	15.2±1.4	15.9±0.7	15.2±1.4	0.43
18:1w9 PL	7.3±1.1	7.4±0.4	6.9±0.9	0.41
18:1w7 PL	2.4±0.3	2.6±0.2	2.6±0.3	0.41
18:2 PL	8.3±1.8	7.8±1.7	8.6±1.5	0.61
20:3w6 PL	5.3±0.6	4.7±0.8	4.5±1.4	0.13
20:4 PL	18.5±2.2	19.0±1.9	18.0±4.2	0.75
20:5 PL	0.16±0.19	0.08±0.12	1.47±5.10	0.48
22:4w6 PL	0.73±0.15	0.69±0.15	0.71±0.35	0.85
22:5w6 PL	1.0±0.3	1.0±0.4	0.8±0.3	0.40
22:5w3 PL	0.45±0.25	0.41±0.19	0.41±0.32	0.88
22:6 PL	6.1±1.8	5.8±0.8	6.3±2.0	0.85
14:0 TG	1.7±0.3	1.8±0.2	1.7±0.3	0.75
16:0 TG	33.5±1.7	33.5±3.1	32.0±3.0	0.26
16:1 TG	5.4±0.9	5.8±0.5	5.4±1.0	0.67
18:0 TG	10.2±4.9	7.6±1.4	7.6±3.3	0.14
18:1w9 TG	26.5±3.1	27.1±3.7	27.0±3.3	0.88
18:1w7 TG	3.2±0.7	3.4±0.7	3.1±0.6	0.65
18:2 TG	11.3±3.2	11.7±2.1	13.8±2.5	0.05[A]
18:3w6 TG	0.26±0.26	0.32±0.32	0.38±0.26	0.52
18:3w3 TG	0.27±0.3	0.20±0.2	0.43±0.2	0.12
20:3w6 TG	0.9±0.8	0.7±0.3	0.8±0.4	0.69
20:4 TG	2.9±1.2	4.1±1.6	3.5±0.9	0.09
20:5 TG	0.08±0.14	0.0±0.0	0.98±0.16	0.30
22:4w6 TG	0.44±0.3	0.85±0.3	0.58±0.3	0.03[B]
22:5w6 TG	0.90±0.4	1.15±0.3	0.99±0.4	0.43
22:5w3 TG	0.11±0.2	0.0±0.0	0.18±0.2	0.11
22:6 TG	1.7±0.9	1.7±0.8	2.0±1.2	0.78
14:0 FFA	0.9±0.3	1.1±0.4	0.9±0.3	0.66
16:0 FFA	36.4±3.3	39.5±2.1	36.3±3.7	0.07
16:1 FFA	2.3±0.8	2.1±0.6	2.5±0.8	0.59
18:0 FFA	22.9±8.4	27.9±5.0	22.1±7.8	0.21
18:1w9 FFA	18.7±6.0	14.6±2.3	19.5±5.2	0.09
18:1w7 FFA	1.5±0.4	1.1±0.1	1.6±0.5	0.09
18:2 FFA	12.0±4.0	9.9±3.4	12.3±3.9	0.35
18:3w3 FFA	0.27±0.3	0.20±0.2	0.43±0.2	0.12
20:3w6 FFA	0.28±0.3	0.17±0.2	0.29±0.3	0.61
20:4 FFA	3.0±1.4	2.0±0.7	2.4±0.9	0.11
22:6 FFA	1.2±0.2	1.0±0.3	1.1±0.4	0.47

14:0 CE	0.8±0.1	0.9±0.1	0.8±0.2	0.61
16:0 CE	21.6±2.1	22.3±2.3	21.2±1.2	0.41
16:1 CE	7.5±1.9	7.9±1.2	7.0±1.3	0.39
18:0 CE	4.0±1.5	3.7±0.4	3.7±1.1	0.67
18:1w9 CE	26.0±3.1	25.8±2.4	26.6±4.1	0.83
18:1w7 CE	3.1±0.5	3.3±0.3	3.3±0.3	0.45
18:2 CE	20.2±6.3	18.6±3.7	20.0±2.5	0.71
18:3w6 CE	0.76±0.1	0.78±0.1	0.66±0.1	0.21
18:3w3 CE	0.07±0.13	0.02±0.06	0.09±0.18	0.49
20:3w6 CE	1.3±0.2	1.3±0.3	1.2±0.3	0.68
20:4 CE	12.8±2.9	13.8±1.3	13.5±2.9	0.66
20:5 CE	0.15±0.16	0.09±0.12	0.12±0.13	0.60
22:5w6 CE	0.04±0.07	0.02±0.06	0.05±0.08	0.67
22:6 CE	1.1±0.4	1.0±0.1	1.2±0.6	0.57
14:0 DG	0.50±1.0	0.87±1.4	0.69±1.2	0.78
16:0 DG	32.2±9.6	28.0±8.7	31.8±6.5	0.49
16:1 DG	0.51±1.0	0.34±0.7	0.20±0.8	0.66
18:0 DG	40.8±18.1	53.6±14.2	42.2±14.2	0.17
18:1w9 DG	20.1±8.3	13.4±6.5	19.4±9.2	0.18
18:1w7 DG	0.30±0.7	0.0±0.0	0.12±0.4	0.48
18:2 DG	5.3±4.2	3.5±1.8	5.8±2.6	0.32
20:4 DG	3.7±1.2	2.0±1.6	3.0±0.7	0.35

Letters indicate Fisher $P<0.05$ - A: Lean vs. Obese; $P=0.01$ - B: Lean vs. Overweight.

CONSIDERAÇÕES FINAIS

Durante a vida intra-uterina, o organismo em formação sofre uma série de influências do ambiente que modificam a expressão de seus genes resultando em diferentes perfis morfológicos, fisiológicos e metabólicos, com repercussões ao longo da vida. Esta plasticidade durante um período de rápido desenvolvimento torna-o especialmente sensível à influência da nutrição e de outros fatores ambientais[9].

Uma intrincada rede de conexões, intracelulares e entre os tecidos, determinam a resistência à insulina. Durante este trabalho observamos que a maior secreção de insulina, o acúmulo de gordura e a produção exagerada de leptina pelo concepto traduzem as modificações sofridas pelos processos metabólicos fetais suscetíveis ao estado nutricional e à composição corporal maternos. Nossa contribuição foi pontual em tão vasto processo restando muitas perguntas para responder.

Em virtude da quantidade de dados ainda a serem analisados e do material restante das coletas, refiro-me ao soro do cordão umbilical e ao RNA das HUVECs, pensei de antemão em algumas perguntas que ainda poderão ser respondidas para a melhor compreensão da resposta adaptativa do feto às condições impostas neste estágio do desenvolvimento. As novas idéias estão estruturadas da seguinte forma:

1. Para esclarecer melhor a respeito do transporte de nutrientes para o feto poderão ser realizadas as dosagens de aminoácidos, de hormônios, e de outros marcadores inflamatórios para observar a troca de informações entre o feto e sua mãe.
2. Após uma análise minuciosa dos dados das *microarrays* e da literatura, investigaremos as vias pós-traducionais que poderiam alterar a expressão dos receptores IR, IGF-I e seus híbridos na membrana celular.
3. Examinaremos também as quatro vias de estresse oxidativo intracelular - a via do poliol, a via da formação dos produtos finais da glicosilação, a via da proteína quinase C (PKC)-diacilglicerol (DAG) e a via da hexosamina - para verificar se algum deles está comprometido nas HUVECs destes recém-nascidos.

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APÊNDICES

APÊNDICES

APÊNDICE A - ABSTRACT ACEITO NO CONGRESSO DA AMERICAN DIABETES ASSOCIATION - PHILADELPHIA, JUNHO/2012

72nd American Diabetes Association. scientificsessions
JUNE 8-12, 2012 • PHILADELPHIA, PA

2546-PO

Maternal BMI is Associated With Infant Umbilical Cord Insulin Levels

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To determine whether maternal obesity is associated with neonatal anthropometrics and metabolic parameters, we prospectively studied healthy pregnant women (n=31). Maternal BMI was assessed at the first prenatal visit (mean 15.0 ± 4.2 weeks) and at 36-40 weeks. Fasting lipids and glucose tolerance (75 g OGTT) were assessed at 30.3 ± 4 weeks and analyzed using ADA criteria; women with GDM were excluded from analysis. Neonatal anthropometrics and cord insulin were measured at birth. Early and late gestational maternal BMI were strongly associated (r=0.89, p<0.0001). Both maternal BMI were associated with maternal glycemia (2-hour OGTT) (r=0.59, r=0.62, both p<0.001) and LDL (r=0.42, r=0.35, p≤0.05). We next assessed relationships between maternal metabolism and fetal growth. Maternal glucose correlated with birth weight (r=0.34, p<0.03). Maternal insulin resistance (HOMA-IR) correlated with head circumference (r=0.55, p<0.01), but not birth weight (r=0.09, p=0.63) or length (r=0.18, p=0.34). Neither maternal BMI correlated with birth weight (r=0.09, r=0.01). However, there was a robust association between both gestation maternal BMI and cord insulin (early: r=0.55, p<0.001; final r=0.65, p<0.0001), which was accounted for in part by association between maternal glucose and cord insulin (r=0.36, p=0.02). There was no correlation between birth weight and cord insulin (r=0.008, p=0.96). Maternal weight gain during pregnancy tended to be inversely related to initial BMI (r=-0.23, p=0.18), as expected, but was not associated with final BMI (r=0.11, p=0.52) or offspring cord insulin (r=0.20, p=0.25). These results demonstrate a strong positive association between maternal BMI, maternal glycemia, and offspring cord insulin levels, even in mothers without GDM. Determining the mechanisms mediating the relationships between maternal obesity and offspring insulin levels will be an important research goal. Optimizing pre-pregnancy BMI and metabolism are key therapeutic goals in women of childbearing age.

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APÊNDICE B - **TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO**

A HIPERINSULINEMIA MATERNA INDUZ O SURGIMENTO DOS RECEPTORES HÍBRIDOS IR/IGF-I NO RECÉM-NASCIDO?

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Dra. Maria Elizabeth Cavalcante Chaves
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A finalidade do estudo é verificar se os recém-nascidos de mães obesas com níveis altos de insulina no sangue, durante a gestação, têm alterações nas células do cordão umbilical. Para isso, acompanharemos as consultas de pré-natal de mães obesas e com peso normal. Será necessária a retirada de sangue para fazer as dosagens de glicose e insulina no começo, no meio e no fim da gravidez. Para fazer os exames, a gestante deverá estar em jejum e durante a coleta do sangue será necessário tomar um copo de água com açúcar dado pela nossa equipe. O incomodo da coleta de sangue é o jejum que começa na noite anterior e a dor à furada. Após o parto receberemos o cordão umbilical da pediatra para retirada do sangue e das células para a pesquisa.

A sua participação é voluntária, e poderá ser cancelada a qualquer momento sem acarretar prejuízo para a sua pessoa. Este estudo não trará gastos financeiros para você, nem tampouco haverá alguma forma de pagamento pela sua participação.

A qualquer momento do estudo você poderá solicitar esclarecimentos necessários.

As informações obtidas através do estudo terão caráter sigiloso, bem como será respeitada a privacidade de seus participantes. Elas poderão ser divulgadas em eventos ou publicações científicas, porém preservando a identidade de seus participantes.

Eu,_____, li o texto acima, compreendi a sua finalidade e dou voluntariamente a permissão para a sua execução.

Recife,___/___/_____

_____ _____

Participante da pesquisa ID: Dra. Suzana Maria Ramos Costa

APÊNDICE C - **FORMULÁRIO DA PESQUISA**

ID.

Data:

Nome:

Endereço:

Data de nascimento (**DN**):

Estudos: 1. nenhum O 2. primário O 3. secundário O 4. universitário O
Estado civil: 1. solteira O 2. união estável O 3. casada O 4. viúva O 5. outros O

Antecedentes Familiares
Diabetes: sim O não O
Hipertensão arterial: sim O não O
Obesidade: sim O não O
Gemelares: sim O não O
Outros: sim O não O

Antecedentes Pessoais
Infecção urinária: sim O não O
Infertilidade: sim O não O
Diabetes: sim O não O
Hipertensão crônica: sim O não O
Cirurgia pélvica uterina: sim O não O
Outros: sim O não O

Antecedentes Obstétricos
Gesta Paridade Abortos Natimortos
Partos vaginais Cesareanas
Data do término da última gestação:
Nascidos vivos Ainda vivem Já morreram
Algum RN pesou menos de 2500g: sim O não O
Nascimento com maior peso g

Gravidez atual
Peso anterior , kg **Estatura** cm
DUM: / / **Dúvidas da DUM**: 0. não O 1. sim O
Antitetânica: prévia: sim O não O Atual: 1ª dose O 2ª dose O 3ª dose O
Hospitalização na gravidez: O. não O 1. sim O

Se sim quantos dias
Grupo Sanguíneo: A B O AB Rh pos neg sensibilizada: sim O não O
Já foi transfundida: sim O não O Quando? Onde?
Ex. clínico normal: sim O não O
Ex. das mamas normal: sim O não O
Ex. odontológico normal: sim O não O
Pélvis normal: sim O não O
Papanicolau normal: sim O não O
Colposcopia normal: sim O não O
Ex. clínico da cérvix normal: sim O não O
Fuma: sim O não O Se sim, quantos cigarros por dias

Patologias nesta gestação (marcar 0 se não ocorrer e 1 se ocorrer)

Gravidez múltipla		Desproporção céfalo-pélvica	
Hipertensão prévia		Hemorragia 1º trimestre	
Pré-eclâmpsia		Hemorragia 2º trimestre	
Eclâmpsia		Hemorragia 3º trimestre	
Cardiopatia		Anemia crônica	
Diabetes		Rutura prematura de membranas	
Infecção crônica		Infecção puerperal	
Outras infecções		Hemorragia puerperal	
Parasitoses		Outra	
Ameaça de parto prematuro		Nenhuma	

Consultas

Cons	1	2	3	4	5	6	7	8	9
Data									
IG									
Peso									
PA									
FU									
Apres									
BCF									
Mov F									

Exames laboratoriais

Exame	data	resultado	data	resultado	conduta
Hb					
Ht					
Urina					
VDRL					
Anti-HIV					
ABO-Rh					
Coombs Ind					

Data	IG DUM	IG USG	Peso fetal	Placenta	Líquido

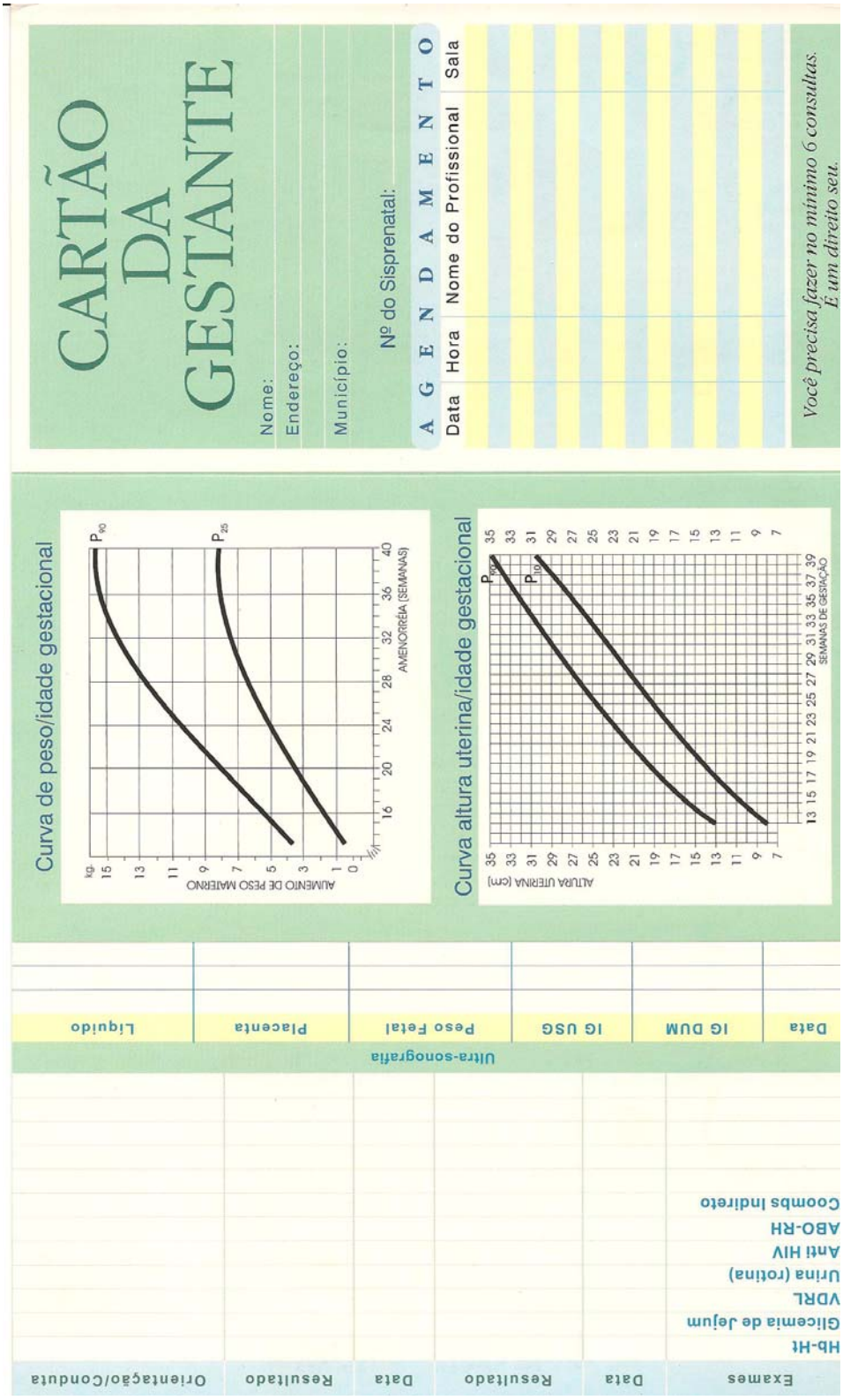
Data	Glicemia	Insulinemia
Basal		
30 minutos		
90 minutos		
120 minutos		
Data	Glicemia	Insulinemia
Basal		
30 minutos		
90 minutos		
120 minutos		

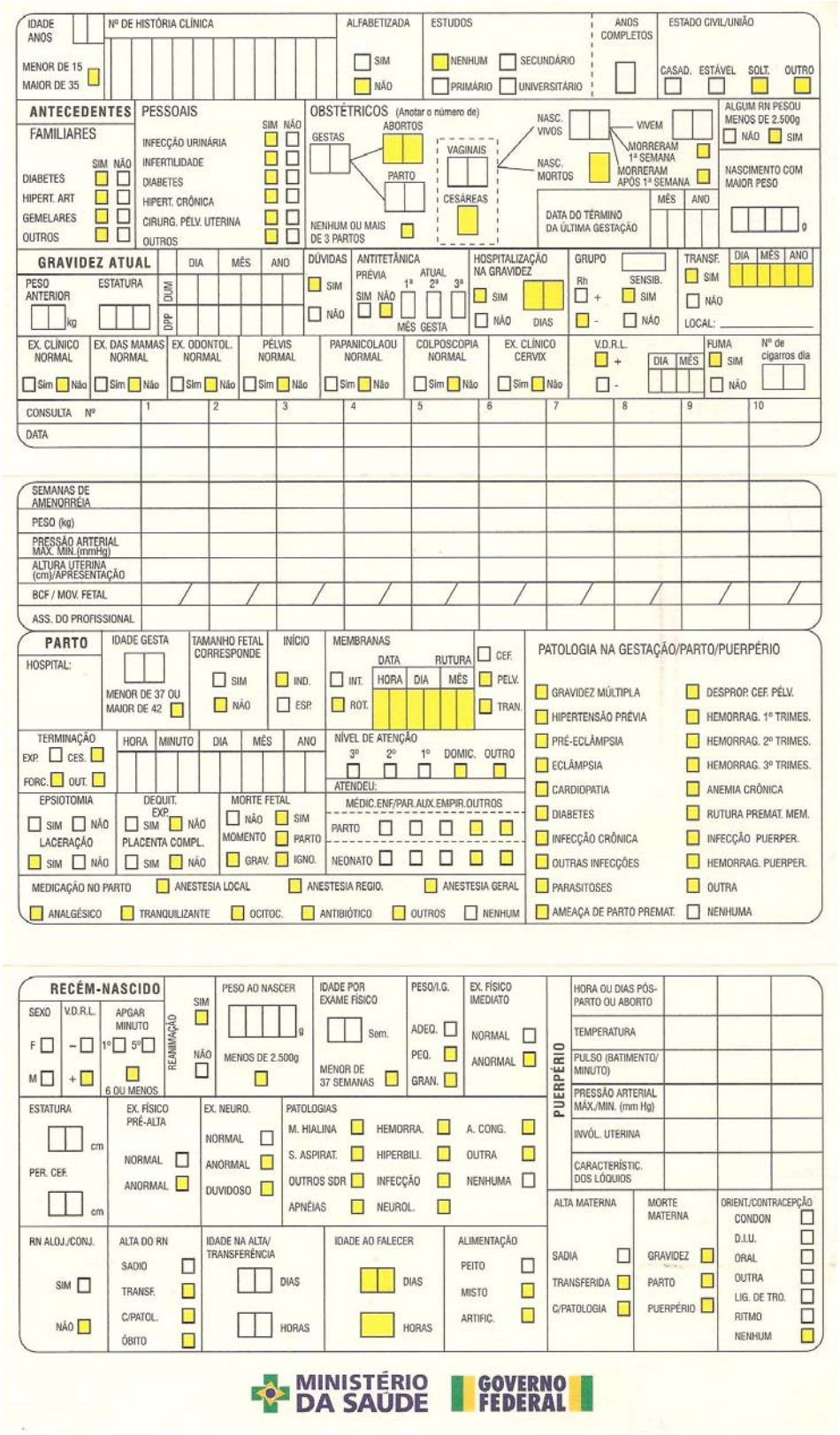
Recém-nascido
Sexo: 1. **F** O 2. **M** O Apgar de 1º min e de 5º min Reanimação: sim O não O
Peso g **Comprimento** , cm
PC , cm **PT** , cm **Capurro** s d
Peso/IG AIG O PIG O GIG O
Ex físico imediato normal: sim O não O
RN encaminhado para alojamento conjunto: sim O não O
RN com patologias: sim O não O
Se sim (marcar um X)

Membrana Hialina		Hemorragia	
SAM		Hiperbilirrubinemia	
Outros SDR		Infecção	
Apnéias		Problema neurológico	
Anomalias congênitas		Outra	

Alimentação: peito O misto O artificial O

APÊNDICE D - **CARTÃO DA GESTANTE**




IDADE ANOS
MENOR DE 15
MAIOR DE 35
Nº DE HISTÓRIA CLÍNICA
ALFABETIZADA
SIM
NÃO
ESTUDOS
NENHUM
SECUNDÁRIO
PRIMÁRIO
UNIVERSITÁRIO
ANOS COMPLETOS
ESTADO CIVIL/UNIÃO
CASAD.
ESTÁVEL
SOLT.
OUTRO
ANTECEDENTES
FAMILIARES
SIM NÃO
DIABETES
HIPERT. ART
GEMELARES
OUTROS
PESSOAIS
SIM NÃO
INFECÇÃO URINÁRIA
INFERTILIDADE
DIABETES
HIPERT. CRÔNICA
CIRURG. PÉLV. UTERINA
OUTROS
OBSTÉTRICOS (Anotar o número de)
GESTAS
ABORTOS
PARTO
NENHUM OU MAIS DE 3 PARTOS
VAGINAIS
CESÁREAS
NASC. VIVOS
NASC. MORTOS
VIVEM
MORRERAM 1ª SEMANA
MORRERAM APÓS 1ª SEMANA
DATA DO TÉRMINO DA ÚLTIMA GESTAÇÃO
MÊS
ANO
ALGUM RN PESOU MENOS DE 2.500g
NÃO
SIM
NASCIMENTO COM MAIOR PESO
g
GRAVIDEZ ATUAL
PESO ANTERIOR
kg
ESTATURA
DUM
DPP
DIA
MÊS
ANO
DÚVIDAS
SIM
NÃO
ANTITETÂNICA
PRÉVIA
SIM NÃO
ATUAL
1ª 2ª 3ª
MÊS GESTA
HOSPITALIZAÇÃO NA GRAVIDEZ
SIM
NÃO
DIAS
GRUPO
Rh
+
-
SENSIB.
SIM
NÃO
TRANSF.
SIM
NÃO
LOCAL:
EX. CLÍNICO NORMAL
EX. DAS MAMAS NORMAL
EX. ODONTOL. NORMAL
PÉLVIS NORMAL
PAPANICOLAOU NORMAL
COLPOSCOPIA NORMAL
EX. CLÍNICO CERVIX
Sim Não
V.D.R.L.
+
-
DIA MÊS
FUMA
SIM
NÃO
Nº de cigarros dia
CONSULTA Nº
1 2 3 4 5 6 7 8 9 10
DATA
SEMANAS DE AMENORRÉIA
PESO (kg)
PRESSÃO ARTERIAL MAX. MIN.(mmHg)
ALTURA UTERINA (cm)/APRESENTAÇÃO
BCF / MOV. FETAL
ASS. DO PROFISSIONAL
PARTO
HOSPITAL:
IDADE GESTA
MENOR DE 37 OU MAIOR DE 42
TAMANHO FETAL CORRESPONDE
SIM
NÃO
INÍCIO
IND.
ESP.
MEMBRANAS
DATA RUTURA
HORA DIA MÊS
INT.
ROT.
CEF.
PÉLV.
TRAN.
PATOLOGIA NA GESTAÇÃO/PARTO/PUERPÉRIO
GRAVIDEZ MÚLTIPLA
HIPERTENSÃO PRÉVIA
PRÉ-ECLÂMPSIA
ECLÂMPSIA
CARDIOPATIA
DIABETES
INFECÇÃO CRÔNICA
OUTRAS INFECÇÕES
PARASITOSES
AMEAÇA DE PARTO PREMAT.
DESPROP. CEF. PÉLV.
HEMORRAG. 1º TRIMES.
HEMORRAG. 2º TRIMES.
HEMORRAG. 3º TRIMES.
ANEMIA CRÔNICA
RUTURA PREMAT. MEM.
INFECÇÃO PUERPER.
HEMORRAG. PUERPER.
OUTRA
NENHUMA
TERMINAÇÃO
EXP. CES.
FORC. OUT.
HORA MINUTO DIA MÊS ANO
NÍVEL DE ATENÇÃO
3º 2º 1º DOMIC. OUTRO
ATENDEU:
MÉDIC. ENF/PAR. AUX. EMPIR. OUTROS
PARTO
NEONATO
EPISIOTOMIA
SIM NÃO
LACERAÇÃO
SIM NÃO
DEQUIT. EXP.
SIM NÃO
PLACENTA COMPL.
SIM NÃO
MORTE FETAL
NÃO SIM
MOMENTO
PARTO
GRAV. IGNO.
MEDICAÇÃO NO PARTO
ANESTESIA LOCAL
ANESTESIA REGIO.
ANESTESIA GERAL
ANALGÉSICO
TRANQUILIZANTE
OCITOC.
ANTIBIÓTICO
OUTROS
NENHUM
RECÉM-NASCIDO
SEXO
F
M
V.D.R.L.
–
+
APGAR MINUTO
1º 5º
6 OU MENOS
REANIMAÇÃO
SIM
NÃO
PESO AO NASCER
g
MENOS DE 2.500g
IDADE POR EXAME FÍSICO
Sem.
MENOR DE 37 SEMANAS
PESO/I.G.
ADEQ.
PEQ.
GRAN.
EX. FÍSICO IMEDIATO
NORMAL
ANORMAL
ESTATURA
cm
PER. CEF.
cm
EX. FÍSICO PRÉ-ALTA
NORMAL
ANORMAL
EX. NEURO.
NORMAL
ANORMAL
DUVIDOSO
PATOLOGIAS
M. HIALINA
S. ASPIRAT.
OUTROS SDR
APNÉIAS
HEMORRA.
HIPERBILI.
INFECÇÃO
NEUROL.
A. CONG.
OUTRA
NENHUMA
RN ALOJ./CONJ.
SIM
NÃO
ALTA DO RN
SADIO
TRANSF.
C/PATOL.
ÓBITO
IDADE NA ALTA/ TRANSFERÊNCIA
DIAS
HORAS
IDADE AO FALECER
DIAS
HORAS
ALIMENTAÇÃO
PEITO
MISTO
ARTIFIC.
PUERPÉRIO
HORA OU DIAS PÓS-PARTO OU ABORTO
TEMPERATURA
PULSO (BATIMENTO/ MINUTO)
PRESSÃO ARTERIAL MÁX./MIN. (mm Hg)
INVÓL. UTERINA
CARACTERÍSTIC. DOS LÓQUIOS
ALTA MATERNA
SADIA
TRANSFERIDA
C/PATOLOGIA
MORTE MATERNA
GRAVIDEZ
PARTO
PUERPÉRIO
ORIENT./CONTRACEPÇÃO
CONDON
D.I.U.
ORAL
OUTRA
LIG. DE TRO.
RITMO
NENHUM
MINISTÉRIO DA SAÚDE
GOVERNO FEDERAL

ANEXOS

ANEXOS

ANEXO A - **PARECER DO COMITÊ DE ÉTICA DO IMIP**

Instituto de Medicina Integral
Prof. Fernando Figueira
Escola de Pós-graduação em Saúde Materno Infantil
Instituição Civil Filantrópica

IMIP

DECLARAÇÃO

Declaro que o projeto de pesquisa **nº 1824** intitulado **"A hiperinsulinemia materna induz o surgimento dos receptores híbridos IR/IGF-IR no recém -nascido?"** apresentado pela pesquisadora **Suzana Maria Ramos Costa** foi APROVADO pelo Comitê de Ética em Pesquisa em Seres Humanos do Instituto de Medicina Integral Prof. Fernando Figueira – IMIP, em reunião de 11 de agosto de 2010.

Outrossim esclarecemos que o projeto, por pertencer a uma área temática especial e ser concluído nos Estados Unidos (Universidade de Harvard) foi enviado à Comissão Nacional de Ética em Pesquisa – CONEP. Conforme o parecer da CONEP o projeto foi Aprovado Com Recomendação e, tendo a pesquisadora atendido adequadamente às solicitações exigidas, o projeto foi aprovado em definitivo por este CEP em reunião ordinária de 10 de agosto de 2011.

Recife, 15 de agosto de 2011.

Dr. José Eulálio Cabral Filho
Coordenador do Comitê de Ética
em Pesquisa em Seres Humanos do
Instituto de Medicina Integral Prof. Fernando Figueira

UTILIDADE PÚBLICA MUNICIPAL - Lei. 9851 de 08/11/67
UTILIDADE PÚBLICA ESTADUAL - Lei. 5013 de 14/05/64
UTILIDADE PÚBLICA FEDERAL - Dec. 86238 de 30/07/81
INSCRIÇÃO MUNICIPAL: 05.897-1
INSCRIÇÃO ESTADUAL - Isento
CNPJ: 10.988.301/0001-29

Rua dos Coelhos, 300 Boa Vista
Recife - PE - Brasil - CEP: 50.070-550
PABX: (81) 2122.4100
Fax: (81) 2122.4722 Cx. Postal 1393
e-mail: imip@imip.org.br
www.imip.org.br

ANEXO B - **PARECER DO CONEP**

CONSELHO NACIONAL DE SAÚDE
COMISSÃO NACIONAL DE ÉTICA EM PESQUISA

PARECER Nº. 387/2011

Registro CONEP 16480 **(Este nº deve ser citado nas correspondências referentes a este projeto)**

Folha de Rosto – 357109 **Processo nº 25000.075385/2011-51**
Projeto de Pesquisa: *"A hiperinsulinemia materna induz o surgimento dos receptores híbridos IR/IGF-IR no recém-nascido".*
Pesquisador Responsável: Suzana Maria Ramos Costa
Instituição: Instituto de Medicina Integral Professor Fernando Figueira - IMIP/PE **(CENTRO ÚNICO)**
CEP de origem: Instituto de Medicina Integral Professor Fernando Figueira - IMIP/PE
Área Temática Especial: Genética humana, Pesquisa com cooperação estrangeira
Patrocinador: CNPq (Processo 472031/2010-4) e Programa CAPES/Fulbright.

Sumário geral do protocolo

A Organização Mundial de Saúde (OMS), a Organização Pan-Americana de Saúde (OPAS) e o Centro de Controle e Prevenção de Doenças (CDC) dos Estados Unidos da América (EUA) reconhecem a obesidade como um dos principais problemas de saúde pública, pois sua prevalência está aumentando em todo o mundo de modo alarmante. A obesidade já é considerada uma pandemia, afetando tanto países desenvolvidos como em desenvolvimento e indivíduos de diferentes classes sócio-econômicas e diferentes faixas etárias.

No Brasil, os resultados da Pesquisa de Orçamento Familiar de 2002-2003 revelaram um crescimento acelerado do excesso de peso, fenômeno bem documentado a partir de 1974. Em 30 anos, sua prevalência entre os homens elevou-se de 16% para 41% e, entre as mulheres, de 29% para 40%. Entre as principais consequências da obesidade observam-se: a predisposição a co-morbidades, aumento do risco de morte e aumento de aposentadoria por incapacidade. Na faixa etária de 25 a 35 anos, a obesidade isolada está associada a um risco 12 vezes maior de morte quando comparado às pessoas magras, sendo maior o risco quanto maior for o peso. Porém, o mais importante, em relação à obesidade, é o aumento da prevalência de diabetes e o maior risco da letalidade cardiovascular. As três enfermidades juntas contribuem com uma grande proporção de morte prematura e diversas condições debilitantes, que repercutem negativamente na qualidade geral de vida do indivíduo. É reconhecido que a epidemia da obesidade no mundo exige uma análise cuidadosa de como a obesidade materna e a resistência à insulina associada também podem contribuir para a perpetuação da obesidade e do metabolismo alterado na prole.

Em virtude da elevada prevalência de obesidade materna entre mulheres em idade fértil e em filhos de mães obesas, segundo os proponentes, é de vital importância para a saúde pública compreender os mecanismos responsáveis pelos efeitos da resistência à insulina associada à obesidade materna sobre a saúde da prole. A obesidade pré-gestacional está associada a um maior risco de morbidade fetal e neonatal.

Está bem estabelecido que a gravidez é um período fisiológico normal de resistência à insulina. A resposta à redução fisiológica de insulina induzida pela gravidez resulta em hiperinsulinemia. Esta condição sobrecarrega o metabolismo já comprometido de mulheres obesas e mudanças no metabolismo energético ocorrem de forma a manter o equilíbrio do ambiente intra-uterino durante a gestação. Estas alterações metabólicas também desempenham um papel importante através da transferência de informações para o feto, desencadeando respostas adaptativas. Anormalidades do metabolismo da glicose e da insulina têm sido demonstradas tão cedo quanto dentro das primeiras 48 horas de vida, tanto em recém-nascidos pequenos como para os grandes para sua idade gestacional.

A resistência à insulina durante a gravidez pode ser devida às mudanças no receptor de insulina e/ou na cascata de processos intracelulares. Um potencial mediador seriam as alterações na sinalização do receptor de insulina devido à formação de receptores híbridos. Parece promissora a pesquisa dos receptores híbridos de insulina/IGF-I e uma possível associação de sua presença e o desenvolvimento da resistência periférica à insulina. A possibilidade de se estudar biomarcadores associados à resistência periférica à insulina em filhos de mães obesas poderá trazer uma contribuição na identificação precoce de recém-nascidos de risco e, com isso, assegurar um seguimento diferenciado que poderá repercutir na saúde e qualidade de vida futura. Dada a dificuldade de obtenção de contribuições a respeito da obesidade materna e da resistência à insulina nos fenótipos da prole em seres humanos, além da necessidade de compreender melhor essa condição e considerando a sua complexidade, um modelo de estudo já desenvolvido com camundongos (heterozigotos para o receptor de substrato IRS-1 da insulina) será utilizado para comparação com os resultados obtidos a partir dos seres humanos.

Assim, o presente estudo visa à compreensão dos mecanismos pelos quais a exposição à resistência à insulina materna pode promover obesidade e resistência à insulina na prole. Especificamente, a hipótese de que a exposição à resistência à insulina materna resulta em níveis elevados de ácidos graxos na prole, ativando a via da hexosamina e aumentando os níveis de modificação pelo substrato doador de N-acetilglicosamina (O-GlcNAc). O aumento da glicosilação do receptor, então, estimula a formação de receptores híbridos. Coletivamente, essas moléculas orquestrariam alterações na sinalização da insulina, crescimento e vias metabólicas. Espera-se com este estudo compreender os mecanismos pelos quais a exposição ao ambiente materno metabolicamente desorganizado pode alterar o metabolismo da prole. Além disso, será verificado se a formação de receptor híbrido pode representar um biomarcador de risco para o desenvolvimento de resistência à insulina.

O estudo foi caracterizado como exploratório, descritivo, com componente analítico. Serão constituídos três grupos, cada qual com 20 indivíduos maiores de idade: gestantes obesas normoinsulinêmicas, gestantes obesas hiperinsulinêmicas e gestantes eutróficas. A Emenda relativa ao protocolo original diz respeito à inclusão da cooperação estrangeira, pois haverá envio de amostras biológicas humanas (células do cordão umbilical e soro) aos EUA para realização de parte das análises no *Joslin Diabetes Center*, tendo em vista a aprovação de uma bolsa de doutorado "sanduíche" do Programa CAPES/Fulbright, para pesquisadora Suzana M. R. Costa, e a disponibilidade de melhor infra-estrutura e técnicas mais avançadas no referido centro estrangeiro. A análise das células endoteliais do cordão umbilical já estava prevista no protocolo original, mas a análise da via biossintética da hexosamina e da metabolômica do soro através de técnica mais avançada é proposta na presente versão do projeto.

Os objetivos apresentados na Emenda ao protocolo original são: Geral - "Determinar os mecanismos pelos quais a resistência à insulina materna durante a gravidez causa resistência à insulina e adiposidade no início da vida na prole" e Específicos - "Determinar se a resistência à insulina materna ativa a via biossintética da hexosamina e aumenta o número de receptores híbridos na descendência" e "Determinar quais são as alterações metabólicas que a resistência à insulina materna promove no feto".

As células endoteliais e soro do cordão umbilical humano estão sendo colhidos como parte do projeto em andamento, com aprovação do CEP IMIP/PE em 11 de agosto de 2010. Para a quantificação e avaliação dos níveis de GLcNAc e do conteúdo de receptor híbrido em células endoteliais serão realizados ELISA e Western-Blot. Para avaliar a metabolômica do soro dos recém-nascidos humanos e dos camundongos será realizada a Cromatografia Líquida de Alta Performance acoplada à Espectrofotometria de Massa em Tandem. Os camundongos para o estudo serão gerados através de técnicas estabelecidas no Joslin Diabetes Center.

Segundo os proponentes, o risco à gestante é o inerente as coletas de sangue realizadas rotineiramente para os exames previstos no pré-natal e preconizados pelo Ministério da Saúde (MS). Quanto ao recém-nascido, não haverá qualquer tipo de risco, uma vez que o material utilizado será o cordão umbilical, que é normalmente descartado. Consta garantia de sigilo e do anonimato da paciente, bem como de aconselhamento genético e clínico, se necessário, sem custos para os sujeitos da pesquisa.

Local de realização

Trata-se de um projeto nacional e unicêntrico, com cooperação estrangeira, a ser realizado no IMIP/PE. A pesquisa constitui o projeto de Doutorado da pesquisadora responsável, inscrita no Programa de Pós-Graduação em saúde da Criança e do Adolescente da Universidade Federal de Pernambuco. Todos os 60 sujeitos de pesquisa serão recrutados no Serviço de Obstetrícia do centro em tela. Além do Brasil, os EUA participarão do estudo, pois parte das análises será realizada no Laboratório da Dra. Mary Elizabeth Patti, localizado no *Joslin Diabetes Center* na *Harvard University*.

Apresentação do protocolo

A Folha de Rosto (FR - 357109) encontra-se preenchida e assinada pela pesquisadora responsável e pela Dra. Nilma G. de Mendonça como responsável institucional. Este documento não foi alterado com vistas a incorporar a modificação imposta pela Emenda em análise. Apesar de constar a indicação de constituição de banco de material biológico humano, a pesquisadora esclarece, no corpo do projeto, que não haverá armazenamento de amostras para utilização em novas pesquisas e que não haverá intervenção para modificação do genoma humano.

O Termo de Consentimento Livre e Esclarecido (TCLE) proposto para o estudo e aprovado pelo CEP foi apresentado.

O orçamento do estudo foi apresentado no protocolo original enquanto os esclarecimentos referentes às fontes de financiamento encontram-se explicitadas na Emenda. O projeto foi aprovado pelo CNPq (Processo 472031/2010-4), prevendo-se R$32.650,00 para as etapas laboratoriais realizadas no Brasil. A bolsa de Doutorado do Programa CAPES/Fulbright financiará as passagens de ida e volta para os EUA, custeará as taxas da *Harvard University*, uma taxa de laboratório para o *Joslin Diabetes Center* (2.000,00 dólares) e uma bolsa de manutenção para a pesquisadora responsável (1.300,00 dólares).

O currículo da pesquisadora responsável, disponível na Plataforma Lattes/CNPq, foi apresentado, observando-se adequação à proposta. Os currículos dos demais integrantes da pesquisa (Giselia Alves Pontes da Silva, Maria Elizabeth Cavalcante Chaves e Mary Elizabeth Rueckel Patti) não foram apresentados.

Segundo o cronograma apresentado anexo à Emenda, o período de permanência nos EUA está estabelecido entre maio de 2011 e abril de 2012. Especificamente, as análises com envolvimento de amostras humanas têm início previsto em junho do ano corrente. Adicionalmente, constam anexados os seguintes documentos: Cartão da Gestante do MS, Formulário da Pesquisa (contendo questões referentes aos antecedentes clínicos e parâmetros clínicos e laboratoriais atuais, da gestante e do recém-nascido) e Parecer Consubstanciado do CEP sobre a Emenda.

Comentários/Considerações:

Parte dos exames que serão realizados no exterior, conforme descrito na Emenda, já constavam do protocolo originalmente aprovado pelo CEP. Adicionalmente, incluiu-se a análise de outros marcadores, em razão do acesso a tecnologias não disponíveis no centro primário da pesquisa. Então, considerando: (a) que as novas análises previstas enquadram-se no Objetivo Geral da pesquisa; (b) a natureza acadêmica da pesquisa, com vistas ao Doutoramento da pesquisadora responsável, com apoio da CAPES e do CNPq; e (c) a relevância dos resultados a serem atingidos, por meio das técnicas incluídas na Emenda, autoriza-se a dispensa de uma emenda ao TCLE aprovado pelo CEP no caso particular do protocolo 16480.

1. A respeito da Folha de Rosto (FR) apresentada:
    a. A mesma não se encontra atualizada (indica que o protocolo se enquadra no Grupo II, não apresentando a informação de que o protocolo passou a pertencer à área temática especial "Pesquisa com cooperação estrangeira" e não informando que haverá participação internacional, a partir da proposição da Emenda). Solicita-se a apresentação de uma nova FR, datada e assinada, com todos os campos corretamente preenchidos.
    b. É informado na FR que o patrocinador do estudo é o Centro de Ciências da Saúde da Universidade Federal de Pernambuco. No entanto, segundo informado na Emenda, o custeio da pesquisa se dará com verba aprovada pelo CNPq (Processo 472031/2010-4) e pelo Programa CAPES/Fulbright. Solicita-se adequar.
2. Apesar do informe na Emenda quanto à realização de parte das análises previstas no Laboratório da Dra. Mary E. Patti no *Joslin Diabetes Center*, mediante a implementação de um doutorado "sanduíche" do Programa CAPES/Fulbright, os devidos documentos comprobatórios não foram apresentados. Solicita-se adequação, em atendimento ao disposto no item II.2 ("explicitar as responsabilidades, os direitos e obrigações, mediante acordo entre as partes envolvidas") da Resolução CNS 292/99.
3. A pesquisadora responsável informa que não constituirá um banco de amostras biológicas para uso em pesquisas futuras. No entanto, não consta, na documentação em análise, declaração equivalente da colaboradora norte-americana. Solicita-se adequação, em atendimento ao disposto no item VII.5 ("Declaração do uso do material biológico e dos dados e informações coletados exclusivamente para os fins previstos no protocolo, de todos os que vão manipular o material") da Resolução CNS 292/99.
4. Adicionalmente, solicitam-se os esclarecimentos cabíveis quanto a destinação do material biológico ao término das análises previstas, no Brasil e no exterior, em

atendimento às disposições contidas nos itens IV.1."g" da Resolução CNS 340/04 e VI.2."n" da Resolução CNS 196/96.

Diante do exposto, a Comissão Nacional de Ética em Pesquisa – CONEP, de acordo com as atribuições definidas na Resolução CNS 196/96, manifesta-se pela aprovação do projeto de pesquisa proposto, devendo o CEP verificar o cumprimento das questões acima, antes do início do estudo.

Situação: **Protocolo aprovado com recomendação.**

Brasília, 27 de junho de 2011.

Gyselle Saddi Tannous
Coordenadora da CONEP/CNS/MS

ANEXO C - **NORMAS PARA PUBLICAÇÃO NA REVISTA** *PEDIATRICS DIABETES*

Author Guidelines

Pediatric Diabetes will consider for publication full-length papers, preliminary communications with important new information, clinical reports and reviews of major topics. Invited editorials and perspectives will be a regular feature. Full-length papers and reviews of major topics should generally not exceed a total of 5000 words (approximately 20 double-spaced typewritten pages) for the text, references, tables, figures, and figure legends, excluding running title page, title page, and abstract. Preliminary communications with important new information, clinical reports, invited editorials and perspectives should generally not exceed 2000 words.

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ACKNOWLEDGEMENTS

Acknowledge only persons who have made substantive contributions to the study, e.g., technical assistance, critical advice, or other assistance. Authors are responsible for obtaining permission from everyone acknowledged by name because readers may infer their endorsement of the data and conclusions. All funding sources supporting the work should be acknowledged.

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Journal articles. Zou L. Burmeister LA, Sperling MA. Isolation of a liver-specific promoter for human growth hormone receptor gene. Endocrinology 1997; 138:1771-1774.
Books and monographs. Sperling MA (ed). Pediatric Endocrinology, 2ND Ed. W.B. Saunders, Philadelphia, 2002.
Book Chapters. Menon RK, Trucco M. Molecular Endocrinology: Relevance to Clinical Management of Hormonal Disorders. In: Sperling MA (ed). Pediatric Endocrinology, 2nd Ed. W.B. Saunders, Philadelphia, 2002, pp 15-32.

References in Articles

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ANEXO D - **NORMAS PARA PUBLICAÇÃO NA REVISTA** *THE JOURNAL OF PEDIATRICS*

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For Articles in Press (online)

Hellems MA, Gurka KK, Hayden GF. A review of *The Journal of Pediatrics*: The first 75 years. J Pediatr (2008). doi:10.1016/j.jpeds.2008.08.049.

For books

Rosenstein BJ, Fosarelli PD. Pediatric pearls: the handbook of practical pediatrics. 3rd ed. St Louis: Mosby; 1997.

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