
Amanda Costa de Lima

# Exposição perinatal a dieta hiperlipídica e hipercalórica promove alterações no desenvolvimento murinométrico, na composição corporal e no consumo alimentar da prole.

Recife

2015

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Orientador: Raul Manhães de Castro.
Co-orientadora: Lígia Cristina Monteiro Galindo.

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Recife

2015

*Aos meus familiares e amigos,*

*a eles dedico todas as minhas vitórias.*

*A eles todo meu amor e gratidão.*

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## RESUMO

O consumo de dieta hiperlipídica e hipercalórica durante o período perinatal induz a disfunções metabólicas como desenvolvimento de adiposidade precoce, alterações de crescimento e hiperfagia. O presente estudo avaliou os efeitos da utilização perinatal de dieta hiperlipídica/hipercalórica sobre gestantes, nutrizes e prole. Ratos *Wistar* foram divididos em dois grupos, de acordo com a manipulação nutricional realizada do 1º dia de gestação ao 21º dia de lactação: Grupo Controle (GC) e Grupo Hiperlipídico/ Hipercalórico (GH). O GC (n=10) foi alimentado com dieta Nuvilab® (calorias: 357,11kcal/100g e lipídios: 5,63g/100g) durante gestação e lactação. O GH recebeu dieta hiperlipídica/hipercalórica durante o mesmo período (calorias: 489,61kcal/100g e lipídios: 25,37g/100g). Nas ratas prenhas e lactantes foram analisados, peso corporal, composição corporal e ingestão alimentar. Na prole, até os 21 dias de vida pós-natal, foram analisados desempenho na lactação, evolução ponderal e medidas murinométricas. Aos 30 dias de vida, os filhotes foram avaliados em relação à composição corporal e medidas murinométricas. Também foi analisado o consumo alimentar dos filhotes após a lactação. O presente trabalho demonstrou que a manipulação nutricional com dieta rica em gorduras não afetou o desempenho da lactação aos dias 7, 14 e 21 dias de vida, porém, levou a alterações nas medidas murinométricas na prole do GH que apresentou menor circunferência torácica, menor circunferência abdominal e menor comprimento naso-anal. Os efeitos tardios da ingestão perinatal de dieta hiperlipídica foram evidenciados com a redução no consumo alimentar aos 30 dias de vida. Em relação às medidas murinométricas no 30º dia pós-natal, o único parâmetro que foi diferente entre os grupos foi o comprimento naso-anal, estando diminuído no GH. Houve maior adiposidade no grupo submetido à dieta hiperlipídica/hipercalórica. Em conjunto, os resultados desse trabalho mostram que um ambiente perinatal composto por um excesso de calorias e lipídeos é capaz de afetar parâmetros relacionados ao desenvolvimento e aumentar a adiposidade corporal, sendo fator de risco para obesidade a longo prazo.



Palavras-chave: Plasticidade celular. Dieta hiperlipídica. Adipogênese. Crescimento & desenvolvimento. Ingestão alimentar.

**ABSTRACT**

The consumption of high fat and high calorie diet during the perinatal period induces metabolic dysfunctions such as development of early adiposity, growth changes and hyperphagia. This study evaluated the effects of perinatal use of high fat / high calorie diet for pregnant women, nursing mothers and offspring. Wistar rats were divided into two groups according to nutritional manipulation performed the 1st day of gestation to 21 days of lactation: Control Group (CG) and hyperlipidic Group / hipercalórico (GH). The GC (n = 10) was fed Nuvilab® diet (calories: 357,11kcal / 100g and lipids: 5,63g / 100g) during pregnancy and lactation. The GH received high fat / high calorie diet during the same period (calories: 489,61kcal / 100g and lipids: 25,37g / 100g). In pregnant and lactating rats were analyzed, body weight, body composition and food intake. In the offspring, up to 21 days of postnatal life, were analyzed performance in lactation, weight gain and murinométricas measures. At 30 days of age, the pups were assessed for body composition and murinométricas measures. Also analyzed the food intake of puppies after lactation. This study demonstrated that nutritional manipulation with high-fat diet did not affect the performance of lactation on days 7, 14 and 21 days of life, however, led to changes in murinométricas measures in the offspring of GH showed lower chest circumference, smaller circumference abdominal and lower naso-anal length. Late effects of perinatal intake of fat diet were highlighted with reduced food intake at 30 days of life. Regarding murinométricas measures on the 30th postnatal day, the only parameter that differed between the groups was the naso-anal length and is decreased in the GH. There was a greater adiposity in the group submitted to high fat / high calorie diet. Taken together, the results of this work show that a perinatal environment composed of an excess of calories and lipids can affect parameters related to the development and increase body fat, being risk factor for long-term obesity.

Keywords: Cellular plasticity. Hyperlipidic diet. Adipogenesis. Growth & Development. Food intake.

# LISTA DE TABELAS

**Tabelas da dissertação**



**Tabelas do artigo original**

# LISTA DE ILUSTRAÇÕES

## Figuras da dissertação



## Figuras do artigo original

## SUMÁRIO

## 1. APRESENTAÇÃO

A alimentação é uma variável ambiental que possibilita um adequado crescimento e desenvolvimento. Este fato é confirmado quando a inadequada oferta de nutrientes no período perinatal (gestação e lactação) acarreta alterações estruturais e funcionais ao feto como redução no tamanho encefálico, alterações na migração e diferenciação celular e em processos como neurogênese, diferenciação neuronal e sinaptogênese (MORGANE, et al., 1993; MANHÃES-DE-CASTRO et al, 2001; BLOOMFIELD, et al., 2013).

Nesse contexto, a plasticidade fenotípica vem sendo estudada e os resultados apresentados por diversos autores mostram a relação entre alterações nutricionais perinatais e o surgimento de doenças na idade adulta (ALFARADHI E OZANNE, 2011; BENTO-SANTOS, et al., 2012; MOURA et al., 2008). O termo plasticidade fenotípica é utilizado para descrever a habilidade que um organismo possui de reagir aos desafios impostos pelo ambiente, permitindo alterações em sua forma, estado e padrão de atividade (WEST-EBERHARD, 1986). Por esse mecanismo o fenótipo do indivíduo pode ser alterado de modo a se adaptar a um ambiente perinatal adverso sendo capaz de aperfeiçoar a utilização de nutrientes.

Diversos estudos relatam tais alterações nutricionais, como o consumo de dieta hiperlipídica durante os períodos de gestação e lactação têm sido associados à atrofia muscular com hipoplasia de fibras e alterações metabólicas em ratos adultos (BAYOL; SIMBI; STICKLAND, 2005; OLIVEIRA, et al., 2011). A ingestão de dietas hiperlipídicas com alto teor de ácidos graxos saturados induz estado inflamatório hipotalâmico com aumento de citocinas inflamatórias e apoptose de neuronal (MILANSKI et al., 2009; MORAES et al., 2009; PIMENTEL, et al., 2012). Evidencias dão suporte à ideia de que o consumo materno desse tipo de dieta, durante a fase fetal precoce, desenvolve na prole o risco aumentado para aparecimento de obesidade e síndrome metabólica na vida adulta. Isto ocorre porque o hipotálamo fetal e de neonatos recebe estímulos nutricionais e hormonais maternos, durante a gestação e lactação (BILBO E TSANG, 2010).

Com a finalidade de investigar os efeitos do consumo de uma dieta rica em gordura durante período perinatal, este trabalho enfoca nas repercussões relacionadas ao

comportamento alimentar e ao desenvolvimento murinométrico em ratos provindos de nutrizes alimentadas com dieta hiperlipídica e hipercalórica durante os períodos de gestação e lactação.

## 2. REVISAO DE LITERATURA

### Nutrição e plasticidade fenotípica

Crescimento e desenvolvimento durante o período inicial da vida são variáveis que dependem do ambiente metabólico, nutricional e hormonal materno. Dessa forma, a adequada alimentação durante a gestação e lactação representa fator imprescindível para que estes processos ocorram de maneira eficaz (DESAI E HALES, 1997). Prova disto é que o aporte nutricional inadequado durante o período inicial do desenvolvimento acarreta repercussões estruturais e funcionais ao feto como: alterações relacionadas ao peso corporal e a características murinométricas; redução no tamanho encefálico; danos a processos como neurogênese, migração, diferenciação neuronais bem como à sinaptogênese (MORGANE, et al., 1993; MANHÃES-DE-CASTRO, et al., 2001; BLOOMFIELD, 2013, CHUNG, et al., 2013).

O período crítico para o desenvolvimento do sistema nervoso é marcado por diferentes e complexos processos que envolvem rápida proliferação, diferenciação e hipertrofia celulares (DESAI AND HALES. 1997). Sendo assim, durante essa fase, os órgãos e sistemas estão mais vulneráveis a injúrias ambientais podendo desenvolver alterações permanentes em sua estrutura e função (DOBBING, 1965). Nos mamíferos e em muitas outras espécies a maior parte desses processos ocorre durante a vida intrauterina, porém, em alguns órgãos e tecidos se dá também após o nascimento. Em humanos, o período crítico de desenvolvimento compreende uma fase pré-natal (a partir do segundo trimestre gestacional) e uma pós-natal (de dois a três anos de vida). Em roedores, corresponde ao período de gestação e lactação (DOBBING, 1970; MORGANE, et al., 1993).

Nesse contexto, a plasticidade fenotípica vem sendo descrita por diversos estudos que demostram a relação entre esses agravos nutricionais durante a gestação e a lactação associados ao surgimento de doenças na idade adulta (BARKER, 1998; MOURA et al., 2008; ALFARADHI E OZANNE, 2011; BENTO-SANTOS, et al., 2012). O termo plasticidade fenotípica é utilizado para descrever a habilidade que um genótipo possui de reagir em resposta aos desafios ambientais, o que pode originar a expressão fenotípica de diferentes estados fisiológicos e/ou morfológicos (WEST-EBERHARD, 1986).

Fundamentando a proposição descrita acima, destacam-se duas teorias. A primeira teoria é conhecida como “fenótipo poupador”. Segundo ela, a má nutrição fetal impõe restrições ao crescimento e ao desenvolvimento, além de mudanças sobre o metabolismo do feto, com finalidade de alcançar economia metabólica (HALES E BARKER, 1992). Essas alterações adaptativas visam distribuição de nutrientes de forma seletiva entre órgãos de forma a preservar o crescimento e desenvolvimento global do encéfalo em detrimento de outros órgãos tais como o fígado e pâncreas. Há ainda adaptação do metabolismo após o nascimento a fim de promover a sobrevivência em condições de escassez alimentar. Entretanto, esse perfil metabólico, benéfico em curto prazo, pode ser prejudicial em condições de oferta nutricional adequada ou de superalimentação (DESAI E HALES, 1997).

A segunda teoria trata do modelo da relação entre “capacidade-carga metabólica” referindo-se à competência dos órgãos responsáveis em manter a homeostase. O fenótipo destes órgãos está diretamente relacionado com o seu crescimento durante a vida fetal e a primeira infância. Tal capacidade metabólica estaria relacionada à “carga metabólica” que se refere à responsabilidade imposta a estes órgãos em manter a homeostase metabólica, refletindo na interação entre massa magra, massa gorda, estatura e ganho de peso com o aparecimento de doenças (WELLS, 2010).

Dessa forma, é clássico o relato de interação entre alterações nutricionais durante o período de crescimento e desenvolvimento e as suas repercussões na prole. Por exemplo, consumo de dieta restrita em proteínas durante o período perinatal leva ao atraso no desenvolvimento da atividade locomotora e a alterações nas fibras musculares de ratos (TOSCANO, et al., 2008). A restrição crônica da oferta calórica na gestação e lactação tem sido associada à redução no peso encefálico da prole, com prejuízo ao desenvolvimento de estruturas encefálicas como hipocampo, núcleo estriado, hipotálamo e córtex (WALKER, 2005). Além disso, estudos epidemiológicos relatam que o baixo peso ao nascer e no primeiro ano de vida está associado à intolerância à glicose, diabetes tipo 2 (HALES E BARKER, 1992) e doenças cardiovasculares na vida adulta (BARKER, 1998).

Por outro lado, a ingestão de dieta hiperlipídica e hipercalórica durante a gestação e lactação tem sido associada à atrofia e hipoplasia de fibras musculares e a alterações metabólicas em ratos adultos (BAYOL; SIMBI; STICKLAND, 2005; OLIVEIRA, et al., 2011). Há ainda associação entre ingestão de dietas hiperlipídicas com alto teor de ácidos graxos saturados e gênese de inflamação hipotalâmica com aumento de citocinas

inflamatórias e apoptose neuronal (MILANSKI et al., 2009; MORAES et al., 2009; PIMENTEL, et al., 2012).

**Obesidade e dieta hiperlipídica e hipercalórica**

A obesidade é considerada um problema de saúde pública no mundo. Dados epidemiológicos revelam que a prevalência estimada para o ano de 2015 é que aproximadamente 2.3 bilhões de indivíduos apresentem sobrepeso e 700 milhões obesidade (WHO, 2013). Sua patogênese envolve complexa interação de fatores que incluem características genéticas, comportamentais e ambientais, tais como acesso e disponibilidade a fontes alimentares, além de fatores físicos ou psicológicos, identidade cultural e nível socioeconômico (MOLLER E KAUFMAN, 2005). Essa condição é caracterizada por estado inflamatório subclínico, que representa fator de risco para o surgimento de resistência à insulina, hipertensão, doenças cardiovasculares e diabetes tipo 2 (WELLEN E HOTAMISLIGIL, 2005, WYMANN E SCHNEITER, 2012).

Os lipídeos são importantes componentes da alimentação humana. Eles fornecem energia e são fonte de ácidos graxos essenciais e vitaminas lipossolúveis. Entretanto, alguns ácidos graxos presentes nas gorduras, em especial os saturados e os *trans*, podem ter efeitos adversos na saúde humana (MENSINK, et al., 2003). Os ácidos graxos saturados são derivados de gorduras de origem animais, enquanto que os ácidos graxos *trans* são encontrados na carne e leite de animais ruminantes (BAUMAN, et al., 2006). A hidrogenação parcial dos ácidos graxos insaturados nos óleos vegetais durante a produção industrial de alguns produtos também produz ácidos graxos *trans* (LEDOUX; JUANÉDA; SÉBÉDIO, 2007).

Atualmente um importante fator que contribui para a elevada prevalência da obesidade atualmente são as escolhas alimentares. Sendo assim, o elevado consumo de dietas ricas em gordura representa mecanismo chave na patogênese dessa doença. A chamada “dieta ocidentalizada”, caracterizada por um elevado consumo de ácidos graxos saturados e ácidos graxos *trans*, associada ao baixo consumo de ácidos graxos ômega-3, está associada a mudanças dietéticas relacionadas ao processo de industrialização e migrações culturais das sociedades modernas (SHARMA; ZHUANG; GOMEZ-PINILLA, 2012). O consumo desse tipo de dieta induz a disfunções metabólicas estando o elevado conteúdo de ácidos graxos

saturados destes alimentos associado ao aumento do estresse oxidativo encefálico, redução da neurogênese, neuroinflamação e aumento da ansiedade (LINDQVIST, et al. 2006; PARK, et al. 2010; THALER, et al. 2012; SOUZA, et al. 2007). Estadella, et al., (2011), avaliando o consumo de dieta rica em ácidos graxos saturados em ratos a partir dos 30 dias de vida, encontrou hiperglicemia e hiperinsulinemia, acompanhada de hiperleptinemia e aumento no conteúdo de lipídeos na carcaça desses animais. Outros estudos associam o consumo deste tipo de dieta ao declínio no aprendizado e na memória e à presença de resistência à insulina (GREENWOOD, WINOCUR, 2005). Chen, et al., (2009), relacionou adiposidade precoce ao crescimento somático rápido e hiperfagia, que foram evidenciados aos 20 dias de vida pós-natal, na prole de mães submetidas a dieta rica em lipídeos durante a gestação.

Estudos em modelos animais mostraram que o consumo aumentado de dietas ricas em lipídeos reduz os níveis encefálicos de ácido docosaexaenoico (DHA) e essa redução está associada ao desenvolvimento de ansiedade (SHARMA; ZHUANG; GOMEZ-PINILLA, 2012). Achados recentes mostram que ratos alimentados com dietas deficientes em ômega-3 durante a gestação e períodos de crescimento pré e pós-natal são mais propensos a desenvolver transtornos de ansiedade em relação aos animais que receberam dieta suplementada com DHA (BHATIA, et al, 2011).

Nesse contexto, o emprego experimental de dieta hiperlipídica e hipercalórica tenta mimetizar o atual padrão alimentar da população, caracterizando-se por aumentado teor de ácidos graxos saturados, carboidratos simples, proteína de origem animal e nutrientes deficitários (MENDONÇA E ANJOS, 2004; NUNES, 2008). Vários estudos estão sendo conduzidos com o emprego deste tipo de dieta de modo a compreender quais as consequências dos hábitos alimentares modernos sobre o desenvolvimento dos organismos (ESTADELLA et al., 2011; OLIVEIRA et al., 2011). Evidencias dão suporte à ideia de que o consumo materno desse tipo de dieta, durante a fase fetal precoce, gera na prole aumento no risco para surgimento de obesidade e síndrome metabólica na vida adulta.

**Dieta hiperlipídica e hipercalórica e comportamento alimentar**

A ingestão de alimentos é controlada por mecanismos homeostáticos e não homeostáticos que promovem o equilíbrio energético do corpo (MAGALHÃES et al, 2010). Os sinais homeostáticos periféricos incluem vários fatores secretados pelo trato grastrointestinal (grelina, peptídeo YY e colecistocinina); pelo pâncreas (insulina); pelo tecido adiposo (leptina) e por sinais provenientes da estimulação de mecanoreceptores intestinais.

No Sistema Nervoso Central (SNC) esses sinais são integrados no tronco cerebral e hipotálamo envolvendo a ação de vários neuropeptídios e de neurotransmissores como a serotonina (CHANDLER-LANEY et al, 2007). Já os sinais não homeostáticos são gerados em várias estruturas encefálicos como núcleo acumbens, usando como um dos principais mensageiros a serotonina (HALFORD E BLUNDELL, 2000).

Dentre as estruturas centrais relacionadas ao controle do comportamento alimentar destacam-se o tronco encefálico e o hipotálamo. O tronco encefálico está associado à mediação da resposta ao reflexo de saciedade através do nervo vago, envolvendo a detecção de flutuações de curto prazo no estado nutricional (LAN et al, 2010).). Ele inicia a resposta motora gastrointestinal, além de receber sinais gustatórios uma vez que a maioria das vias gastrointestinais do paladar fazem sinapse nessa área (LAN et al, 2010). O hipotálamo é uma das estruturas responsáveis pela regulação homeostática do comportamento alimentar e do peso corporal. Ele atua integrando informações sobre armazenamento de energia a longo prazo e outros fatores fisiológicos e ambientais para formular respostas à alimentação. Estudos tem identificado essa região cerebral como local de produção, distribuição e atuação de algumas moléculas que estimulam ou inibem a ingestão de alimentos (KALRA et al, 1999).

Durante o período pré ou pós-natal do desenvolvimento, estímulos ambientais podem alterar o hipotálamo gerando modificações tardias no comportamento alimentar (LEVIN E DUNN-MEYNELL, 2000; BILBO E TSANG, 2010). Por exemplo, a supernutrição pós-natal resulta em hiperleptinemia e aumenta os níveis de neuropeptídeo Y (NPY) e pró-opio-melanocortina POMC hipotalâmicos, levando ao aumento da ingestão alimentar (IKENASIO-THORPE et al, 2007). Sullivan, et al., (2010) postula que a exposição perinatal a obesidade ou ao consumo materno de dieta rica em lipídeos, resulta em rompimento na regulação homeostática e na capacidade de detecção de nutrientes nos circuitos hipotalâmicos, com consequente hiperfagia.

O núcleo acumbens também é responsável pelos mecanismos neurais nos quais "motivação" é traduzida em "ação" uma vez que recebe sinais de estruturas límbicas sendo responsável por integrar características motivacionais do comportamento alimentar influenciando no controle do apetite (MOGENSON et al, 1980). Estudos mostram que o consumo materno de dieta de cafeteria, rica em lipídeos, atua nesse sistema alterando a sinalização dopaminérgica e aumentando a ingestão de gordura na prole até os 90 dias de vida

pós-natal (ONG E MUHLHAUSLER, 2011). Já Erlanson-Albertsson (2005), mostrou que o consumo de alimentos palatáveis, ricos em gorduras, inibem os sinais de saciedade promovendo fome e estimulando os centros de recompensa.

Atualmente o foco do estudo sobre o comportamento alimentar são os tipos e subtipos de receptores serotonérgicos que regulam a alimentação, uma vez que a serotonina exerce sua função através da interação com uma variedade de receptores (BARNES E SHARP, 1999). Sendo os receptores da família 5-$HT_1$ e 5-$HT_2$ os mais relacionados com o controle da ingestão alimentar, e o subtipo 5-$HT_{2C}$ o mais importante na relação entre ingestão alimentar e balanço energético. Camundongos desprovidos desse receptor tornam-se obesos, enquanto que o uso de agonistas com atividade nesse receptor gera diminuição da ingestão alimentar (WARD et al, 2008).

Agindo sobre receptor 5-$HT_{2C}$ no hipotálamo, a serotonina ativa a clivagem do POMC. Adicionalmente, a interação entre serotonina e o receptor 5-$HT_{1B}$ inibe, no núcleo arqueado o NPY e a proteína relacionada à agouti (AGRP), deprimindo a transmissão gabaérgica da alfa-melanocortina (alfa-MSH) e do transcrito regulado por cocaína e anfetamina (CART). Esses mecanismos associados produzem estímulo à saciedade e aumento na termogênese. Quando atua sobre o receptor 5-$HT_{1B}$, a serotonina modula a liberação endógena de ambos os agonistas e antagonistas dos receptores da melanocortina que são componentes do sistema de controle da homeostase do peso corporal (GARFIELD E HEISLER, 2009). A hiperfagia e obesidade geradas após depleção de serotonina, através de um inibidor da enzima triptofano hidroxilase ou após lesões neurotóxicas de neurônios serotoninérgicos, reforçam o entendimento da relação entre sistema serotoninérgico e captação/utilização de energia (GARFIELD E HEISLER, 2009).

**Dieta hiperlipídica, hipercalórica e regulação da homeostase da lactação**

A produção de leite varia em torno do número de células mamárias secretoras e da atividade por célula. O número de células secretoras é determinado em grande parte pelo aumento da glândula mamária. Durante a puberdade e gravidez, a atividade funcional individual de células epiteliais mamárias é controlada pelo sistema endócrino e por fatores parácrinos bem como pela taxa de sucção de leite durante a lactação (CAPUCO E ELLIS, 2010). O pico na produção de leite é atingido no início do ciclo de lactação e é seguido por declínio na sua produção até que cesse sua produção. Esta redução na produção de leite pode ser de até 50% quando o pico de produção de leite é alcançado. A homeostase lactacional é

definida como o processo pelo qual o fluxo de leite constante a partir da glândula mamária é mantido durante todo o período de lactação (KNIGHT, PEAKER, WILDE, 1998).

A diminuição na produção de leite vista durante o avanço da lactação ocorre devido ao declínio no número de células secretoras, bem como na sua função. Tal declínio ocorre via apoptose (CAPUCO E ELLIS, 2010). Outros fatores como nutrição, estresse oxidativo imposto pelo metabolismo e hormônios reprodutivos (estradiol e progesterona), além da frequência de ordenha influenciam a persistência de lactação através da regulação do apoptose. Mastite, estresse, gravidez concomitante e diminuição da frequência de ordenha podem diminuir a persistência da lactação, aumentando a apoptose (CAPUCO E ELLIS, 2010).

O consumo de dietas ricas em gorduras e o desenvolvimento de obesidade gestacional também são associados a alterações na lactação. Estudos em diferentes espécies de mamíferos associam a obesidade ao desenvolvimento mamário durante esse período e demostram um atraso na lactogênese que está relacionado ao acúmulo de gotículas de gordura dentro das células epiteliais, com diminuição da expressão gênica da proteína do leite (RASMUSSEN, KJOLHEDE, 2004). Um estudo utilizando modelo de obesidade pré-gestacional em ratas, demonstrou redução da resposta a amamentação induzida pela prolactina e interrupção no desenvolvimento normal das glândulas mamárias durante a gestação (FLINT, et al, 2005). O consumo de dieta hiperlipídica durante gestação e lactação tem demonstrado alterações na lactogênese com redução na produção de leite pelas lactentes que consumiam esse tipo de dieta, principalmente nos primeiros dias de lactação (MATSUDA et al, 2004; HERNANDEZ et al, 2012).

Diante do exposto, é visto que a manipulação nutricional durante períodos críticos do desenvolvimento é capaz de causar alterações no comportamento alimentar e aumentar o risco de obesidade. Com isso, justifica-se o presente estudo com o objetivo de compreender os efeitos de uma supernutrição em períodos iniciais da vida e suas repercussões metabólicas e comportamentais, através da exposição materna a uma dieta hiperlipídica e hipercalórica.

A dissertação encontre-se composta por um artigo original intitulado: *The use of high-fat diet during pregnancy and lactation promotes changes in murinometrics development and adiposity in young rats.*

## 3. HIPÓTESE

O consumo materno de dieta hiperlipídica e hipercalórica durante o período perinatal atrasa o desenvolvimento murinométrico, reduz o consumo alimentar e aumenta a deposição adiposa na prole.

# 4. OBJETIVOS

## 4.1.GERAL

Investigar os efeitos do consumo de uma dieta hiperlipídica e hipercalórica durante a gestação e lactação sobre os parâmetros murinométricos, consumo alimentar e deposição adiposa da prole.

## 4.2.ESPECÍFICOS

**Em ratas durante a gestação e lactação, avaliar:**

- A evolução ponderal de peso corporal;

- O consumo alimentar;

-A deposição adiposa.

**Na prole, avaliar:**

- A ingestão láctea aos 7, 14 e 21 dias de vida;

- A evolução ponderal do peso até os 30 dias de vida;

- O consumo alimentar aos 30 dias de vida;

- As determinações murinométricos, índice de massa corporal e índice de Lee aos 7, 14, 21 e 30 dias de vida;

- Deposição adiposa aos 30 dias de vida.

# 5. METODOS

## 5.1. ANIMAIS

Foram utilizados 80 ratos machos (provenientes de 10 ninhadas) da linhagem *Wistar* selecionados aleatoriamente, provindos de ratas primíparas e peso corporal entre 220g-260g. Os animais foram obtidos na colônia do Departamento de Nutrição da Universidade Federal de Pernambuco (UFPE). Todos os animais foram mantidos em gaiolas de polipropileno (46cmx34cmx20cm), em ambiente com ciclo invertido de luz (18:00 às 06:00h) e escuridão (06:00 às 18:00 h), temperatura 22± 2°C, com livre acesso à água e ração.

Para acasalamento foi utilizado um rato macho para duas fêmeas (1:2), com idade entre 90 e 120 dias. O diagnóstico da prenhez foi realizado através do teste de esfregaço vaginal com constatação de espermatozóides na secreção vaginal e acompanhamento do ganho de peso corporal. Imediatamente após a identificação da prenhez, as ratas foram divididas em dois grupos experimentais: Grupo controle (GC) e Grupo Hiperlipídico (GH). Um dia após o nascimento de filhotes, as ninhadas foram ajustadas para 8 filhotes. Foram incluídos no estudo filhotes com peso entre 6 e 8 gramas no primeiro dia pós-natal (DPN1). Todos os procedimentos foram realizados de acordo com a aprovação do Comitê de Ética em Experimentação Animal (CEEA) da Universidade Federal de Pernambuco (23076.056342/2013-29).

## 5.2. GRUPOS EXPERIMENTAIS

Os grupos experimentais foram formados a partir do primeiro dia após o nascimento. O total de 80 animais foi utilizado para atingir o número total de amostras. Dois grupos experimentais foram organizados de acordo com a manipulação dietética (Figura 1):

Grupo Controle (Normolipídico/Normocalórico) (GC) (n= 40 filhotes). Todos os filhotes amamentados por nutrizes que receberam dieta comercial (Nuvilab®, CR1, Brasil);

Grupo Hiperlipídico/Hipercalórico (GH) (n = 40 filhotes). Constituído por ratas e sua prole que receberam dieta hiperlipídica/hipercalórica desde o primeiro 1º dia de gestação até o 21º dia de lactação), A dieta utilizada foi constituída de uma mistura de alimentos hipercalóricos contendo ração comercial (Nuvilab ®, CR1, Brasil), amendoim torrado,

chocolate ao leite e biscoito maizena, na proporção de 3:2:2:1 (ESTADELLA et al., 2004). Todos os componentes foram triturados, misturados e ofertados em forma de péletes.

A dieta hiperlipídica e hipercalórica foi confeccionada no Departamento de Nutrição da Universidade Federal de Pernambuco (UFPE). A composição centesimal das dietas normolipídica e normocalórica, hiperlipídica e hipercalórica foi realizada no Laboratório de Experimentação e Análise de Alimentos Nonete Barbosa (LEAAL) (Tabela1). O perfil de ácidos graxos da dieta hiperlipídica e hipercalórica foi realizado no Instituto de Tecnologia de Alimentos (ITAL-SP) (Tabela 2). As dietas foram oferecidas durante o período de gestação e lactação.

No 22º dia após o nascimento, os filhotes foram desmamados, e alocados em gaiolas com um total de quatro filhotes por gaiola, onde passaram a receber dieta Nuvilab® adotada como padrão do biotério até o final do experimento.

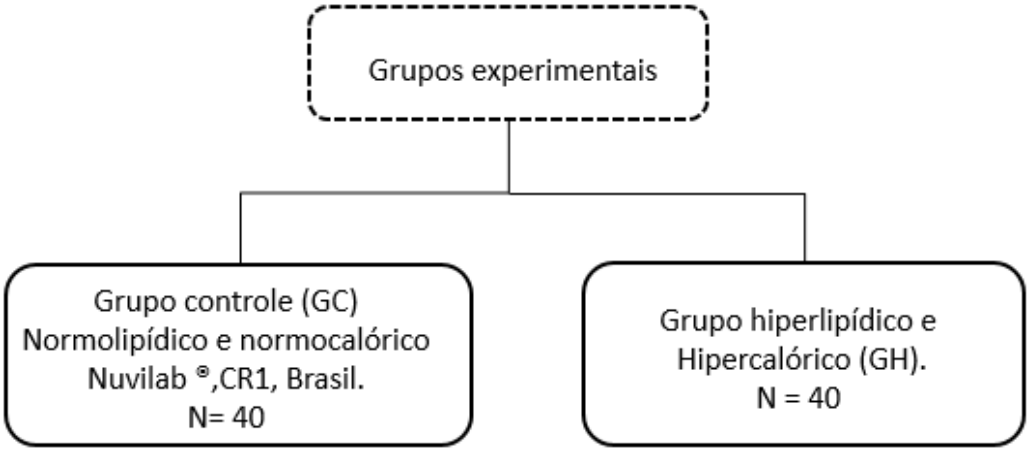


**Figura1: Representação esquemática dos grupos experimentais.**

## 5.3.ANÁLISE CENTESIMAL DAS DIETAS EXPERIMENTAIS

**Tabela 1: Composição centesimal das dietas hiperlipídica, hipercalórica e Nuvilab.**

| Nutrientes | Dieta hiperlipídica e hipercalórica (g/100g) | Dieta Normolipídica e normocalórica (Nuvilab ®, CR1, Brasil – g/100g) |
|---|---|---|
| **Carboidratos** | 38,08 | 47,76 |
| **Proteína** | 27,24 | 28,85 |
| **Lipídios** | 25,37 | 5,63 |
| **Cinzas** | 4,88 | 7,91 |
| **Umidade** | 4,43 | 9,85 |
| **Energia (Kcal/g)** | 489,61 | 357,11 |

- Adolfo Lutz para umidade e substâncias voláteis, proteínas, lipídeos e cinzas;

- Por cálculo/ASCAR, 1985 para carboidratos;

**Tabela 2: Perfil de ácidos graxos da dieta hiperlipídica e hipercalórica.**

| Ácidos graxos | Resultados g/100g |
|---|---|
| **Saturado** | 6,97 |
| **Monoinsaturado** | 11,76 |
| **Poli-insaturados** | 4,32 |
| **Ômega-3** | 0,10 |
| **Ômega-6** | 4,22 |
| **Trans – isômeros totais** | ND < 0,01 |
| **NI** | 0,04 |

ND: não detectado

NI: não identificado

## 5.4. CONSUMO ALIMENTAR DAS GESTANTES

O consumo alimentar das gestantes foi avaliado durante toda a gestação e lactação, durante o início do ciclo escuro. A ração foi pesada e ofertada a cada 2 dias. O total da ingestão de alimentos foi a diferença entre o peso da ração ofertada e o rejeito a cada dia.

### 5.5. EVOLUÇÃO PONDERAL E COMPOSIÇÃO CORPORAL DAS GESTANTES.

O peso corporal das gestantes de ambos os grupos experimentais foi mensurado ao final de cada semana gestacional, sempre no mesmo horário, no início do ciclo escuro.

Após o período de amamentação, as ratas foram pesadas e eutanasiadas por decapitação. O tecido adiposo gonadal e retroperitoneal foi dissecado e pesado em balança digital (Marte® S-100; sensibilidade/precisão 0,01g).

### 5.6. EVOLUÇÃO PONDERAL NA PROLE

O peso corporal dos filhotes foi obtido no 1º, 7º, 14º, 21º e 30º dias de vida pós-natal. A prole foi pesada sempre no mesmo horário, durante o início do ciclo escuro e o peso foi registrado utilizando balança digital (Marte® S-100; sensibilidade/precisão0,01g)

### 5.7. TESTE DE INGESTÃO LÁCTEA NA PROLE

O dia seguinte ao nascimento dos filhotes, foi considerado primeiro dia de vida pós-natal da prole (1º DPN). No 3º DPN, as ninhadas foram ajustadas para 8 filhotes/nutriz. As avaliações foram realizadas 3 horas pós o início da fase escura no ciclo de luz. O desempenho na lactação foi avaliado nos 7º, 14º e 21º DPN. Logo após a retirada dos filhotes do ninho, foi realizada estimulação nas regiões abdomino-pélvica e genital para favorecer a excreção de urina e fezes. Em seguida, os filhotes foram pesados e permaneceram em incubadora aquecida a 33ºC durante 60 minutos. Após os 60 minutos de jejum, os filhotes foram devolvidos ao ninho e permaneceram outros 60 minutos com a nutriz. Após este período foram novamente pesados e o ganho líquido de peso dos filhotes foi considerado o consumo alimentar neste período. Este é um procedimento de pesagem-mamada-pesagem modificado (PERILAN, 2007).

### 5.8. CONSUMO ALIMENTAR E DETERMINAÇÕES NUTRICIONAIS NA PROLE

Quando os animais completaram 30 dias de vida pós-natal o consumo alimentar foi avaliado durante 5 dias consecutivos, sempre ao início do ciclo escuro. A ingestão total de alimentos foi dada pela diferença entre o peso da ração ofertada e o seu rejeito em 24h. O consumo médio foi o valor do consumo total dividido pelo número de animais na gaiola.

Com base na alimentação e ingestão calórica, foi calculado o seguinte parâmetro nutricional:

O consumo energético (kJ/dia) = média do consumo de alimentos x energia metabolizável dietética (NOVELLI,2007).

## 5.9. DETERMINAÇÕES ANTROPOMÉTRICAS E COMPOSIÇÃO CORPORAL NA PROLE

A circunferência abdominal (CA) (imediatamente anterior ao ante pé), circunferência torácica (imediatamente atrás da pata dianteira), comprimento do corpo (comprimento do nariz-ânus) e peso corporal, foram determinados na prole masculina aos dias 7, 14, 21, 30 dias de idade. As medidas foram obtidas em centímetros utilizando trena antropométrica (1.5 metros Physical® com trava e precisão de 1 mm.) O peso corporal e comprimento do corpo foram usados para determinar os seguintes parâmetros antropométricos:

Índice de massa corporal (IMC) = peso corporal (g) / comprimento² (cm2)

Índice de Lee = raiz cúbica do peso corporal (g) / comprimento nariz - ânus (cm) (BERNARDIS, 1968).

Aos 30 dias de vida, após a avaliação do consumo alimentar, os animais foram sacrificados e a gordura corporal (gonadal e retroperitoneal) foi dissecada e pesada.

## 6. ANÁLISE E PROCESSAMENTO DE DADOS

Para verificar a distribuição dos dados segundo critério de normalidade foi utilizado o teste de Kolmogorov-Smirnov. Os dados que apresentaram distribuição normal foram apresentados em média e desvio padrão. O teste t não pareado foi utilizado para a comparação entre os grupos em cada idade. ANOVA *two-way* para medidas repetidas foi utilizada para as comparações entre os grupos ao longo do tempo, seguida pelo Bonferroni Posttest. Foi utilizado o software GraphPad Prism versão 4.0 2004. O nível de significância foi mantido em 5% ($p < 0,05$).

# 7. RESULTADOS E DISCUSSÃO

## 7.1. Artigo Original

Os resultados e a discussão deste trabalho serão apresentados na forma de artigo científico intitulado: **“The use of high-fat diet during pregnancy and lactation promotes changes in murinometrics development and adiposity in young rats”.** Este artigo foi submetido como artigo original à *Revista Nutrition Research*. Qualis B1

# The use of high-fat diet during pregnancy and lactation promotes changes in murinometrics development and adiposity in young rats

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**Abstract**

Changes in perinatal nutritional environment can influence the phenotypic expression of factors related to the acquisition and use of energy, both the deficit and excesso of nutrients can signal to the greatest risk of obesity at long-term. 80 albinos Wistar rats were used (from 10 litters), divided into two groups, according to nutritional manipulation performed the 1st day of gestation to 21 days of lactation, control (n = 5) and high fat/ high calorie (n = 5), composed of pups (n = 10) receiving, respectively, control diet (calories: 357,11 kcal / 100g and lipids: 5,63g / 100g) and high fat/ high calorie diet (calories: 489,61kcal / 100g and lipids: 25,37g / 100g). Were analyzed in pregnant and lactating rats body weight and body composition and food intake. In the offspring up to 21 days of postnatal life was analyzed the lactation performance, the weight gain and murinometrics measures, and in the 30 days of life were analyzed body composition and food intake. In this study maternal exposure to high fat / high calorie diet during pregnancy and lactation did not affect the performance of lactation, but led to changes in murinometrics measures in the offspring of hyperlipidic group with smaller thoracic circumference and lower naso-anal length. Late effects of perinatal intake of high fat / high calorie diet were detected at the 30 days of postnatal life, with the reduction in food intake and naso-anal length and increased adiposity in offspring exposed to high calorie and high fat diet compared the control group. Together, these findings suggest that the nutritional environment, in a study composed by the excess calories and lipid, can disrupt the murinomtrics development and be associated with the emergence of adiposity in the offspring.

*Keywords*: Phenotypic plasticity, hyperlipidic and hypercaloric Diet, Body Adiposity, murinometrics parameters.

## 1. Introduction

Obesity is a public health problem in the world. Epidemiological data show that the estimated prevalence for the year 2015 is that approximately 2.3 billion individuals have overweight and 700 million obese (1). Its pathogenesis involves a complex interaction of factors including genetic, behavioral and environmental characteristics such: access and availability to food sources; physical or psychological factors; cultural identity; level of education and socioeconomic status (2). This condition is characterized by proinflammatory condition, which is a risk factor for the onset of insulin resistance, hypertension, cardiovascular disease and type 2 diabetes (3, 4). The high consumption of diets rich in lipids presents key mechanism in the pathogenesis of obesity (5). The call "Westernized diet," characterized by a high intake of saturated fatty acids and trans fatty acids, associated with low consumption of omega-3 fatty acids is associated with dietary changes related to the industrialization and cultural migrations of modern societies (6).

The consumption of this type of diet induces metabolic disorders being the high content of saturated fatty acids of these foods associated with reduced neurogenesis; to increased oxidative stress in the brain, neuroinflammation and increased anxiety (7- 10). Estadella (11), evaluating the consumption of a diet rich in saturated fatty acids in rats after 30 days of age find alterations in glucose metabolism accompanied by hyperleptinemia and increased lipid content in the carcass of such animals. Other studies have linked consumption of this type of diet to the decline in learning and memory as well as insulin resistance (12). Chen (13) related to the rapid growth early adiposity and hyperphagia, which were detected at 20 days of postnatal life in the offspring of rats submitted to diet high in fat during pregnancy. Intake of diets high in saturated fatty acids also induces the inflammatory state on the hypothalamus with increased inflammatory cytokines and apoptosis of neurons (14-16).

The consumption of diets rich in lipids and development of gestational obesity is also associated with changes in lactation. Studies in different species of mammals associate obesity with deficit in the lactogenic, since in these cases there was accumulation of fat droplets in the epithelial cells of the mammary glands leading to a decrease in gene expression of milk protein (17). Other pre-pregnancy obesity model in rats showed reduction in the response to breastfeeding induced prolactin and interruption of the normal development of the mammary glands during pregnancy (18).

Is known that diets rich in lipids can influence processes related to growth, development, lactogenesis, in addition are capable to promote hypothalamic resistance to the main anorexigenic hormones. Thus, this study aims to investigate the effects of using a high-fat and high calorie diet during the perinatal period on the murinometrics development and the lactation performance of the offspring. To investigate the late effects of this dietary manipulation were also assessed food intake and fat deposition after lactation.

## 2. Methodology

### 2.1. Animals

80 albinos Wistar rats were used (from 10 litters), randomly selected stemmed of primiparous rats weighing between 220g and 260g-. The animals were obtained in the colony of the Department of Nutrition at the Federal University of Pernambuco (UFPE). All animals were housed in polypropylene cages (46cmx34cmx20cm) environment using a reversed light cycle (18:00 to 06: 00) and darkness (6:00 to 18:00 h), temperature 22 ± 2 ° C, free access to water and feed. For mating were used one male mouse for two females (1: 2), aged 90 and 120 days. The diagnosis of pregnancy was performed by the vaginal smear test with finding sperm in vaginal secretion and monitoring to the body weight gain. Consanguineous crossings were made. Immediately after the identification of the pregnancy, the rats were divided into two groups: control group (CG) and high fat / high caloric Group (GH). One day after the birth (PND 1), litters were adjusted to 8 puppies (males) weighing between 6 and 8 grams. All procedures were performed in accordance with the approval of the Ethics Committee for Animal Experimentation (ECAE) of the Federal University of Pernambuco (Protocol 23076.056342 / 2013-29).

### 2.2. Experimental groups

The day after the birth of the puppies was considered the first day of postnatal life of the offspring (PND 1). In the 1rd PND, the litters were adjusted to 8 puppies / nursing. 80 animals were used to compose the two groups of the study.

Control Group (Normolipidc / normocaloric) (CG) (n = 40 pups). All puppies suckled by nursing who received commercial diet (Nuvilab®, CR1, Brazil);

High fat / high caloric Group (GH) (n = 40 pups). Composed of rats and their offspring who received high fat / high calorie diet from the first day of gestation to day 21 of

lactation. The diet consisted of a mix of high-calorie foods containing comercial feed (Nuvilab®, Brazil), roasted peanuts, milk chocolate and biscuit maizena, in proportion 3: 2: 2: 1 (11). All components were triturated, mixed and offered in form of pellets. The high fat and high calorie diet was made in the Department of Nutrition at the Federal University of Pernambuco (UFPE). Chemical composition of normolipdic and normocaloric, high fat and high calorie diets was held in the Experimentation Laboratory of Food Analysis Nonete Barbosa (LEAAL) (Table 1).

The fatty acid profile of the high fat and high calorie diet was held at the Food Technology Institute (ITAL-SP) (Table 2). The diets were offered during the period of pregnancy and lactation. After 22 days after birth, the pups were weaned, and allocated in cages, with a total of four pups per cage, which have received Nuvilab® diet adopted as the standard of bioterium until the end of the experiment. The diet high fat/high caloric diet contained 46,63% of lipids, and these 12,8% was saturated fatty acids, 21,6% monounsaturated fatty acids and 7,94% polyunsaturated fatty acids, with relationship omega-6: omega:3 of 4,2: 0,1.

**Table 1: Centesimal composition of high fat, high caloric diets and Nuvilab.**

| Nutrients | High fat, high caloric diet (g/100g) | Normolipidic and normocaloric diet (Nuvilab ®, CR1, Brasil – g/100g) |
|---|---|---|
| **Carbohydrates** | 38.08 | 47.76 |
| **Protein** | 27.24 | 28.85 |
| **Lipids** | 25.37 | 5.63 |
| **Ashes** | 4.88 | 7.91 |
| **Humidity** | 4.43 | 9.85 |
| **Energy (Kcal/g)** | 489.61 | 357.11 |

- Adolfo Lutz to humidity and volatile substances, proteins, lipids and ashes;
- For calculation / NASCAR 1985 for carbohydrates;

**Table 2: Fatty acid profile of the high fat and high calorie diet.**

| Fat acids | Result g/100g |
|---|---|
| **Saturated** | 6.97 |
| **Monounsaturated** | 11.76 |
| **Polyunsaturated** | 4.32 |
| **Omega-3** | 0.10 |
| **Omega-6** | 4.22 |
| **Trans - Total isomers** | ND < 0.01 |
| **NI** | 0.04 |

ND: not detected; NI: Unidentified

### 2.3. Body Weight, Food Intake and adiposity in Pregnancy and Lactation

Dietary intake of pregnants rats was assessed throughout the entire pregnancy and lactation, the feed was weighed and offered every 2 days. Total dietary intake was the difference between the offered food and its reject.

The gestational body weight in both experimental groups was measured at the end of each gestational week, always at the same time, at the beginning of the dark cycle. After the nursing period, the rats were weighed and euthanized by decapitation. The gonadal and retroperitoneal adipose tissue was dissected and weighed on a digital balance (Marte® S-100; sensitivity / precicion 0.01g).

### 2.4. Body weight of offspring

The body weight of the pups has been obtained 1, 7, 14, 21 and 30 days of postnatal life. The offspring was always measured at the same time, during the beginning of the dark cycle and the weight was registered using a digital scale (Marte® S-100; sensitivity / accuracy 0.01g).

### 2.5. Intake testing in offspring

During lactation, food intake assessment was performed using the lactation performance that occurred through the test of weighing-Breastfeeding-weighing. The test

occurred 3 hours after the onset of the dark phase of the light cycle on the 7th, 14th and 21st DPN. Immediately after the removal of the litter, stimulation was performed in the regions abdominal-pelvic and genital to promote the excretion of urine and feces. Then, the pups were weighed and kept in incubator heated to a temperature of 33 ° C for 60 minutes. After 60 minutes of fast, pups were returned to the litter and stayed another 60 minutes sucking. After this period, were weigh again and the liquid gain of weight in the offspring was considered the food consumption during this period. This test is called modified-weighed weigh-feeding (19).

### 2.6. Nutritional determinations and food consumption in offspring after Lactation

From 30 days of life, food intake was assessed for 5 days. The total dietary intake was the difference between the offered food and its reject in 24h. The average consumption was the total consumption divided by the number of animals in the cage. Based on food and caloric intake was calculated energy consumption (kJ / day) was given by the mean food intake multiplied by dietary metabolisable energy (20).

### 2.7. Determinations Murinometrics and adiposity in offspring

Abdominal Circumference (AC) (obtained by measurement of the region immediately before of the forefoot), thoracic circumference (obtained by measuring the area immediately behind the front leg), the body length (obtained by the total length of the space between the nose and anus) and body weight were determined in male offspring on days 7, 14, 21, 30 days of age. Measurements were obtained in centimeters using tape Anthropometric (1.5 meters Physical® with lock and precision of 1 mm), and the weight was measured on digital balance (Marte® S-100; sensitivity / accuracy 0.01g) in grams. Body weight and body length were used to determine the anthropometric parameters:

Body mass index (BMI) = body weight (g) / length (cm) $^2$

Lee index = body weight cubic root (g) / anal naso length (cm) (21).

At 30 days of age the animals were sacrificed by decapitation for removal of abdominal and retroperitoneal fat content. Immediately after its removal, adipose tissue was weighed on a digital balance (Marte® S-100; sensitivity / accuracy 0.01g).

### 2.8. Statistical analysis

To verify the distribution of the data using normal criteria we used the Kolmogorov-Smirnov test. The data show a normal distribution were expressed as mean and standard deviation. The unpaired t test was used for comparison between groups at each age. Two-way ANOVA measures repeated was used for comparisons between groups over time, followed by Bonferroni post-test. Was used software GraphPad Prism Version 4.0 2004. The significance level was maintained at 5% ($p < 0.05$)

## 3. Results

### 3.1. Body Weight and Food Intake in Pregnancy and Lactation

On the analysis of body weight of pregnant rats, the Two Way ANOVA Test for Repeated Measurements showed no interaction between body weight and dietary manipulation ($F = 2.83$) (Figure 1A). There was no difference in food consumption among pregnant in CG and GH (Figure 1B).

During lactation, there was no weight difference among nursing rats to the both groups, but, food intake was lower in rats belonging to GH will be shown (CG: 224.17 ± 11.93Kcal / g, n = 5 vs GH: 101.09 ± 15.22Kcal / g, n = 5) ($P < 0.01$) (Figures 1C and 1D).

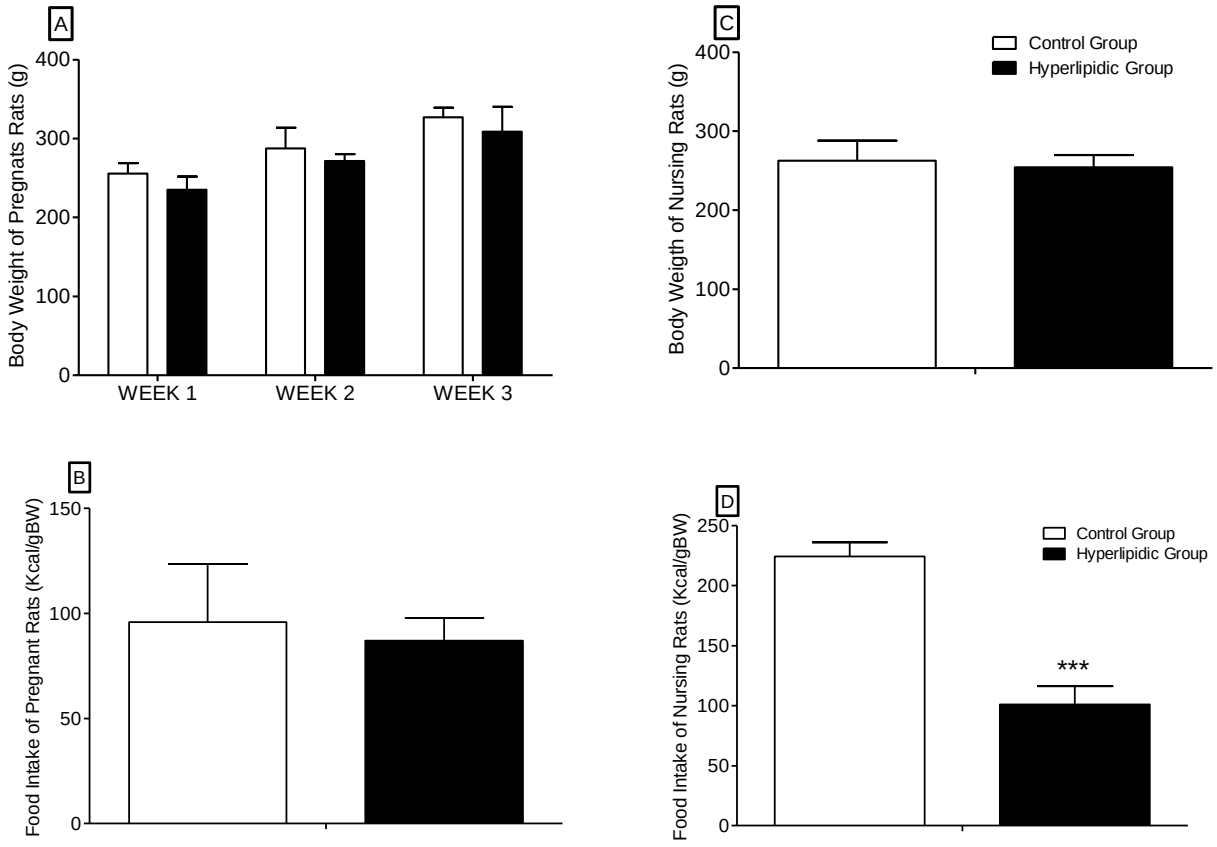


**Figure 1: Body weight of pregnant rats (A); Food intake of pregnant rats (B); Body Weight of Nursing rats (C); Food Intake of Nursing rats (D).** The dams were fed a diet control (C, N = 5, 14.1% kcal from lipids) or high fat/high caloric diet (GH, N = 5; 46.6% kcal from lipids) during pregnancy and the lactation. Body weight was measured weekly during pregnancy and lactation. Food consumption was performed every two days during the pregnancy and lactation periods. A: Two Way ANOVA repeated measures, Bonferroni Post Test. $P \leq 0.01$. B, C, D: t-Test Student. * Means difference between the groups. *** $P < 0.01$.

### 3.2. Adiposity in female rats after Lactation

The rats belonging to high fat / high caloric group had higher abdominal fat deposition that the rats belonging to the control group (Table 3).

**Table 3: Body composition of pregnant in the control group and high fat/ high caloric diet.**

| | **CG (n=5)** | **GH (n=5)** | **Significance** |
|---|---|---|---|
| **Retroperitoneal fat (g)** | 2.38 ± 0.44 | 8.07 ± 0.87 | *** |
| **Gonadal fat (g)** | 3.32 ± 1.03 | 9.62 ± 0.92 | *** |

P< 0.01, *Student´s t- Test*

### 3.3. Lactational Intake, Body Weight and Body Mass Index (BMI) in the offspring during lactation

The intake testing in offspring was the food intake of the pups during lactationa period. The Two Way ANOVA repeated measures showed a trend towards interaction between performance during lactation and the high fat / high calorie diet during the lactation period (F1.36 = 4.28) (P = 0.053). Although there this tendency, there was no difference between groups in each age (Figure 2A).

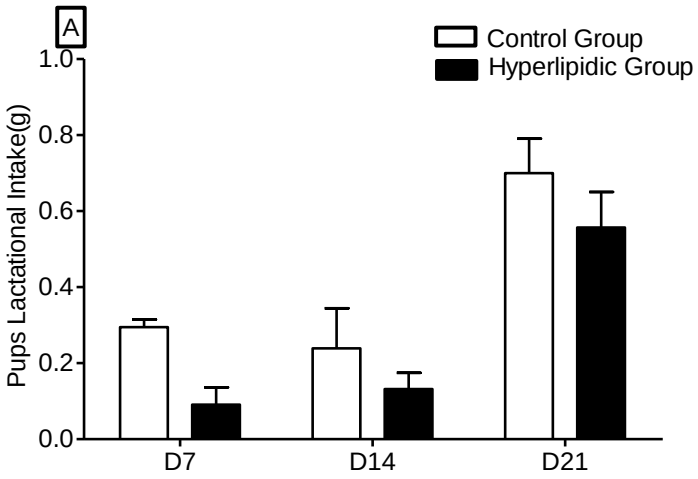


**Figure 2: (A) Pups Lactational Intake.** The female rats were fed at diet control (CG, N = 5, 14.1% kcal from lipids) or high fat/high caloric diet (HG, N = 5; 46.6% kcal from lipids) during pregnancy and the lactation. Pups lactational intake have been assessed at the ages 7, 14 and 21 days. ANOVA Two Way repeated measures, Bonferroni Post Test.

There was interaction between body weight and dietary manipulation (F (1.54) = 27.9) (P <0.01) with GH presenting lower body weight at 14 days (CG: 28.0 ± 2.5, n = 10 vs GH: 31.2 ± 5.7, n = 10) and 21 days (CG: 41.1 ± 2.8, n = 10 vs. GH: 31.2 ± 5.7, n = 10) (Figure 3A). However, there no was interaction between the diet used and BMI at any age (Figure 3B).

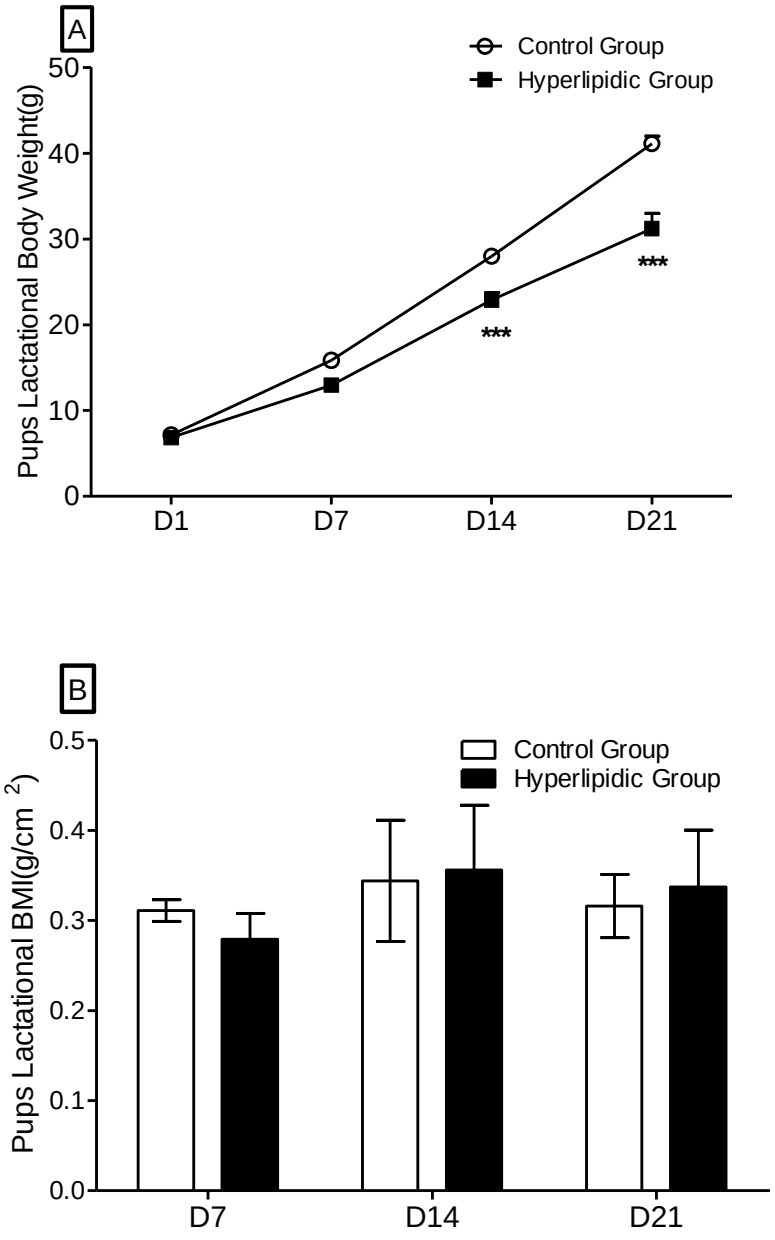


**Figure 3: (A) Body Weight; (B) Body Mass Index, during lactation.** The female rats were fed at diet control (CG, N = 5, 14.1% kcal from lipids) or high fat/high caloric diet (GH, N = 5; 46.6% kcal from lipids) during pregnancy and the lactation. Dietary intake and offspring BMI have been assessed at ages 7, 14 and 21 days. Body weight was measured on days 1, 7, 14 and 21 of lactation. ANOVA Two Way repeated measures, Bonferroni Post Test. * Means difference between *** P <0.01.

### 3.4. Murinometric assessment in the offspring during lactation

There was interaction between thoracic circumference and diet (F (1.36) = 38.61). The GH showed lower thoracic circumference than the CG at 7 days (P <0.01) and 21 days (P <0.01) (Figure 4A). The abdominal circumference in GH group was lower than the CG only at 21 days of life F (1.36) = 6.05; P = 0.02 (Fig 4B). The nose-anus length also showed interaction with dietary manipulation F (1.36) = 44.57; P <0.01, with significant differences between the groups at 14 (CG: 9.1 ± 0.6, n = 10 vs GH: 8.0 ± 0.5, n = 10) and 21 (CG: 11.0 ± 0.6 vs GH: 9.9 ± 0.5, n = 10) days of age (Figure 4C).

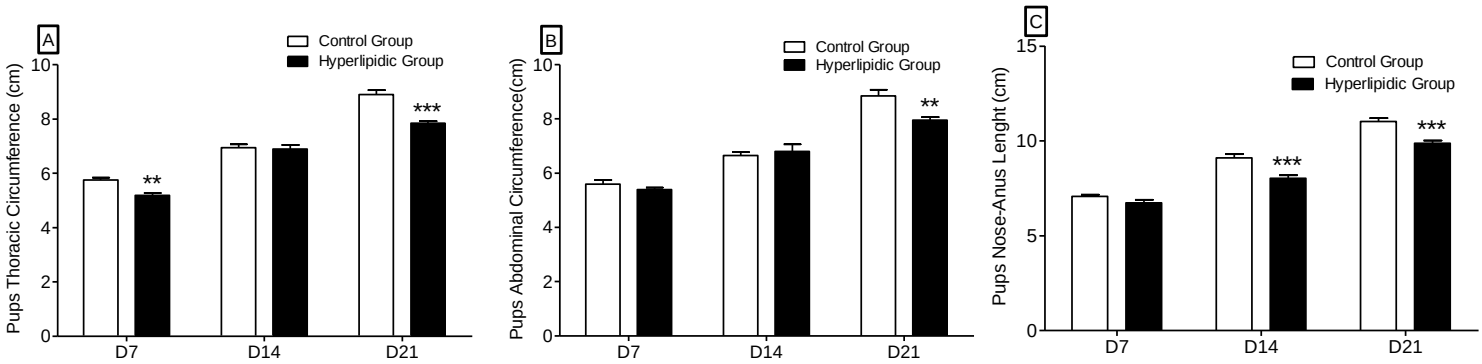


**Figure 4: (A) Thoracic circumference, (B) Abdominal Circumference and Nose-Anus length of the offspring during lactation.** The female rats were fed a diet control (CG, N = 5, 14.1% kcal from lipids) or high fat/high caloric diet (GH, N = 5; 46.6% kcal from lipids) during pregnancy and the lactation. The evaluations were performed at 7, 14 and 21 days of life. ANOVA Two Way repeated measures, Bonferroni Post Test. * Means difference between the groups. ** P <0.01); *** P <0.01.

### 3.5. Food Intake, Body Weight and Body Mass Index in the offspring after Lactation

Food intake of offspring at 30 days of life was lower at the GH group than in the CG (CG: 245.5 ± 38.0, n = 5 litters vs GH: 150.0 ± 66.4, n = 5 litters) (Figure 5A). There no was difference between the groups regarding body weight and BMI (Figures 5B and 5C).

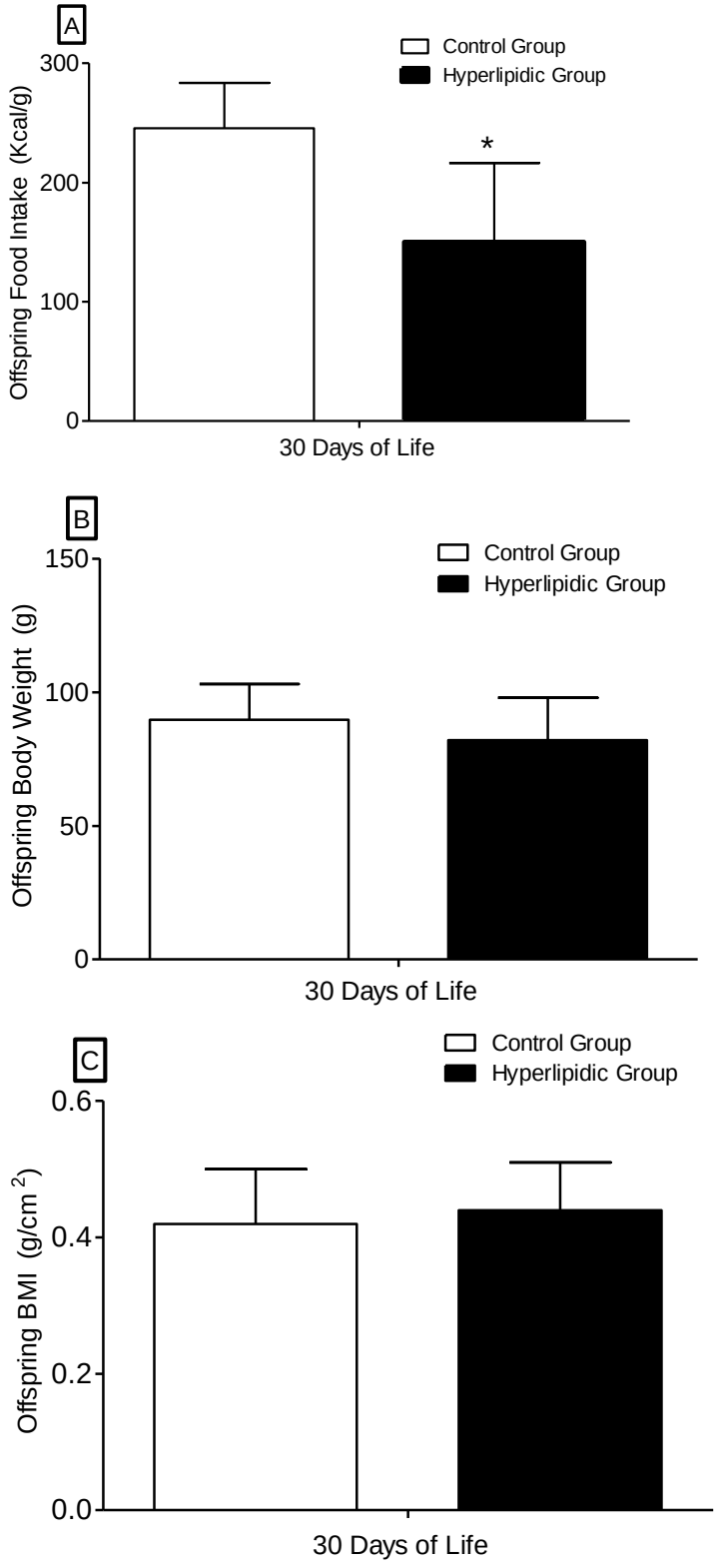


**Figure 5: (A) Food Intake; (B) Body weight and, (C) Body Mass Index (BMI) in the offspring after lactation.** The female rats were fed a diet control (CG, N = 5, 14.1% kcal from lipids) or high fat/high caloric diet (GH, N = 5; 46.6% kcal from lipids) during pregnancy and the lactation. The evaluations were performed at 30 days of life. For analysis of the Food Intake at Litter, 4 litters were used in CG and 5 litters in GH. To analyze Body Weight and BMI was used n = 10 / group; Student's t test. * Indicates difference between the groups. * P <0.01.

### 3.6. Murinometric evaluation and adiposity after Lactation

No differences were found on the parameters of abdominal circumference and thoracic circumference between groups at the 30 days of life (Figures 6A and 6B), however, the nose-anus length has decreased at the 30 days in the offspring belonging to GH (Figure 6C). It was found higher gonadal and retroperitoneal fat in GH at 30 days of age (Table 4). The lee index values for the control and high fat / high caloric group was 0,3 ± 0,0 and 0,3 ± 0,0 respectively (N=10).

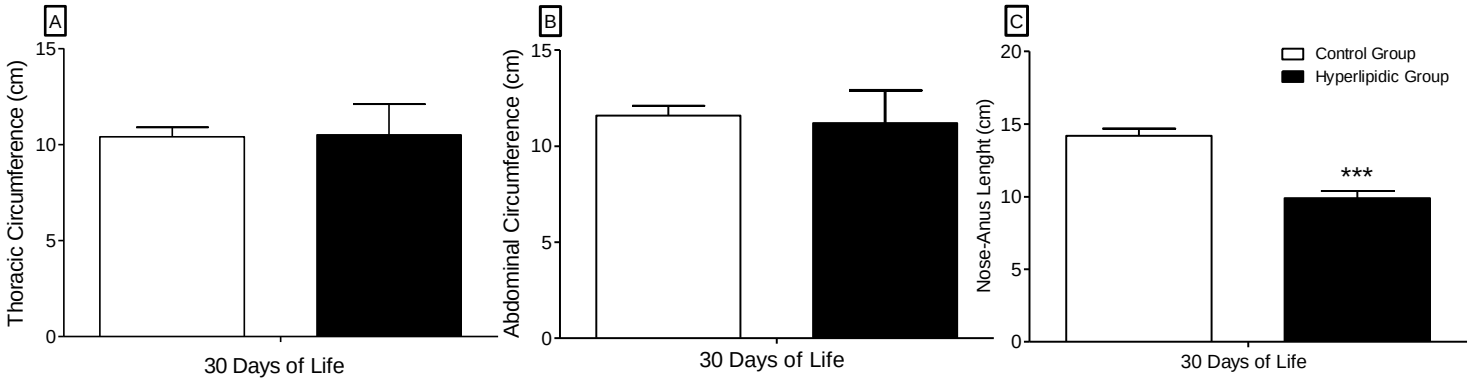


**Figure 6: (A) Thoracic circumference; (B) Abdominal Circumference, and (C) Nose-Anus length at the Prole after lactation.** The female rats were fed a diet control (CG, N = 5, 14.1% kcal from lipids) or high fat/ high caloric diet (GH, N = 5; 46.6% kcal from lipids) during pregnancy and the lactation. The evaluations were performed at 30 days of life. Student's t test. * Indicates difference between the groups. *** P <0.01.

**Table 4: Adiposity in the offspring of the group control and high fat / high caloric diet after lactation.**

| | CG (n=10) | GH (n=10) | Significance |
|---|---|---|---|
| **Retroperitoneal fat (g)** | 0.3 ± 0.1 | 0.6 ± 0.1 | *** |
| **Gonadal fat (g)** | 0.4 ± 0.1 | 0.7 ± 0.1 | *** |

Results are expressed as mean ± SD. Student t-test, significance level was P <0. 05.

## 4. Discussion

Intrauterine and early postnatal environments are considered critical for the development of organisms. Previous studies have reported that high fat diets used during pregnancy and lactation may have metabolic consequences from weaning to adulthood (22, 23). The maternal obesity is associated with the development of gestational diabetes and may predispose the offspring to metabolic side effects (24). In this study maternal exposure to hyperlipidic and hypercaloric diet during pregnancy and lactation induced in these dams and their offspring the following changes: reduction in food intake and increased body fat in nursing mothers; reduced body weight and changes in murinometrics measures in offspring during the neonatal period; reduction in food intake and body length of the young offspring with increased adiposity.

Our results showed that dams body weight do not differ between the groups during the pregnancy and lactation. The same was found by Giriko et al. (25) that also used high-fat diet during pregnancy and lactation. Samuelsson et al. (26) found in their study that dams fed with high-fat diet during pregnancy and lactation exhibited higher body weight than the animal fed with standard diet. In these rats, the accumulation of abdominal fat was four times higher and there was hyperleptinemia, hyperinsulinemia, hyperglycemia and hypercholesterolemia expression (26). Even though no differences in pregnancy, lactation feed intake in dams exposed to dietary manipulation was lower than that of dams that received standard diet. Findings related to food consumption coincide with those described in a study that also used high-fat diet during pregnancy and lactation (25). During this period, pregnant rats that consumed a high fat diet reduced their food intake to maintain similar caloric intake as control group (27). Keesey and Hirvonen (28) proposed the existence of a "set-point" for body weight in rodents and in humans. According the “set-point” the body weight increases or decreases, leading to changes in food intake or energy expenditure to return to the target weight. Thus, mechanisms related to body weight control may explain the effects of high fat diet on body weight and on food intake in this study. However, in this study, higher gonadal and retroperitoneal fat pad found in females fed with high-fat/ high calorie diet suggest nutritional environment effects on dams, after lactation period.

Obesity and overweight in animal models are related to the potential decrease in flow of breast milk and lactogenesis having a negative impact on the development of the mammary glands during pregnancy (18, 29). However, in this study were not found significant results

that associate the consumption of high-fat/high-caloric diet with changes in lactogenesis. In a study relating reduced fat diet to lactogenesis decrease there were changes in breast glands parenchyma associated with increased local inflammation (30). Clinical studies report that obese mothers show risk of developing increasing breastfeeding difficulties as a result of delay in lactogenesis (31). Thus, the milk supply would be reduced resulting in lower availability of essential amino acids to support tissue growth and contributing to decrease in body weight during the nursing period. In contrast, Purcell et al. (23) showed that in the first week of postnatal life the pups exposed to a hyperlipidic diet during pregnancy had higher intake of milk than the control group. This fact could contribute to long-term metabolic changes in these animals (32). Coincidentally, the development of hypothalamic circuits that play a key role in the control of food intake and body weight regulation which may be related to our findings occurs in this period (33).

The pups from dams that were fed with high-fat diet during pregnancy and lactation exhibited no differece in body weight at birth and 7th day of life, in relation to chow fed pups. The difference was expressed only in animals on the 14th and 21st day of life. These results coincide with findings where pups exposed to high-fat diet during pregnancy and lactation had lower body weight and lower leptin levels in early lactation and adult obesity (34). Other studies have shown that adult rats from mothers that were fed with high fat diet developed hypertension, hypercholesterolemia, overeating and overweight when compared to control animals (26, 35).

In our study, the animals exposed to high-fat diet did not differ in relation to body weight at 30 days of life, but exhibited greater body fat pad. This may be associated with the possible development of late metabolic alterations associated with perinatal exposure to excessive consumption of saturated fatty acids, omega-6 and deficient intake of omega-3 fatty acids. The deficiency in the intake of omega-3 contributes to the onset of insulin resistance, metabolic syndrome (36, 37) hepatic steatosis and nonalcoholic fatty liver disease (38).

Our results demonstrated changes in murinometrics development of offspring exposed to high fat/high calorie diet during pregnancy and lactation. There was thoracic circumference reduction (PND7 and PND14); abdominal circumference reduction (PND21) and lower nose-anus length (PND14 and PND21). The lower nose-anus length, an important indicator of somatic development, lasted until the 30 days of life. Santillán et al. (39), using a high-fat diet supplemented with omega-6 during pregnancy and lactation, found lower body length in the

offspring of the 7th day of postnatal life until weaning. Giriko et al., (25) showed cranial axes reduction in offspring exposed to highfat diet during pregnancy and lactation. In addition, other experimental study has shown the influence of maternal nutrition during the perinatal period in the growth of the body and cranial axes (40). For example, the offspring from females fed with restricted diets protein show reductions in skull axes lengths (40, 41). Thus, maternal obesity during pregnancy and lactation associated with excessive intake of saturated fatty acids can be as damage for development as well malnutrition.

Related to obesity predictors index, Bernardis and Patterson (21) suggest that the Lee index greater than 0.3g/cm can be used as an indicator of excess body fat. However, none of our groups showed values above 0.3 g/cm at 30 days of life. Similarly, Novelli et al. (20) suggested that BMI can also be used for evaluation of body fat pad in rats being an indicator of lipid disorders. They suggest that the average for non-obese rats varies between 0.45 and 0.68 g/cm². In this study there was no evidence of changes in BMI, maybe because we used young rats.

At 30 days of life, offspring exposed to nutritional manipulation with high fat/high caloric diet during perinatal period showed food consumption reduction, however, there no was difference related to body weight that was lower in group undergoing nutritional manipulation. Oliveira et al. (35) found different results, with na increase in food intake and body weight in offspring exposed to nutritional manipulation with high fat and high calorie diet during pregnancy and lactation. The evaluations were performed at 90 days of life. Bayol et al., (42) using cafeteria diet during the perinatal period found that the offspring exposed to this type of diet showed hyperphagia as well as a greater preference for palatable foods in the 10th week of postnatal life, presenting increased risk to develop obesity.

Changes in perinatal nutritional environment can alter the phenotypic expression of factors related to the acquisition and use of energy and that both deficit and excess of nutrients may signal the greatest long-term risk of obesity. This study demonstrated that the use of high fat/high calorie diet during the perinatal period was associated to lower body weight and nose-anus length, the later being an important somatic development indicator. Moreover, we evidenced that is a recovery in body weight between 21º and 30º days of life. This fact suggest a possible catch-up growth associated to higher gonadal and retroperitoneal adiposity in offspring exposed to dietary manipulation.

Together, these findings suggest that the nutritional environment under study consists of the excess lipids and calories can disrupt the murinometric development and be associated with the development of adiposity in the offspring.

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## 8. CONSIDERAÇÕES FINAIS

Nesse estudo o consumo materno de dieta hiperlipídica e hipercalórica durante a gestação e lactação foi associado a prejuízos no peso corporal e redução de parâmetros murinométricos, em especial do comprimento corporal, um importante indicador de desenvolvimento. Além disso, durante fases mais tardias foi evidenciado recuperação do peso corporal da prole com aumento da adiposidade, apesar desses animais apresentarem uma menor ingestão alimentar. Em conjunto, os resultados desse trabalho mostram que um ambiente perinatal composto por um excesso de calorias e lipídeos é capaz de afetar parâmetros relacionados ao desenvolvimento e aumentar a adiposidade corporal, sendo fator de risco para obesidade a longo prazo. Porém é necessário a realização de mais estudos, avaliando o perfil lipídico e glicêmico desses animais com finalidade de enterder melhor o seu perfil metabólico.

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**APÊNDICE A – Artigo de Revisão**

# Nutritional Neuroscience

## Perinatal exposure to hypercaloric and high-fat diet: effects on the serotonergic system and lactation homeostasis.

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| Abstract: | The role that balanced nutrition plays to provide adequate growth and development is well established. This fact is confirmed when the imbalance in the supply of nutrients during periods of pregnancy and lactation causes structural and functional changes to the fetus. The consumption of diets with high levels of saturated fatty acids induces an inflammatory state in the hypothalamus with increased inflammatory cytokines and apoptosis of neurons and this exposure been known to cause disturbances in the development of the serotonergic system. The review consisted of searches of electronic databases, MEDLINE / PubMed, Lilacs and Bireme using the terms as descriptors in English: serotoninergic system, developmental plasticity, high fat diet, feeding behavior and Lactogenesis. Searches were conducted from March to August 2014. The perinatal consumption of high calorie and high fat diet triggers an inflammatory state in the hypothalamus leading to dysfunction of this structure with loss of homeostatic control of food intake and energy metabolism. In addition, the consumption of this type of diet is also related to disorders in Lactogenesis. However, more studies need to be conducted to identify the relationship between the consumption of a high fat diet in critical periods of development and its impact on Lactogenesis and in brain levels of serotonin. |

Title page

# Perinatal exposure to hypercaloric and high-fat diet: effects on the serotonergic system and lactation homeostasis.

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Perinatal exposure to hypercaloric and high-fat diet: effects on the serotonergic system and lactation homeostasis.

## ABSTRACT

The role that balanced nutrition plays to provide adequate growth and development is well established. This fact is confirmed when the imbalance in the supply of nutrients during periods of pregnancy and lactation causes structural and functional changes to the fetus. The consumption of diets with high levels of saturated fatty acids induces an inflammatory state in the hypothalamus with increased inflammatory cytokines and apoptosis of neurons and this exposure been known to cause disturbances in the development of the serotonergic system. The review consisted of searches of electronic databases, MEDLINE / PubMed, Lilacs and Bireme using the terms as descriptors in English: serotoninergic system, developmental plasticity, high fat diet, feeding behavior and Lactogenesis. Searches were conducted from March to August 2014. The perinatal consumption of high calorie and high fat diet triggers an inflammatory state in the hypothalamus leading to dysfunction of this structure with loss of homeostatic control of food intake and energy metabolism. In addition, the consumption of this type of diet is also related to disorders in Lactogenesis. However, more studies need to be conducted to identify the relationship between the consumption of a high fat diet in critical periods of development and its impact on Lactogenesis and in brain levels of serotonin.

***Keywords:*** *serotonergic system*, *development plasticity*, *fat diet*, *eating behavior*, *Lactogenesis.*

## INTRODUCTION

In human societies, the advent of globalization has brought remarkable transformation of eating habits of individuals, and this fact is observed in all social classes. Currently, considering the practicality and convenience, there is an increase in the consumption of high fat and high calorie diet, called the Westernized diet (1).

Despite the current situation, the role that good nutrition plays to provide growth and development is confirmed when an imbalance in the supply of nutrients during the pregnancy and lactation is associated with structural and functional changes to the organism (2-4). In this context, the phenotypic plasticity has been studied and the results reported by several authors demonstrate the relationship between these nutritional changes in the critical period of development and the increase of diseases in adulthood (5-7).

Several studies have reported the relationship between the inadequate supply of nutrients during the critical period of growth and development and its long-term repercussions. Such consumption of high fat and high calorie diet in these periods have been associated with muscle atrophy and hypoplasia of fibers and metabolic alterations in adult rats (8, 9). However, the intake of high fat diet with high levels of saturated fatty acids induces an inflammatory state in the hypothalamus with increased inflammatory cytokines and apoptosis of neurons (10-12). Evidence suggests that maternal consumption of this type of diet in early pregnancy leads to increased risk of obesity development and metabolic syndrome in the offspring when in the adulthood
(13).

Furthermore, exposure to inflammatory cytokines triggered by the consumption of high fat and high calorie diet is known to cause disturbances in the development of serotonergic system (14). Additionally, recent studies reveal that the westernized diet perinatal consumption is associated with changes in the homeostasis of lactation, via serotonergic system (15, 16).

Thus, modulation of the serotonergic system through nutrition has become the research field to explain the development of diseases, such as obesity. The present study aims to investigate the damage induced by a high fat and high calorie diet during the perinatal period on the serotonergic system and its effects on feeding behavior, energy metabolism and homeostasis of lactation with the purpose to better understand how the binomial food and serotonergic system works.

# METHODS

The literature review was performed with the aim to characterize the impact of high calorie and high fat diet consumption during the critical period of development in the serotonergic system. This process involved electronic research in databases, MEDLINE / PubMed, Lilacs and Bireme using the terms as descriptors in English: serotonergic system, developmental plasticity, hight fat diet, feeding behavior.

Researches were conducted from March to August 2014. Between the selected articles, classic studies of "phenotypic plasticity" dated from 1964 and current studies on phenotypic plasticity are included. For discussion of serotonergic system, high fat diet, feeding behavior, energy metabolism and homeostasis of lactation, were used articles published between 2000 and 2013.

## Nutrition and phenotypic plasticity

Fetal growth and development are variable depending on the metabolic, nutritional and maternal hormonal environment. Thus, adequate nutrition during pregnancy and lactation is essential factor for these processes to occur efficiently (17). Evidence of this is that inadequate nutritional intake during the early period of development causes structural and functional effects to the fetus, such as: changes related to body weight and murinometrics characteristics; reduction in brain size and damage to processes such as neurogenesis, migration, neuronal differentiation and the synaptogenesis (2-4, 18).

Different complex processes involving rapid cell proliferation, differentiation and cellular hypertrophy characterize the critical period for development of the nervous system (17). Therefore, during this phase, the organs and systems are more vulnerable to environmental insults and may develop permanent changes in their structure and function (19). In mammals and many other species, most of these processes occur during intrauterine life, but in some organs and tissues also occur after birth. In humans, the critical period of development comprises a prenatal phase (from the second trimester of pregnancy) and postnatal (two to three years of life). In rodents, it is the period of pregnancy and lactation (2, 20).

In this context, phenotypic plasticity has been described by several studies that demonstrate the relationship between these nutritional deficiencies during pregnancy and lactation associated with the development of diseases in adulthood (5-7, 21). The phenotypic plasticity term is used to describe the ability of a genotype has to react in response to environmental challenges, which can lead to the phenotypic expression of different physiological and / or morphological state (22).

Basing the proposal described above, we highlight two theories. The first theory is known as "thrifty phenotype". It said that the fetal malnutrition imposes restrictions to growth and development, as well as changes on the metabolism of the fetus, with the purpose of achieving metabolic economy (23). These adaptive changes intended distribution selectively nutrients between organs to maintain growth and overall development of the brain in detriment to the other organs such liver and pancreas. The metabolism is also adapted in postnatal to promote survival under conditions of food shortage. However, this metabolic profile, in short term beneficial, may be harmful in adequate nutritional supply conditions or overfeeding (17). The second theory is the model of the relationship between "metabolic capacity-load" referring to the competence of bodies to maintain homeostasis. The phenotype of these organs is directly related to their growth during fetal life and infancy. Such metabolic capacity is related to "metabolic burden" that the liability imposed on these bodies in maintaining metabolic homeostasis, reflecting the interaction between lean mass, fat mass, height and weight gain with the onset of diseases (24).

Thus, it is the classic story of changes in nutritional interactions during the period of growth and development and their impact on children. Such consumption of diet restricted in protein during the perinatal period leads to a delay in the development of locomotor activity and changes in muscle fibers of rats (25). Chronic restriction of caloric intake during pregnancy and lactation has been associated with a reduction in brain weight of the offspring, with damage to the development of brain structures such as the hippocampus, striatum, hypothalamus and cortex (26). In addition, epidemiological studies have reported that low birth weight in the first year of life are associated with impaired glucose tolerance, type 2 diabetes (23), and cardiovascular diseases in adulthood (21).

Furthermore, studies have associated the intake of hyperlipidic and hypercaloric diet during pregnancy and lactation with atrophy and muscle fiber hypoplasia and metabolic disorder in adult rats (8, 9). There is also an association between intake of hyperlipidic diets with high levels of saturated fatty acids and genesis of hypothalamic inflammation with increased inflammatory cytokines and neuronal apoptosis (10-12).

## Serotonergic system, nervous plasticity and development

The serotonergic system consists of the neurotransmitter serotonin (5hydroxytryptamine, 5-HT) and its specific receptors located pre and postsynaptic neurons and the serotonin transporter (SERT or HTT-5). Serotonin is synthesized in large quantities in the enterochromaffin cells of the gastrointestinal tract, and to a lesser extent, in the central nervous system (CNS), in the raphe nuclei of the brain stem (27). In the CNS, 5-HT is released along of the neuraxis and acts as a neurotransmitter modulator being associated with the regulation of mood and anxiety, cognitive and various autonomic functions, including the maintenance of homeostasis to reproduction (28).

Serotonin is synthesized in two stages from the essential amino acid tryptophan, which is obtained in the diet. Tryptophan is first hydroxylated in position 5 of the indole ring by tryptophan hydroxylase, the rate-limiting enzyme in the serotonin production, yielding 5-hydroxytryptophan. This product is then decarboxylated by aromatic Ldecarboxylase amino acid, which leads to the formation of 5-hydroxytryptamine (5-HT, serotonin). Both steps of the synthesis of serotonin occur within the serotonergic neurons (29).

Once synthesized, serotonin is packaged in synaptic vesicles in preparation for exocytosis. Serotonin released at synapses sends signals via serotonin receptors and their signaling is terminated by uptake of serotonin synapses by the serotonin transporter (5-HTT or SERT). After uptake, 5-HT is metabolized in two steps: oxidative deamination by monoamine oxidase (MAO), to give the 5-hydroxyindole-3acetaldehyde and for the aldehyde dehydrogenase enzyme oxidation of 5hydroxyindoleacetic acid (28).

Nine distinct populations within the raphe nuclei of the brain stem form the neurons that synthesize serotonin. These populations are designated B1-B9. The flow cell groups, B1-B4, provide the downward projections serotonin, while groups rostral cells, B5-B9, give rise major upward projections (30). The downward projections of the targets serotonin include the cerebellum, midbrain, pons, and most spinal cord segments. The serotonin projections converge upward in the middle region of the forebrain, including cortex, hippocampus, thalamus, hypothalamus, striatum and amygdala (31).

The serotonin signaling is determined by a variety of receptors that are divided into seven families based on their evolutionary lineage and intracellular effects homology, designated 5-HT1 to 5-HT7 (32). With the exception of 5-HT3 receptor that belongs to the family of ligand-gated ion channels receptors (ionotrophic) selectively permeable to sodium (Na +), potassium (K +) and calcium (Ca +), the remaining receptors are included in the superfamily of G-protein coupled receptors (metabotropic). Its stimulation affects several enzymatic systems, including adenylate cyclase, phospholipase A and C channels cations, particularly K + and Ca ++ channels, through specific G protein activation (33).

There are at least 14 different subtypes of serotonergic receptors (34). The 5HT1 family includes receptors that are negatively coupled to adenylate adenililato and includes the 5-HT 1A receptors, 1B, 1D, 1E and 1F. This receptor operates in the raphe nuclei as self-receptor, while the target areas of serotoninergic neurons in the hippocampus corresponds to heteroreceptors located on postsynaptic terminals (35).

The 5-HT1B receptor is expressed both as serotoninergic and not serotonergic neurons, acting as autoreceptor and heteroreceptor, respectively. This receptor is distributed into different regions of the CNS and is predominantly found at the presynaptic level (36). The 5-HT1D receptor is known for its inhibitory functions serving as the serotonin autoreceptor terminals (37), inhibiting serotonin release on raphe midbrain, the hippocampus and frontal cortex (34). The 5-HT2 receptor family has three receptor subtypes they are classified as 5-HT2A, 5-HT2B and 5-HT2C. They act by activating phospholipase C increasing intracellular Ca ++ levels (37).

The 5-HT3 receptor, as previously mentioned, is an ion channel selectively permeable to Na +, K + and Ca ++, being distributed both centrally and peripherally in tissues (37). The 5-HT4 receptors, 5-HT6 and 5-HT7 41 are Gs-protein coupled which activate adenylate adenililato. 5-HT5 receptor family contains two 5-HT5A and 5HT5B subtypes. It is believed that 5-HT5A type is negatively coupled to adenylate adenililato while the 5-HT5B effector is connected to another system not well characterized (37).

The SERT is primarily responsible for regulating the levels of serotonin in the synaptic cleft, once engaged in their reuptake thus enabling its inactivation and / or degradation within the presynaptic

neuron. This protein can also control the synthesis of serotonin, by modulating the activity of the enzyme tryptophan hydroxylase (38).

Serotonergic neurons are the primary cortical neuronal system board. These neurons are the first to go through the process of differentiation and play an important role in neurogenesis (39). Moreover, the distribution of the terminals and the diversity of brain serotonin receptors have generated the hypothesis that the serotonergic system may influence almost all neuronal plasticity mechanisms, for example, autonomic, neuroendocrine, behavioral and cognitive mechanisms (31).

During the period of development, the expression of serotonin is related to the formation and differentiation of neurons, synaptogenesis, and the self-regulation of serotonergic neurons and the development of target-tissues (40). In rats, the first serotonergic neurons appears between the 12th and the 14th days of gestation (41).

Neurons upper tier contain serotonin, since the day 12 of gestation, while the bottom group is the 5-HT and it is found two days after (42). Serotonergic axons appear in the upper tier of the brain medial beam on the 14th gestational day and innervate the hippocampus, thalamus and neocortex on day 18. Around the 19th day gestational all projections of the raphe nucleus reach their targets (43). In the human brain, the serotonergic neurons are first evidenced in the fifth week of pregnancy, number of rapidly increasing until the tenth week and are found predominantly in the dorsal raphe nucleus (44). During brain development, higher levels of serotonin are needed, and any decrease in these levels can leads to serious consequences for the nervous system at the end of maturation (44).

Changes in serotonin function still occur during the development. Initially, its role is related to cell survival, however, after the maturation of the brain, serotonin is passed to serve on the migration and cell differentiation. It is postulated that even in the adult brain, serotonin also acts to maintain the same synaptic plasticity and mechanisms used during development (44).

## Serotonergic system and energy metabolismo

Food intake is controlled by homeostatic and non-homeostatic mechanisms that promote energy balance of the body (45). The peripheral homeostatic signals include several factors secreted by the gastrointestinal tract (ghrelin, peptide YY and cholecystokinin); the pancreas (insulin); by adipose tissue (leptin) and signals from the mechanoreceptors of intestinal stimulation. In the CNS such signals are integrated in the hypothalamus and brainstem involving the action of several neuropeptides and neurotransmitters such as serotonin (46). The non-homeostatic signals are generated in various encephalic structures, such as nucleus accumbens, using serotonin as a main messenger (47).

The nucleus accumbens is also responsible for the neural mechanisms in which "motivation" is translated into "action" as it receives signals from limbic structures, which are responsible for

integrating motivational characteristics of feeding behavior influence on appetite control (48). Stimulation of serotonin receptors in this brain region induces demand for palatable foods (45).

Between the central structures related to the control of feeding behavior, brainstem and hypothalamus are highlighted. The brain stem is associated with the mediation of the response to satiety reflex via the vagus nerve, involving short-term fluctuations detection in nutritional status. It starts with gastrointestinal motor response, and receive gustatory signals, since the most gastrointestinal pathways of taste make synapse in this area (28). The hypothalamus is one of the structures responsible for homeostatic regulation of feeding behavior and body weight. It acts integrating information on long-term energy storage and other physiological and environmental factors to formulate answers to food. Studies have identified brain region such as a place of production, distribution and activity of some molecules that stimulate or inhibit food intake (49). During the pre or post-natal development, environmental stimuli can alter the hypothalamus generating late changes in eating behavior (50).

The motivational aspects of food have been associated with activity in mesolimbic circuits. Serotonin, which diffusely innervates most of the neuraxial, is ideally placed to coordinate or influence these responses influencing both the brainstem reflex center, as hypothalamic centers involved in the control of food intake. Serotonin has an inverse relationship with food intake as the increased availability of 5-HT is associated with decreased food intake and increased availability of this, the increase in food intake (28).

Currently, the focus of the study on the eating behavior are the types and subtypes of serotonergic receptors that regulate feeding, since serotonin exerts its function through interaction with a variety of receptors (37). Being family-HT1 receptors 5-HT2 and 5 the most related to the control of food intake, and the 5-HT 2C subtype the most important in the relationship between food intake and energy balance. Mice lacking this receptor become obese, whereas the use of agonists of this receptor activity results in decreased food intake (51).

Acting on 5-HT2C receptor in the hypothalamus, serotonin activates the cleavage of pro-opiomelanocortin (POMC). Additionally, the interaction between serotonin and 5-HT1B receptor inhibits the arcuate nucleus neuropeptide Y (NPY) and agouti-related protein (AGRP), depressing the GABAergic transmission of alphamelanotropin (alpha-MSH) and the regulated transcript cocaine and amphetamine (CART). These associated mechanisms produce stimulating satiety and increase in thermogenesis. When acting on the 5-HT 1B receptor, the serotonin modulates the endogenous release of both agonists and antagonists of melanocortin receptors that are components of the control system of body weight homeostasis (52). The hyperphagia and obesity generated after depletion of serotonin, via a tryptophan hydroxylase enzyme inhibitor or after neurotoxic injury

serotonergic neurons, enhance the understanding of the relationship between the serotonergic system and uptake / utilization of energy (52).

## Obesity, hyperlipidemic diet and serotonergic system

Epidemiological studies have shown that the prevalence estimated for 2015 is that approximately 2.3 billion individuals have overweight and 700 million obese (53). The pathogenesis of obesity is elaborated by the complex interaction of factors that involve hereditary genetic, behavioral, environmental characteristics, such as access and availability of food sources, physical or psychological factors, cultural identity, education level and socioeconomic (54). This pathological condition is characterized by a proinflammatory condition, which is a determining factor for the onset of insulin resistance and type 2 diabetes (55).

Lipids are important components of human nutrition. They provide energy and are a source of essential fatty acids and fat-soluble vitamins. However, some fatty acids present in the fat, particularly saturated and trans, can provoke adverse effects on human health (56). Saturated fatty acids are derived from animal sources, while trans fatty acids are found in meat and ruminant animals milk (57). The partial hydrogenation of unsaturated fatty acids in vegetable oils for industrial production of some products also produces trans fatty acids (58).

Increased dietary intake of trans and saturated fatty acids, typical eating habits of western populations, favors an inflammatory pro-state that contributes to the development of insulin resistance. The role of these fatty acids has been demonstrated in various inflammatory processes and resulting imbalance of lipid signaling pathways contribute to the progression of chronic inflammation, autoimmune disease, allergy, cancer, atherosclerosis, hypertension, and cardiac hypertrophy, as well as metabolic and degenerative diseases (59). The consumption of hyperlipidic and high calorie diets have also been associated with the development of hypothalamic inflammatory processes leading to their dysfunction (60). Inflammatory mediators important in this response include saturated fatty acids and various cytokines, including tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). Inflammation of the hypothalamus, later leads to a central resistance to hormones responsible for its regulation (leptin and insulin) and loss of homeostatic control of food intake and energy expenditure, leading to weight gain (61).

In this context, the hyperlipidic and hypercaloric diet experimental use try to mimic this current food patterns of this population, characterized by increased content of saturated fatty acids, simple carbohydrates, animal protein and nutrients deficit (1, 62). Several studies are being conducted with the use of this type of diet in order to understand the consequences of modern eating habits on the development of organisms (9, 63). In rats, there were changes in lipid metabolism, accompanied by hyperleptinemia, hyperinsulinemia, normoglycemia and insulin resistance (9, 63). Evidence

supporting the idea that consumption of such maternal diet during early fetal life, carries in the offspring increased risk for development the obesity and metabolic syndrome in adult life, because the hypothalamus fetal and the newborns is important target to maternal nutritional and hormonal stimuli during pregnancy and lactation (13). Previous studies have demonstrated that postnatal overnutrition and hyperleptinemia results in increased levels of hypothalamic NPY and POMC (64).

Exposure to cytokines have also been linked to disturbances in serotonergic system (14). Maternal dietary hyperlipidic intake results in increased genetic expression of the tryptophan hydroxylase enzyme bound for the synthesis of 5-HT, and increased self inhibitory receptor 5-HT1A receptors in the raphe nucleus rostral, which is the primary source of serotonin to the hypothalamus (65). Together, these data indicate that maternal consumption of this type of diet causes changes in fetal serotonergic system. Furthermore, the increase in 5-HT1A receptor expression in the fetal age and decreased serotonin in cerebrospinal fluid in juvenile age suggest that serotonergic system is suppressed in the offspring of mothers who consume high-fat diet. Therefore, the increased expression of tryptophan hydroxylase was a compensatory response to increasing 5-HT 1A receptor expression in the rostral nucleus of the raphe (66).

## Serotonin and regulation of lactation homeostasis

Milk production number varies around the mammary secretory cells and the activity per cell. The number of secreting cells is largely determined by the increase in mammary gland. During puberty and pregnancy, individual functional activity of mammary epithelial cells is controlled by paracrine and endocrine factors as well as the suction rate of milk during lactation (67). The peak in the milk yield is achieved early in the lactation cycle and is followed by a decline in production to cease production. This reduction in milk production can be up to 50% when the milk production peak is reached. Lactational homeostasis is defined as the process by which the steady flow of milk from the mammary gland is maintained throughout the lactation period (68).

The decrease in milk production seen in the advancing lactation occurs due to a decline in the number of secretory cells, as well as a decline in their function. This decline occurs via apoptosis (67). Other factors such as nutrition, oxidative stress imposed by metabolism and reproductive hormones (estradiol and progesterone), and the milking frequency, influences the persistence of lactation through the regulation of apoptosis. Mastitis, stress, pregnancy and concomitant decrease in milking frequency can decrease the persistency of lactation, increasing apoptosis (67).

Serotonin has been identified as one of the chemical signals of mammary epithelial cells and produce inhibitory response is association with feedback during lactation (15). The regulatory functions by alveolar feedback synthesis and secretion of milk control the degree of strain within the lobe-alveolar units and adjust milk secretion for the offspring demand. Individual glands are regulated by cellular

signals inside the unit. It was reported that 5-HT milk inhibits protein synthesis and causes epithelial apoptosis (15). Serotonin is one of many hormones also involved in the neuroendocrine regulation of prolactin and was proposed to be a component of autocrine-paracrine feedback homeostatic functions to inhibit the development of the mammary gland and milk synthesis (69). In addition, an intrinsic system 5-HT has been found in the mammary gland in rats. A study evaluating the overall gene expression in mice that express the super pituitary prolactin gene found that TPH-1 expressed in the mammary glands was prolactin dependent (15) It was also reported that knockout mice TPH-1 were more sensitive to stimulation by the pituitary prolactin (15)).

It was also found that serotonin adversely affects the transepithelial electrical resistance as it promotes an increase in the permeability of tight junctions (70). The tight junctions are impermeable during lactation, which allows milk to be stored between periods of care without leakage of components from the lumen (70). At low concentrations and earlier times, serotonin potentiates the transmembrane epithelial resistance, and higher concentrations that occur at late times, this neurotransmitter decreases cell transepithelial resistance and stops tight junction type. In addition to acting at these junctions, 5-HT reduces the expression of milk protein gene mRNA. The same phenomenon occurs when using a selective serotonin reuptake inhibitor (15)

Studies correlating high-fat diet consumption during pregnancy and lactation and serotonin levels have shown alterations in lactogenesis, since this would be the neurotransmitter responsible for accelerating the involution of the mammary glands in various mammalian species via the 5-HT7 receptor. This would lead to a reduction in the production of milk by infants consuming this type of diet, especially in the first days of lactation (15, 16).

# CONCLUSION

Feeding is essential to maintain proper growth and development. However, currently, the eating patterns of global population have undergone changes with increased consumption of Westernized diets high in fat and calories. Use of this type of diet, especially during the perinatal period is associated with hypothalamic inflammation. As the hypothalamus is an important regulatory center of the feeding behavior and energy metabolism, their dysfunction may be associated with the development of obesity. Other studies have shown that inflammatory state generated by the consumption of this type of diet can suppress the serotonergic system. Because serotonin is intrinsically related to the acquisition/ use of energy, disturbances in serotonergic system may also result in obesity onset. Additionally, studies have linked the consumption of diets rich in fat with altered homeostasis of lactation. This process has also been associated with the serotonin neurotransmitter since it is responsible for the regression of mammary glands causing a reduction in

milk production. However, the studies linking perinatal use of hyperlipidic diets to serotonergic system and its repercussions have yet to be deepened, thus there will be more evidence of this interaction.

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## APÊNDICE B – Densidade proteica do Trasnportador de Serotonina (SERT)

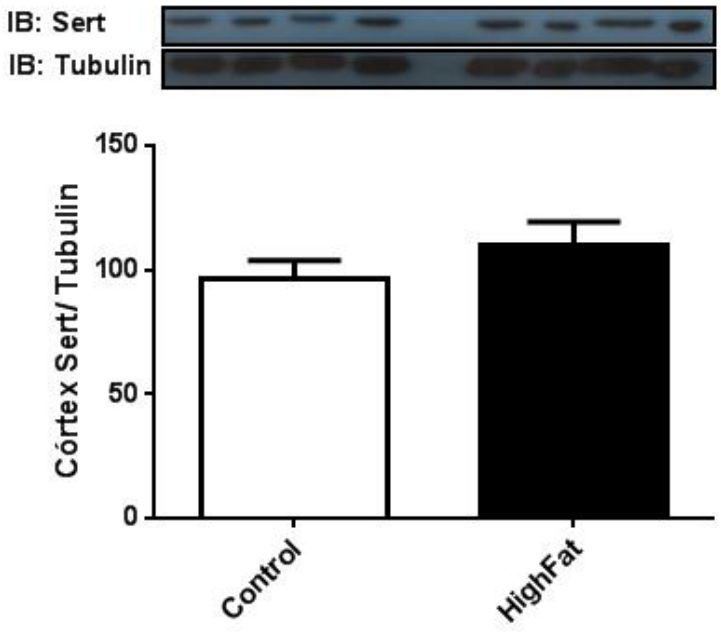


**Figura 1: Densidade proteica do SERT no Córtex.** As ratas foram alimentadas com Dieta Controle (GC, N=5; 14,1% kcal proveniente de lipídeos) ou com Dieta Hiperlipídica e Hipercalórica (GH, N=5; 46,6% kcal proveniente de lipídeos) durante a gestação e a lactação. A densidade proteica do SERT foi avaliada na prole aos 90 dias de vida. Media da coluna A, n=4; 96,2 ± 7,602; Média da coluna B, n=4; 109,8 ± 9,519; Student *t- Test.* P=0,3081.

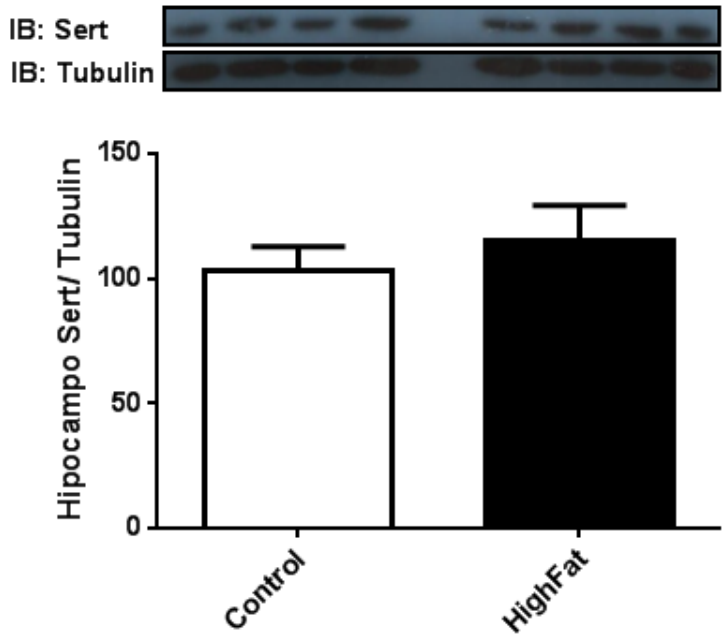


**Figura 2: Densidade proteica do SERT no Hipocampo.** As ratas foram alimentadas com Dieta Controle (GC, N=5; 14,1% kcal proveniente de lipídeos) ou com Dieta Hiperlipídica e Hipercalórica (GH, N=5; 46,6% kcal proveniente de lipídeos) durante a gestação e a lactação. A densidade proteica do SERT foi avaliada na prole aos 90 dias de vida. Media da coluna A, n=4; 103,3 ± 9,305; Média da coluna B, n=4; 115,5 ± 13,85; Student *t-Test.* P=0,4955.

## ANEXO A - Documentação de encaminhamento do artigo ao periódico

Nutrition Research
Title: The use of high-fat diet during pregnancy and lactation promotes changes in murinometrics development and adiposity in young rats
Authors: AMANDA C LIMA; WENICIUS F CHAVES; MARIA EDUARDA A LUCENA; LIGIA M GALINDO; RAUL M CASTRO

Dear Ms. AMANDA COSTA LIMA,

The PDF for your submission, "The use of high-fat diet during pregnancy and lactation promotes changes in murinometrics development and adiposity in young rats" has now been built and is ready for your approval. Please view the submission before approving it, to be certain that it is free of any errors. If you have already approved the PDF of your submission, this e-mail can be ignored.

To approve the PDF please login to the Elsevier Editorial System as an Author:

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Then click on the folder 'Submissions Waiting for Author's Approval' to view and approve the PDF of your submission. You may need to click on 'Action Links' to expand your Action Links menu.

You will also need to confirm that you have read and agree with the Elsevier Ethics in Publishing statement before the submission process can be completed. Once all of the above steps are done, you will receive an e-mail confirming receipt of your submission from the Editorial Office. For further information or if you have trouble completing these steps please go to: http://help.elsevier.com/app/answers/detail/a_id/88/p/7923.

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Your submission will be given a reference number once an Editor has been assigned to handle it.

Thank you for your time and patience.
Kind regards,
Editorial Office
Nutrition Research

**********
For further assistance, please visit our customer support site at http://help.elsevier.com/app/answers/list/p/7923. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

**ANEXO B - Parecer do Comitê de Ética em Pesquisa**

**Universidade Federal de Pernambuco**
**Centro de Ciências Biológicas**

Av. Prof. Nelson Chaves, s/n
50670-420 / Recife - PE - Brasil
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Recife, 17 de dezembro de 2013.

Ofício nº 660/13

Da Comissão de Ética no Uso de Animais (CEUA) da UFPE
Para: **Profª. Amanda Costa Lima**
Universidade Federal de Pernambuco
Departamento de Nutrição
Processo nº 23076.056342/2013-29

Os membros da Comissão de Ética no Uso de Animais do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (CEUA-UFPE) avaliaram seu projete de pesquisa intitulado, **"Consumo perinatal de dieta hipercalórica e hiperlipídica pelo binômio mãe-filhote altera o desenvolvimento dos níveis encefálicos de serotonina?".**

Concluímos que os procedimentos descritos para a utilização experimental dos animais encontram-se de acordo com as normas sugeridas pelo Colégio Brasileiro para Experimentação Animal e com as normas internacionais estabelecidas pelo National Institute of Health Guide for Care and Use of Laboratory Animals as quais são adotadas como critérios de avaliação e julgamento pela CEUA-UFPE.

Encontra-se de acordo com as normas vigentes no Brasil, especialmente a Lei 11.794 de 08 de outubro de 2008, que trata da questão do uso de animais para fins científicos e didáticos.

Diante do exposto, emitimos **parecer favorável** aos protocolos experimentais a serem realizados.

Origem dos animais: Biotério; Animal: ratos heterogênicos; Linhagem: Wistar; Idade: 1-90 dias; Peso: 1-260 g; Sexo: machos e fêmeas; Nº Total de Animais: 95.

Profª Tania Rieger
Presidente do CEUA/CCB-UFPE
SIAPE 2306924