UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS:
BIOQUÍMICA

ESTRESSE NO PERÍODO PRÉ-PÚBERE COM ACESSO A DIETA HIPERLIPÍDICA: COMPORTAMENTO DO TIPO DEPRESSIVO E ALTERAÇÕES METABÓLICAS A LONGO PRAZO EM RATOS MACHOS E FÊMEAS

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Bioquímica da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do Grau de Mestre em Ciências Biológicas: Bioquímica.

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Porto Alegre – RS

2014

Aos meus amados pais, que apesar da distância, sempre me deram apoio incondicional e incentivo constante durante essa minha trajetória.

AGRADECIMENTOS

A Deus por permitir que esse caminho fosse trilhado.

À minha querida orientadora, Profª. Drª. Carla Dalmaz, pela confiança, pela forma acessível e sempre disposta a auxiliar, pela compreensão, pelos ensinamentos e por ser a grande profissional que é.

À minha coorientadora, Drª Simone Nardin Weis, pelos ensinamentos de pesquisa, pela paciência ao ensinar e também pelos conselhos como amiga e colega.

Aos professores da banca examinadora, por sua cordialidade e disponibilidade na avaliação deste trabalho.

Aos professores do Departamento de Bioquímica, pelo aprendizado e contribuição para a minha formação.

A uma grande amiga e exemplo de solidariedade, Rachel Krolow, pelo incentivo, pelos ensinamentos, pela paciência, pela parceria, pela amizade e por sempre trazer consigo um otimismo que estimula a acreditarmos em nós mesmos. Além do grande caráter que tem como profissional e como pessoa.

A todos os colegas do laboratório 37, pela amizade, pela convivência, parceria e ajuda nos experimentos. Em especial à Danusa Mar Arcego, pela grande ajuda em todos os momentos dessa caminhada, pela força, amizade e respeito. Com grande carinho, agradeço também à Carine Lampert, pela amizade, pela parceria, pelo otimismo e pelo grande incentivo. À Camilla Lazzaretti, por sempre estar disposta a ajudar e pela amizade.

Aos colegas do laboratório 35, pela amizade e convivência.

À Letícia Pettenuzzo pela ajuda e pela e parceria em experimentos.

Aos funcionários do Programa de Pós-graduação.

À minha família que é minha base pra tudo por estar lá sempre que precisei e por sempre trazer o conforto quando necessário.

À Universidade Federal do Rio Grande do Sul e ao Departamento de Bioquímica pela oportunidade de realizar o mestrado.

A CAPES pela bolsa de mestrado.

SUMÁRIO

RESUMO

A pré-puberdade é um período crítico para maturação dos circuitos neuronais que controlam a homeostase energética e as respostas ao estresse, além de ser um período de grande desenvolvimento emocional. A exposição a fatores ambientais como o estresse e dietas ricas em gordura durante esse período, podem modificar os processos de maturação neural causando alterações comportamentais e neuroquímicas que podem repercutir em disfunções e patologias na idade adulta. Dessa forma, o objetivo desse estudo foi investigar os efeitos da exposição ao estresse por isolamento social durante o período da pré-puberdade em ratos machos e fêmeas com ou sem o acesso crônico a uma dieta rica em gordura e seus efeitos a longo prazo sobre parâmetros de consumo, deposição de gordura e hormônios como leptina e adiponectina. Além disso, nosso estudo investigou se a exposição ao estresse durante o período pré-púbere com ou sem acesso a dieta rica em gordura pode levar a um comportamento do tipo depressivo e se esse comportamento estaria relacionado com parâmetros inflamatórios. Os ratos machos isolados recebendo apenas ração padrão apresentaram menor ganho de peso que seus controles, efeito revertido pelo acesso à dieta rica em gordura que aumentou o peso dos animais na semana em que foram submetidos ao estresse. Na semana do isolamento, a dieta rica em gordura diminuiu a eficiência calórica nos animais. Diferentemente do que ocorreu na idade adulta, quando o consumo da dieta rica em gordura aumentou a eficiência calórica nos animais, sendo mais pronunciada nos machos do que nas fêmeas. Além disso, foi observado que a dieta rica em gordura aumentou os níveis de leptina e os níveis de adiponectina na idade adulta. Tanto os animais estressados quanto aqueles que receberam a dieta rica em gordura exibiram comportamento do tipo depressivo, que não parece estar associado aos parâmetros inflamatórios avaliados. Esses resultados sugerem que intervenções como o estresse por isolamento durante o período pré-púbere associado ao acesso crônico a uma dieta rica em gordura podem causar modificações no metabolismo de forma sexo-específica e levar ao comportamento do tipo depressivo a longo prazo.

ABSTRACT

The pre-puberty period is critical for the maturation of neural circuits that control energy homeostasis and stress responses, besides being a period of emotional development. Exposure to environmental factors, such as stress and high-fat diet, during this period can modify the neural maturation process causing behavioral and neurochemical changes that may impact in disorders in adult age. Thus, the aim of this study was to investigate the effects of exposure to isolation stress during the prepubertal period in male and female rats with or without chronic access to high-fat diet and its long-term effects on consumption parameters, fat deposition and on hormones such as leptin and adiponectin. Furthermore, our study investigated whether exposure to stress during prepubertal period with or without access to high-fat diet would lead to depressive-like behavior and whether this behavior would be related to inflammatory parameters. Stressed male rats receiving standard chow had less body weight gain than their controls, effect reversed by access to high-fat diet that increased the body weight gain of animals during first week, when they were submitted to stress. During the isolation week, the access to high-fat diet decreased caloric efficiency in all animals. Differently, in adulthood high-fat diet consumption increased caloric efficiency in animals, being more pronounced in males than females. Moreover, it was observed that high-fat diet increased leptin levels and adiponectin levels in adulthood. Both stressed animals and those receiving high-fat diet exhibited depressive-like behavior that seems to be not associated with evaluated inflammatory parameters. These results suggest that interventions such as isolation stress during prepubertal period and chronic access to a high-fat diet can cause changes in a sex-specific manner and lead to depressive-like behavior in the long-term.

LISTA DE ABREVIATURAS

HHA - Hipotálamo-hipófise-adrenal

CRH - Hormônio liberador de corticotropina

ACTH - Hormônio adrenocorticotrópico

SNC – Sistema nervoso central

RG – Receptor de glicocorticoides

GCs – Glicocorticoides

IL-1 - interleucina -1

IL-6 - interleucina-6

TNF-α – fator de necrose tumoral- alfa

NF-kB - fator nuclear kappa B

1. INTRODUÇÃO

1.1 Estresse

O estresse pode ser definido como um desafio interno ou externo ao indivíduo que tem o potencial de perturbar a manutenção da homeostase, estimulando respostas adaptativas no organismo (Chrousos & Gold, 1992). As respostas fisiológicas do organismo à exposição ao estresse são variadas, incluindo respostas neurovegetativas, imunológicas e comportamentais (Tsigos & Chrousos, 2002) além da ativação do sistema simpato-adrenomedular levando a liberação de catecolaminas e da ativação do eixo hipotálamo-hipófise-adrenal (HHA) e consequentemente, liberação de glicocorticoides (GCs) (Kvetnansky *et al.*, 1995). Ao ser ativado, o eixo HHA, inicialmente libera o hormônio liberador de corticotropina (CRH) dos núcleos paraventriculares no hipotálamo, que irá estimular a hipófise anterior a liberar o hormônio adrenocorticotrópico (ACTH). O ACTH, por sua vez, estimula a liberação de glicocorticoides (cortisol em humanos e corticosterona em roedores) pelo córtex das adrenais (McCormick *et al.*, 2010).

Os glicocorticoides possuem diversas ações em todo organismo. Em resposta a um estressor, eles aumentam a disponibilidade de substratos energéticos (aumentam a produção de glicose e a liberação de ácidos graxos a partir de triacilglicerois circulantes) e tem ação imunossupressora (Chrousos, 1995; Barnes, 1998; Peckett *et al.*, 2011). Porém uma exposição prolongada a altos níveis de glicocorticoides tem efeitos nocivos em vários sistemas, incluindo o sistema nervoso central (SNC) (McEwen, 2000). Por isso, para limitar a exposição aos glicocorticoides é importante que haja um mecanismo de regulação inibitória na atividade do eixo HHA, que é realizada por um sistema de retroalimentação negativa feita pela interação do glicocorticoides com seus

receptores (RG) em estruturas como córtex pré- frontal medial e hipocampo (Charmandari *et al.*, 2003).

1.2 Estresse e período pré-pubere

Períodos de desenvolvimento são caracterizados por um intenso remodelamento cerebral e intervenções durante esses períodos podem perdurar a longo-prazo. A pré-puberdade corresponde dos 21° ao 30° dias de vida pós-natal (Eiland & Romeo, 2013) e é considerado um período sensível do desenvolvimento. Durante este período há intensa maturação de circuitos neuronais que controlam a homeostase energética e as respostas ao estresse (McCormick & Mathews, 2007), processos acompanhados por um grande desenvolvimento emocional e crescimento físico. Algumas estruturas cerebrais, como o córtex pré- frontal estão em plena maturação durante a pré-puberdade o que as confere uma grande vulnerabilidade ao estresse (Gogtay *et al.*, 2004; Buwalda *et al.*, 2011).

A exposição a estressores durante essa fase do desenvolvimento pode ter efeitos a longo prazo sobre aspectos emocionais, comportamentais, metabólicos, de crescimento, reprodutivos e imunológicos que podem ser irreversíveis (Pervanidou & Chrousos, 2012). Sabe-se que as interações sociais são recompensadoras para roedores jovens (Panksepp & Lahvis, 2007), por outro lado, o isolamento social é considerado aversivo e por esse motivo, um potente estressor durante essa fase (Weiss *et al.*, 2004). A exposição ao isolamento social pode causar alterações comportamentais, anatômicas e neuroendócrinas que podem modificar a atividade do eixo HHA na idade adulta (Weiss *et al.*, 2004; Ferdman *et al.*, 2007) com diferentes consequências para machos e fêmeas (McCormick & Mathews, 2007; Krolow *et al.*, 2013).

1.3 Estresse e Comportamento Alimentar

Fatores internos e externos influenciam no apetite, quantidade e escolha dos alimentos (Torres & Nowson, 2007). O estresse, dentre os fatores externos, pode causar tanto um aumento quanto uma diminuição na ingestão de alimentos dependendo da duração e intensidade da exposição ao estressor (Marti *et al.*, 1994).

A preferência pelo consumo de alimentos altamente palatáveis está associada ao excesso de glicocorticoides liberados em resposta a um estímulo crônico do eixo HHA pelo estresse (Pecoraro *et al.*, 2004). O aumento da ingestão de alimentos confortantes "comfort foods" tem como função reduzir a resposta do eixo HHA ao estresse (Pecoraro *et al.*, 2004). A ativação exagerada do eixo HHA, devido a uma sensibilidade elevada a estressores está associada a obesidade e transtornos de compulsão alimentar (Gluck *et al.*, 2004). Estudos em humanos mostram que indivíduos altamente reativos ao estresse ingerem mais calorias e de forma compulsiva (Epel *et al.*, 2001; Freeman & Gil, 2004). Nessa mesma linha, camundongos com maior sensibilidade aos efeitos do estresse, apresentaram uma preferência pela dieta rica em gordura quando expostos ao agente estressor (Teegarden & Bale, 2008).

Os efeitos do estresse sobre o consumo de alimentos altamente calóricos podem ser sexo- específicos (Zylan & Brown, 1996; Liang *et al.*, 2007). Estudos do nosso grupo de pesquisa mostraram que ratos machos e fêmeas submetidos a um estresse crônico com acesso a chocolate foram diferentemente afetados. O consumo de chocolate preveniu o aumento do peso relativo da glândula adrenal, causado pela exposição ao estresse crônico, somente nas fêmeas mostrando uma inibição da atividade do eixo (Fachin *et al.*, 2008). A influência dos hormônios sexuais sobre as respostas do eixo HHA ao estresse (Young & Altemus, 2004; Liang *et al.*, 2007), o tipo, a intesidade

e a duração do estressor podem influenciar na diferença de comportamento em resposta ao estresse (Liang *et al.*, 2007).

O tipo de dieta consumida influencia diferentemente a atividade do eixo HHA. Dietas palatáveis, ricas em carboidrato e gordura, parecem reduzir a atividade do eixo HHA frente ao estressor crônico., (Pecoraro *et al.*, 2004) entretanto, a exposição contínua à dietas ricas em gordura realçam os níveis de glicocorticoides basais e os níveis induzidos pelo estresse, agindo como um fator estressor (Tannenbaum *et al.*, 1997). O aumento do consumo de dietas ricas em gordura como resposta ao estrsse está dietamente associado ao aumento do ganho de peso e da gordura abdominal, com indução da obesidade (Hariri & Thibault, 2010) e resistência à insulina e leptina, fatores associados à síndrome metabólica (Park *et al.*, 2005; Morrison *et al.*, 2009) . Além disso, como as dietas ricas em gordura exacerbam a respostas do eixo HHA, o consumo crônico dessas dietas estão relacionados ao aumento da ansiedade e de comportamentos do tipo depressivo (Sharma & Fulton, 2013).

1.4 Exposição ao estresse e a uma dieta rica em gordura - associação com o comportamento do tipo depressivo.

A depressão é uma das mais prevalentes patologias psiquiátricas e fatores genéticos (Heim & Nemeroff, 2001) e ambientais (Schmidt *et al.*, 2010) podem contribuir para o seu estabalecimento (Kendler *et al.*, 1995). O estresse é um dos principais fatores ambientais de risco para o desenvolvimento da depressão (Belmaker & Agam, 2008) e alguns estímulos estressores podem facilitar o desenvolvimento da depressão em indivíduos predispostos geneticamente a esta patologia (Fava & Kendler, 2000; Heim & Nemeroff, 2001; Caspi *et al.*, 2003). Transtornos psiquiátricas estão

relacionados com mudanças no eixo HHA (Juruena *et al.*, 2004). Pacientes depressivos apresentam uma atividade aumentada do eixo HHA e do sistema nervoso simpático. A hipersecreção de cortisol, que em parte é devido a um prejuízo na retroalimentação negativa do eixo HHA, que pode ser atribuída a uma resistência aos glicocrticoides (Juruena *et al.*, 2004; Boyle *et al.*, 2005; Pariante, 2009). Dados têm sugerido que a resistência aos glicocorticoides na depressão pode ser ocasionada por uma diminuição na expressão ou na função dos receptores de glicocorticoides (Juruena *et al.*, 2004; Boyle *et al.*, 2005; Pariante, 2009). Esta resistência aos glicocorticoides pode levar a uma ativação de rotas inflamatórias (Zunszain *et al.*, 2013) que, aprecem estar relacionadas com a patofisiologia da depressão (Tagliari *et al.*, 2011), já que pacientes depressivos apresentam elevados níveis de citocinas pró-inflamatórias (Howren *et al.*, 2009).

Estudos em animais e em humanos sugerem que o estresse durante fases precoces do desenvolvimento pode induzir alterações persistentes na capacidade do eixo HHA em responder ao estresse na vida adulta. Estas alterações podem causar uma maior susceptibilidade ao desenvolvimento de alterações psiquiátricas, como a depressão (Glover & O'Connor, 2002). Na infância, um estressor, como o isolamento social, no pode influenciar no desenvolvimento da depressão na idade adulta (Weiss *et al.*, 2004).

O aumento da disponibilidade e do consumo de alimentos altamente calóricos são fatores que contribuem para a obesidade (Shin *et al.*, 2010), que por sua vez está positivamente correlacionada com o alto risco de desenvolvimento da depressão (Dong *et al.*, 2004). De fato, dietas hiperlipídicas induzem obesidade em animais e em humanos (Hariri & Thibault, 2010) e também podem contribuir para o estabelecimento da depressão devido a sua característica de exacerbar a atividade do eixo HHA (Akbaraly *et al.*, 2009; Sharma & Fulton, 2013).

1.5 Exposição ao estresse e a uma dieta rica em gordura– associação com a inflamação.

Em condições normais, os glicocorticoies são considerados anti-inflamatórios, imunosupressores e imunomoduladores (Sorrells & Sapolsky, 2007). Para exercer estas funções os GC precisam se ligar aos seus receptores (RG). Os RG encontram-se na forma inativa no citoplasma, ligados a um complexo de proteínas chaperonas. Após a ligação GC-RG os receptores de glicocorticoides são ativados e se dissociam do complexo de proteínas de choque térmico, sofrem uma modificação conformacional e translocam pra o núcleo. No núcleo, os RG se dimerizam e interagem com elementos responsivos no DNA ou fatores de transcrição como o fator nuclear- kappa B (NF-kB), inativando rotas pró-inflamatórias (Zunszain *et al.*, 2013). Contudo em situações de estresse (Pace *et al.*, 2007; Cohen *et al.*, 2012) e na depressão (Juruena *et al.*, 2004), falhas no funcionamento dessa resposta e a diminuição da expressão de RG, resultando na resistência aos GCs, induz a ativação de rotas inflamatórias (Pace *et al.*, 2007), que se reflete em uma maior liberação de citocinas. As citocinas inflamatórias, por sua vez podem prejudicar o funcionamento dos RG assim como diminuir sua expressão (Pace *et al.*, 2007).

Pacientes que sofreram eventos estressores durante a infância são mais propensos a desenvolverem distúrbios neuroendócrinos e inflamatórios que podem antecipar o desenvolvimento de um fenótipo inflamatório na idade adulta (Chida *et al.*, 2007).

A ativação de fatores pró-inflamatórios está intimamente relacionada com a obesidade tanto em humanos quanto em animais. Dietas hiperlipídicas estimulam a atividade do eixo HHA elevando os níveis de glicocorticóides, um dos possíveis mecanismos de indução da obesidade (Shin *et al.*, 2010). Na obesidade, a liberação de

citocinas pró-inflamatórias (Bullo *et al.*, 2003; Weisberg *et al.*, 2003) pode alterar a função dos RG diminuindo sua sensibilidade aos glicocorticoides formando assim um ciclo vicioso que está relacionado a diversas patologias. O tecido adiposo produz várias proteínas inflamatórias (adipocinas) envolvidas no balanço energético, metabolismo de lipídeos, pressão sanguínea, homeostase e angiogênese (Trayhurn, 2005). Entre essas adipocinas estão incluídas a adiponectina e a leptina, que além dos seus papéis na inflamação, agem no cérebro regulando o balanço energético (Henry & Clarke, 2008).

1.6 A relação da Inflamação com a depressão

Dados sugerem que a ativação do sistema imunológico com a liberação de citocinas pró-inflamatórias são fatores associados à patofisiologia da depressão maior (Pace & Miller, 2009). Pacientes com depressão maior apresentam níveis aumentados de marcadores inflamatórios principalmente de citocinas como IL-1, IL-6 e TNF-α e também exibem um aumento nas respostas imunes induzidas pelo estresse incluindo aumento na ligação do NF-kB ao DNA (Pace & Miller, 2009).

A hiperativação do eixo HHA associada ao aumento dos glicocorticoides circulantes e a resistência a esses hormônios são fatores que estão envolvidos na depressão (Juruena *et al.*, 2004; Boyle *et al.*, 2005; Pariante, 2009). A resistência aos glicocorticoides devido ou a uma diminuição da quantidade ou da função dos RG impede a inibição das rotas inflamatórias, aumentando assim, a liberação de citocinas inflamatórias (Raison & Miller, 2003). As citocinas, por sua vez, influenciam diminuindo a função e a expressão dos RG (Pace *et al.*, 2007) formando um ciclo vicioso.

2. OBJETIVOS

2. 1 Objetivo geral

Avaliar a influência da exposição ao estresse por isolamento social no período pré-púbere associado ao acesso crônico a uma dieta rica em gordura sobre o comportamento do tipo depressivo, alterações metabólicas e sobre parâmetros inflamatórios que podem estar relacionados com o comportamento do tipo depressivo considerando diferenças sexo-específicas para todos os parâmetros analisados.

2. 2 Objetivos específicos

- Analisar os efeitos da exposição a um estressor sub-agudo (isolamento) no período pré-púbere associado ao consumo crônico de uma dieta rica em gordura sobre o peso corporal e sobre o consumo calórico total (dieta rica em gordura e/ou de ração padrão) da pré-puberdade até a idade adulta em ratos machos e fêmeas;

- Verificar os efeitos da exposição a um estressor sub-agudo (isolamento) no período pré-púbere associado ao consumo crônico de uma dieta rica em gordura sobre a porcentagem de calorias provindas apenas da dieta rica em gordura e sobre a eficiência calórica da pré-puberdade até a idade adulta em ratos machos e fêmeas;

- Avaliar os efeitos da exposição a um estressor sub-agudo (isolamento) no período pré-púbere associado ao consumo crônico de uma dieta rica em gordura sobre a deposição de gordura abdominal e sobre hormônios (leptina e adiponectina) na idade adulta em ratos machos e fêmeas;

- Verificar os efeitos da exposição a um estressor sub-agudo (isolamento) no período pré-púbere associado ao consumo crônico de uma dieta rica em gordura

sobre o comportamento na tarefa do nado forçado, avaliando o comportamento do tipo depressivo na idade adulta em ratos machos e fêmeas;

- Analisar os efeitos da exposição a um estressor sub-agudo (isolamento) no período pré-púbere associado ao consumo crônico de uma dieta rica em gordura sobre parâmetros inflamatórios que possam estar relacionados com o comportamento do tipo depressivo em ratos machos e fêmeas.

3. MATERIAIS, MÉTODOS E RESULTADOS

O material e métodos e resultados dessa dissertação estão apresentados a seguir, da seguinte forma:

- Capítulo 1: Artigo a ser submetido para publicação na revista *Appetite.*

3.1 Capítulo 1

DEPRESSIVE-LIKE BEHAVIOR AND METABOLIC CHANGES AFTER PREPUBERTAL STRESS EXPOSURE WITH CHRONIC ACCESS TO A HIGH FAT DIET

Artigo a ser submetido para publicação na revista *Appetite*.

DEPRESSIVE-LIKE BEHAVIOR AND METABOLIC CHANGES AFTER PREPUBERTAL STRESS EXPOSURE WITH CHRONIC ACCESS TO A HIGH FAT DIET

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Abstract

The prepubertal period is crucial to the final maturation of neuronal circuits that control energy homeostasis and stress responses and exposure to environmental factors such as stress and / or high-fat diets at this point of development can have long term effects on emotion, behavior and metabolism. The aim of this study was to investigate the effects of isolation stress during the prepubertal period in male and female rats with or without a chronic high-fat diet and its long-term effects on parameters related to food consumption, abdominal fat and on hormones such as adiponectin and leptin. Furthermore, our study investigated whether exposure to stress during the prepubertal with or without access to a high- fat diet (HFD) would lead to a depressive-like behavior and whether this behavior would be related to inflammatory parameters. Caloric efficiency was reduced in animals with access to HFD during the isolation period, but during the following weeks they showed increased caloric efficiency. HFD increased leptin levels as well as adiponectin levels. Additionally, stressed animals and those receiving HFD exhibited depressive-like behavior, which was not associated with inflammatory parameters. These results suggest that interventions such as exposure to stress during the prepubertal period or access to a HFD may program metabolism in a sex - specific manner, and lead to a depressive-like behavior in the long term.

Key-words: High-fat diet; Isolation stress; Pre- pubertal period; Depression; Sex-differences.

Abbreviations: HFD - high fat diet., HPA - hypothalamic–pituitary–adrenal ., GCs – glucocorticoids., GR - glucocorticoid receptors., IL-1 - interleukin -1., IL-6 - interleukin-6., TNF-α - tumor necrosis factor-alpha., NF-kB - factor nuclear kappa B., PND – postnatal day., FST - forced swim test

1. Introduction

The growing epidemy of obesity in childhood and adolescence is a major challenge for public health in the 21st century (Ogden *et al.*, 2012; Wang & Lim, 2012). The rapid increase in the prevalence of obesity in youth is attributed to a modern "obesogenic environment" (Kaur *et al.*, 2003), in which the consumption of high-fat diets (HFD) (Stein & Colditz, 2004; Hariri & Thibault, 2010) and the exposure to stress are highly involved (Huneault *et al.*, 2011). Additionally, the development of chronic diseases and depression has been atributed to obesity in children (Renders *et al.*, 2004).

Environmental factors, such as consumption of palatable diets and exposure to stress during the prepubertal period, can program the animal metabolism during the development in a sex-specific manner (Krolow *et al.*, 2013). The prepubertal period (immediately prior to the onset of puberty) is critical for development, and to the maturation of neuronal circuits that control energy homeostasis and stress responses (McCormick & Mathews, 2007). During this period, growth and changes leading to sexual maturity occur, accompanied by emotional developmental and great brain plasticity (McCormick & Mathews, 2007; Pervanidou & Chrousos, 2012). Therefore, exposure to stressors at this point of development can have long term effects on emotion, behavior, and metabolism (Pervanidou & Chrousos, 2012). An important stressor for rodents during the prepubertal period is the social environment (Panksepp *et al.*, 2007). Social interactions are considered rewarding to rodents (Douglas *et al.*, 2003; Douglas *et al.*, 2004; Panksepp & Lahvis, 2007) and social isolation is considered aversive.

The physiological responses to situations of stress involves the activation of the sympatho-adrenal system, leading to release of catecholamines, and activation of the

HPA axis, leading to glucocorticoids (GCs) release (Kvetnansky *et al.*, 1995). The GCs, through glucocorticoid receptors (GR), regulate the activity of the HPA axis in the hippocampus and medial prefrontal cortex by negative feedback (Charmandari *et al.*, 2005; McEwen, 2008). A reduction in GR in these structures may cause resistance to GCs and a deficiency in the negative feedback of the HPA axis. Studies have shown that, as a result of this resistance, excess of circulating GCs may be directly related to depression (Carvalho & Pariante, 2008).

Metabolic responses to stress include long-term fat accumulation, particulary visceral adipose tissue, arterial hypertension, metabolic syndrome, type 2 diabetes mellitus (Pervanidou & Chrousos, 2012), inflammatory processes (Black, 2003), episodes of depression (Kendler *et al.*, 1999), and changes in feeding behavior (Ely *et al.*, 1997). In fact, animal and human studies have shown that stress can cause both increases and decreases in food intake (Ely *et al.*, 1997; Silveira *et al.*, 2000; Pecoraro *et al.*, 2004; Groesz *et al.*, 2012). Of particular importance is the association of increased GC levels in response to stress in rats and higher consumption of "comfort foods" (sucrose and fat) (Epel *et al.*, 2001; Dallman *et al.*, 2003; Dallman *et al.*, 2005). In rats, especially the chronic consumption of high-fat diets increase neuroendocrine stress responses resulting in increased levels of circulating corticosterone (Tannenbaum *et al.*, 1997).

Glucocorticoids are known to have anti-inflammatory effects, and these effects are thought to occur through their action on factors involved in the regulation of cytokines and other immune responses, such as NF-kB (Revollo & Cidlowski, 2009). On the other hand, high GCs circulating levels can activate inflammatory pathways with the release of inflammatory cytokines (Zunszain *et al.*, 2013). Patients with major depression have increased concentrations of IL-1, IL-6 and TNF-α (Raison *et al.*, 2006).

Considering that the effects of stressors in childhood may be sex- specific and that many psychopathologies have a different prevalence in men and women, and considering the increased consumption of HFD in the prepubertal period, including its possible consumption as a comfort food, the aims of the present study are to evaluate effects of isolation stress during early life in males and females with or without chronic exposure to a high fat diet on body weight, caloric consumption and abdominal fat, as well as on hormones from the adipose tissue. Additionally, we investigate if these factors, HFD and isolation stress, will lead to a depressive-like behavior and altered signaling related to inflammatory processes in prefrontal cortex.

2. Materials and Methods

2.1. Subjects

All animal proceedings were performed in strict accordance to the recommendations of the Brazilian Society for Neurosciences (SBNeC), Brazilian Law on the use of animals (Federal Law 11.794/2008) and were approved by the Institutional Ethical Committee (CEUA-UFRGS 23467).

Wistar rats were housed in Plexiglas cages (65 x 25 x 15 cm) with the floor covered with sawdust and maintained on a standard 12h dark/light cycle (lights on between 7:00h and 19:00h), temperature of 22 ± 2°C. At postnatal day (PND) 21, males and females were weaned and separated according to sex. Half of the animals were housed in standard cages in groups of 3 to 5 animals (control); the other animals were submitted to stress by social isolation (isolated in a smaller home cage, 27x17x12 cm). Only one male and one female per litter were used in each group. Animals were weighed and then subdivided according to the diet offered: (a) standard lab chow; (b)

both standard chow and HFD. These last animals were free to choose between standard chow and HFD. Therefore, four groups of each sex were obtained: (1) controls + standard chow (17 males and 18 females); (2) controls + standard chow and high-fat diet (16 males and 15 females); (3) isolated + standard chow (16 males and 17 females) and (4) isolated + standard chow and HFD (13 males and 15 females). During 40 days, beginning on PND 21, both HFD and standard chow were offered *ad libitum*, according to the groups, and the daily consumption was measured for each diet. Isolation stress was performed between PND 21 to 28. On PND 28, isolated animals were returned to regular home cages in groups of three to five.

2.2 Diets

The nutritional compositions of standard lab chow and high-fat diet used are displayed in Table 1. The HFD was enriched with fat (42%) from lard and soy oil. In addition, the diet contained vitamins and a salt mixture, purified soy protein, methionine, lysine and starch (Ziegler *et al.*, 2002). This ratio soy oil/lard has larger amounts of saturated and monounsaturated fatty acids, to reproduce the consumption of fat in western diets.

2.3. Evaluation of food consumption

Predetermined equal amounts of standard lab chow and HFD were offered to animals and the remaining pellets were removed from cages and weighed. Food consumption was measured per cage and the amount of food consumed was divided by the number of animals per cage to determine mean consumption. To verify the amount of kilocalories consumed, the amount of food ingested was multiplied by the caloric

content per gram of chow or diet. Standard lab chow had a caloric content of 3.24 kcal/g and HFD had a caloric content of 5.8 kcal/g (79% more caloric than standard chow).

2.4. Abdominal fat and adrenal gland dissection

At PND 60-62, part of the animals were killed by decapitation around 13:00 h, after 6h of fasting. The two major portions of abdominal fat (gonadal and retroperitoneal adipose tissue depots) and adrenal glands were carefully dissected and weighed. Adrenal weight is expressed in relation to the body weight. Results from abdominal fat are shown in grams.

2.5 Biochemical analysis

2.5.1 Blood sampling, plasma assays and prefrontal cortex

The animals were killed by decapitation and trunk blood was collected in heparinized tubes on ice for leptin and adiponectin determination. Plasma was separated and frozen at -80°C. The brains were quickly dissected on ice to remove prefrontal cortex, that were immediately stored at -80°C until use.

2.5.2 Plasma levels of leptin and adiponectin

Plasma leptin was measured by Rat Leptin Elisa assay, Kit Invitrogen, (nº cat KRC2281) and adiponectin was measured by Rat Adiponectin Elisa, Kit Abcam, (nº cat ab108784).

2.5.3. Quantification of cytokines

Prefrontal cortex was homogenized 1:10 (w:v) with buffer containing 1mM EDTA, 1 % Triton X-100, 10mM Tris-HCl and 1% protease inhibitor cocktail (PIC), pH 7.4. Samples were then centrifuged at 12.000 g for 25 min at 4°C and the supernatant was separated. Simultaneous quantification of IL-10 and TNF was achieved by the cytometry based method Cytometric Bead Array®. Specific functional beads were assembled by conjugation of BD OptEIA® antibodies against IL-10 (nº cat. 555134) and TNF (nº Cat. 558535) to BD beads (nº cat. 558585 and 558586), following manufacturer instructions. Samples were analyzed on ACCURI C6 flow cytometer and data from 300 events were acquired by each bead type. Flow cytometry acquisitions and analyses were performed using BD FCAParray 3.0.

2.5.4. Quantification of Interleukin -1

Interleukin-1 levels in prefrontal cortex were measure by ELISA (eBiosciences nº cat. 88-6010) according to the manufacturer instructions.

2.5.5. Western Blot Analysis

2.5.5.1 Immunoblotting to glucocorticoid receptor (GR)

Prefrontal cortex was homogenized in ice-cold lysis buffer pH 7.9 containing 2.5M KCl, 10mM HEPES, 0.6mM EDTA, 0.4 % SDS and 1% protease inhibitor cocktail (PIC). Homogenized samples were centrifuged at 1.000g/10min/4°C and the supernatant (cytosolic fraction) was used. Protein was measured by Lowry modified

according to (Peterson, 1979) with bovine serum albumin as the standard. Equal protein concentrations (60 μg/lane) were loaded onto 8% polyacrylamide gels, analyzed by SDS-PAGE and transferred (MiniVE Electrophoresis System Amersham Biosciences) to nitrocellulose membranes (1 h at 25V in transfer buffer containing 48 mM Trizma, 39 mM glycine, 20% methanol, and 0.25% SDS (Valentim *et al.*, 2001). The blot was then washed for 10 min in Tris-buffered saline (TBS) (0.5 M NaCl, 20 mM Trizma, pH 7.5), followed by 2 h incubation in blocking solution with 5% powdered milk in Tris-buffered saline plus 0.1% Tween-20. Membranes were incubated overnight at 4 °C in TBS-T (TBS plus 0.1% Triton X-100) solution containing the primary antibody: anti-glucocorticoid receptor (1:500; Sigma-Aldrich), followed by 2h incubation with peroxidase-conjugated anti-rabbit IgG secondary antibody (1:1000, Sigma-Aldrich) at room temperature. β-actin (1:1000; Sigma -Aldrich) antibody was used as control. Membranes were developed using a chemiluminescence ECL kit (Amersham, Oakville, Ontario). Chemiluminescence band were detected using X-ray films and densitometry analyzes were performed using the Image J® Software.

2.5.5.2. Immunoblotting to NF-kB

Prefrontal cortex was homogenized in ice-cold lysis buffer pH 7.9 containing 10 mM KCl, 10 mM Hepes, 1mM EDTA, 1.5mM MgCl, 1 mM dithiothreitol and 1 mM phenylmethanesulfonyl fluoride (PMSF). A detergent IGEPAL 10% was then added and the homogenate were left on ice 15 min and centrifuged at 20800g/30s/4°C. The pellet was resuspended with buffer pH 7.9 containing 20mM Hepes, 1.5mM MgCl2, 400mM NaCl, 0.25mM EDTA, 25% glycerol, 1 mM dithiothreitol and 0.5mM PMSF and after 40 min of intermittent mixing, the material was centrifuged at 12.000g /10min.

This pellet (nuclear fraction) was resuspended, protein concentration was determined using the method described by Bradford (Bradford, 1976). Proteins (18 μg) were separated by SDS-PAGE on 10% (w/v) acrylamide, 0.275% (w/v) bisacrylamide gels and electrotransfered onto nitrocellulose membranes. Membranes were incubated in TBS-T (20 mmol/L Tris–HCl, pH 7.5, 137 mmol/L NaCl, 0.05% (v/v) Tween 20) containing 5% (w/v) albumine. Membranes were incubated overnight at 4 °C TBS-T solution containing the primary antibody: anti- NF-kB (1: 5.000; Cell Signaling Technology) rinsed with TBS-T and exposed to horseradish peroxidase-linked anti-IgG antibodies (1:5.000; Cell Signaling Technology) for 2 h at room temperature. Lamin B2 was used as control. Chemiluminescent bands were detected using X-ray films, and densitometry analyses were performed using Image-J® software.

2.6. Forced Swimming Test

The forced swim test (FST) was based on the original procedures of Porsolt et al. (1977) with some modifications. The test is used to assess depressive-like behavior. At PND 60, animals (7-12 / group) were placed in the test environment room for 30 min for habituation. Afterwards, rats were placed individually in Plexiglas cylinders (height of 50cm, diameter of 20cm) filled with water (25 $\pm$ 1°C) to a depth of 33 cm for 15 min. During the swimming session, immobility and swimming time was measured in blocks of 5 min. The swimming time was considered computing escape behaviors, such as diving, circling the cylinder and clambering at the walls. Immobility or floating was considered when animals stayed immobile without fight and making only movements to keep the head above the water. Increased immobility time indicates that the animal may be prone to a depressive-like behavior (Leussis & Andersen, 2008).

2.7 Statistical Analysis

Data are expressed as mean ± SE of the mean and analyzed using three-way ANOVA, with *isolation stress, diet* and *sex* as factors. For body weight and caloric intake, repeated measures ANOVA were used (the within subjects factor was *time*; the between subjects factors were *stress, diet* and *sex*). With regard to repeated measures ANOVA, the Greenhouse-Greisser correction was applied when necessary, considering the violation of the sphericity assumption, as shown by the Mauchly´s Test. All analyses were performed using SPSS software and a $P \leq 0.05$ was considered significant.

3. Results

3.1 Body weight and food consumption

Caloric consumption and body weight gain were analyzed during the first week of treatment, when the animals were subjected to isolation and until PND 60. In relation to body weight gain during PND 21-28 (period of isolation stress), a three-way ANOVA showed an effect of *sex* [F (1,118) = 7. 9, P= 0.006], and an interaction between *stress* and *diet*, [F (1,118) = 4.0, P=0.048] (Figure 1A), since isolated male animals receiving lab chow had lower weight gain than controls, while those with access to high fat diet had increased weight gain. With regard to the caloric consumption during the first week (PND 21-28), animals showed increased consumption over time [F(4.77, 338.89)=35.18, P<0.001] and there was an interaction between *time* and *diet* [F(4.77, 338.89) = 5.41, P< 0.001], since animals with access to the high-fat diet consumed more calories from the beginning than those receiving only standard lab chow and this consumption tends to reach similar values between the

groups as the time goes by. Additionally, the *diet* main effect indicated that animals with access to HFD had increased caloric intake [three-way ANOVA; F (1, 71) = 11.91, P=0.001], and the same effect was observed for exposure to isolation stress [three-way ANOVA; F (1, 71) = 7.06, P = 0.01] (Figure 1C). Besides, since the animals receiving HFD had free access to both HFD diet and lab chow, the percentage of calories from HFD consumed during the isolation period was evaluated. We observed an effect of *stress* [three-way ANOVA; [F (1, 32) = 10.92, P = 0.002], since both males and females exposed to isolation stress had a greater increase of the percentage of calories from HFD, suggesting that the increased consumption induced by stress was mainly from an increase in the consumption of HFD (data not show). In addition, the caloric efficiency (ratio of body weight gain divided by caloric intake) in the first week decreased in animals with access to HFD [F (1, 70) = 5.18, P = 0.026] (Figure 1B).

At PND 28, isolated animals were returned to groups. Until PND 60 the mean caloric consumption and the body weight gain was calculated and analyzed using repeated measures ANOVA. The body weight gain from PND 28 to 60 increased over time [F (2.55, 301.73) = 6227.26, P < 0.001], with interactions between *time* and *sex* [F (2.55, 301.73) = 470.19, P< 0.001] and between *time* and *diet* [F (2.55, 301.73) = 5.39, P= 0.002]. There was a *sex* effect on body weight gain [F (1,118) = 363.80, P< 0.001] (Figure 2A), as expected. Regarding caloric consumption from PND 28 to 60, animals showed increased consumption over time [F(4,112)= 81.92, P<0.001], and there was an interaction between *time* and *diet* [F(4,112)= 4.78, P= 0.001], and an interaction between *time* and *sex* [F(4,112)= 24.83, P<0.001]. The ANOVA showed a *sex* effect [F (1, 28) =95.61, P<0.001], since males had greater increase in caloric consumption than females (Figure 2C). Considering the percentage of HFD consumed, no effect was detected between groups (P>0.05, data not show). Caloric efficiency between PND 28

and 60 was increased by access to HFD [F (1, 28) =7.37, P=0.011]. There was also a *sex* main effect [F (1, 28) =63.58, P<0.001], since females had lower caloric efficiency than males (Figure 2B).

3.2 Abdominal fat and adrenal weight

Adrenal gland weight and fat deposition were analyzed in adult male and female rats (Table 2). Regarding the relative weight of adrenal glands, it was observed a significant effect of *sex* [F (1, 70) = 42.18, P< 0.001], with females presenting higher relative weight of adrenals than males. There was an effect of *diet* on gonadal fat [(F (1, 70) = 78.70, P< 0.001]. For retroperitoneal fat deposition, an interaction between *diet* and *sex* was detected [F (1, 70) = 11.86, P= 0.001].

3.3 Basal plasma levels of leptin and adiponectin during adulthood

Both plasma leptin and adiponectin levels were increased by access to HFD [leptin: F (1, 40) = 39.21, P< 0.001; adiponectin: F (1, 34) = 7.89, P= 0.008]. A main effect of *sex* was also detected on both hormones [leptin: F (1, 40) = 11.53, P= 0.002; adiponectin: F (1, 34) = 4.95, P= 0.033] (Table 3).

3.5. Forced Swimming Test

During test exposure, animals showed increased immobility over time [F (1.83, 124.83) = 528.89; P<0.001] with main effects of *stress* [F (1, 68) = 4. 10; P= 0.047] and *diet* [F (1, 68) = 6.30; P 0.014], both increasing immobility (Figure 3A males and 3B females).

3.6. Quantification of GR, NF-kB, TNF-α, and IL-1 in prefrontal cortex

There were no alterations in the immunocontent of citosolic GR (Figure 4) and nuclear NF-kB (Figure 5), as evaluated by Western Blotting analysis. Similarly, no differences were observed in the contents of TNF-α (detection range 1 pg/ml), and IL-1 (detection range 2,74pg/ml para IL-1) (measured by flow cytometry and ELISA, respectively) ($P>0.05$ for all factors) (Table 4). IL-10 levels were bellow detection range using this technique.

4. Discussion

In the current study we observed differences between the sexes regarding chronic access to HFD (animals had free choice between the HFD and standard chow), associated or not with stress by isolation during the prepubertal period. Our findings show that the exposure to isolation stress during PND 21-28 decreased body weight gain in males receiving lab chow; however, this effect was reverted when HFD was provided. Exposure to stress also increased total caloric consumption, effect also observed in animals with access to HFD. After the period of isolation, all animals receiving HFD had increased caloric efficiency. Free access to HFD lead to high abdominal fat deposition and increased circulating leptin and adiponectin levels. Both stress by isolation and chronic HFD induced a depressive-like behavior.

Previous data show that the severity, type and duration of exposure to a stressor can differently influence changes in body weight (Tannenbaum *et al.*, 1997; Harris *et al.*, 2006). There was a decrease in body weight gain in isolated males receiving lab

chow when compared to their respective controls. This result corroborates literature data obtained in adult male rats in which stressors caused body weight loss in male rats given standard lab chow, including restraint (Harris *et al.*, 2006), social defeat (Tamashiro *et al.*, 2004; Pulliam *et al.*, 2010), or chronic variable stress (Ulrich-Lai *et al.*, 2007), probably due to an imbalance between low intake and great energy expenditure during the stress period (Harris *et al.*, 2006). On the other hand, during the first week, when animals where submitted to isolation, the consumption of HFD not only attenuated the stress-related weight loss but also increased weight gain in isolated males. These stressed animals increased caloric consumption from HFD, indicating a preference for fat during isolation, fact already observed in other study using chronic variable stress (Teegarden & Bale, 2008). Our results reinforce the theory that stress conditions lead to increased consumption of comfort foods (Dallman *et al.*, 2003; Pecoraro *et al.*, 2004).

High fat diet is known to enhance both basal and stress-induced HPA axis activity (Tannenbaum *et al.*, 1997). Prolonged exposure to HFD increases basal activity of HPA axis leading to great production of GC and resulting in high endocrine response to stress, making the HFD a chronic stressor (Tannenbaum *et al.*, 1997). Glucocorticoids are the final effectors of the HPA axis and participate in the control of whole body basal homeostasis and act on the termination of stress response (McCormick *et al.*, 2010). Therefore, a vicious cycle may have occurred, in which stress conditions enhance the consumption of comfort food, as the HFD, that in turn, when chronically available, increase HPA axis activity leading to high GC production and overreacted stress response (Juruena *et al.*, 2004). Additionally, glucocorticoids are known to interfere with thyroid function and affect circulating thyroid hormone levels depending on the developmental stage at which exposure to GCs occurred (Charmandari *et al.*, 2005; Van der Geyten & Darras, 2005). It is possible that high

GCs levels somehow decreased thyroid function leading to low energy metabolism, high abdominal fat deposition and increased caloric efficiency, which was observed in the present study, mainly in adult males fed HFD.

It is known that energy expenditure per kilogram of body mass during resting conditions is greater in children than adults and varies with pubertal status (Goran *et al.*, 1995; Bitar *et al.*, 1999), probably due to multifactorial causes including growth and puberty, differences in body mass and the greater proportional amount of internal organs in children (Daniels *et al.*, 1978; Cooke *et al.*, 1991; Roemmich *et al.*, 2000). Puberty increases muscle mass, especially in boys, which explains the higher energy expenditure in boys than in girls (Bitar *et al.*, 1999). Here, observed a decrease in caloric efficiency during the first week in HFD fed rats when compared to those who receive standard lab chow, i.e. animals did not efficiently convert calories from HFD diet into body mass. In addition, studies have shown that excess consumption of the cafeteria diet increases thermogenesis, especially in young mice, functioning as an adaptive mechanism to increase caloric consumption (Rothwell & Stock, 1979). Thus, we can speculate that excessive consumption of high-fat diet may be acting similarly to the cafeteria diet, rats fed HFD can have a marked energy loss leading to less caloric efficiency.

Differently from the prepubertal period, in adulthood, HFD increased caloric efficiency in the animals and furthermore, sex-specific differences were observed as male rats showed higher caloric efficiency than females. Several studies have indicated sex differences in energy balance that can be linked to sexual hormones (Richard, 1986). In females, estrogen correlates with serum leptin levels and it is known that females are more sensitive to the effects of leptin than males (Clegg *et al.*, 2003; Clegg *et al.*, 2006). A study with females fed standard chow ad libitum show that women have

a higher proportion of active metabolically active organs per unit of body mass and therefore higher basal energy expenditure in addition to greater activation of thermogenesis compared with males (Valle *et al.*, 2005). In relation to increased thermogenesis, studies show that leptin increases energy loss through thermogenesis (Rahmouni & Morgan, 2007), suggesting that females are more sensitive to the effects of leptin than males and therefore have higher energy loss as heat and lower power consumption leading to less caloric efficiency compared to males.

Chronic consumption of HFD led to high abdominal fat deposition (retroperitoneal and gonadal fat). When chronically receiving HFD, a positive energy balance takes place with substantial portions of food energy stored as lipids, contributing to obese and metabolic syndrome development. On the other hand, adipose tissue releases a variety of adipokines, among them adiponectin and leptin, which appear to control metabolic, vascular, immune and endocrine processes (Kyrou *et al.*, 2006; Korner *et al.*, 2007; Wozniak *et al.*, 2009).

Leptin is secreted in proportion to adipose stores (Trayhurn & Bing, 2006; Badman & Flier, 2007) and is a mediator of long-term regulation of energy balance, suppressing food intake and thereby inducing weight loss (Klok *et al.*, 2007; Tomiyama *et al.*, 2012). Experiments using animal models suggest male and female brains are differently sensitive to effects of leptin (Clegg *et al.*, 2003). The consumption of HFD increased leptin levels in both male and female rats, being more pronounced in males, probably related to their high content of abdominal fat and great caloric efficiency (Arcego *et al.*, 2013). However, high levels of leptin were accompanied by high caloric consumption and body weight gain. A low sensitivity to leptin in the hypothalamus could have taken place in which high plasma leptin concentrations do not lead to reduction in food intake, probably suggesting a resistance to the effects of endogenous

leptin (Zhang & Scarpace, 2006). In contrast, females present lower increase in leptin and consumed fewer calories than males, suggesting that leptin may be acting in both food intake and energy balance (Fungfuang *et al.*, 2013). Elevated levels of leptin, a characteristic process of resistance to this adipokine, are found in obesity and metabolic syndrome (Zhang & Scarpace, 2006).

Studies in rodents showed that high adiponectin levels decreased the negative aspects of obesity, whereas low plasma levels are linked to metabolic syndrome (Badman & Flier, 2007). Interestingly, chronically consumed HFD increased plasma levels of adiponectin, an adipocitokine with anti-inflammatory, anti-atherogenic and anti-diabetic properties (Matsuzawa, 2006; Okamoto *et al.*, 2006), mainly in females, suggesting that they may be less susceptible to metabolic syndrome. Other studies have also shown that adiponectin levels in females may be increased by interventions during development, suggesting a protection by this adipocitokine in females (Krolow *et al.*, 2013).

It is important to emphasize the association of leptin and adiponectin with depression (Taylor & Macqueen, 2010). Animal and human studies shown that leptin (Lu, 2007) and adiponectin (Leo *et al.*, 2006) insufficiency are associated with depression. We observed that when submitted to stress by isolation during the prepubertal period or when feed with HFD, male and female rats showed increased time of immobility, a depressive-like behavior. When the animal stop to trying to escape by swimming and floats on the surface of the water it is considered to have "given up". An animal that gives up relatively quickly is thought to be displaying characteristics similar to human depression (Castagne *et al.*, 2009).

Regarding human studies, patients with major depression have HPA axis hyperactivity with a lower negative feedback, glucocorticoid resistance and thus elevated circulating cortisol levels (Juruena *et al.*, 2004; Boyle *et al.*, 2005; Pariante, 2006). Under normal conditions, these hormones are considered anti-inflammatory, immunosuppresive and immunomodulatory. However, in the central nervous system, high concentrations of GCs may cause inflammatory disorders (Sorrells & Sapolsky, 2007). The GCs exert their effects by binding to a cytoplasmatic glucocorticoides receptor GR, which is subsequently activated and is thus able to translocate into the nucleus, where it acts as a transcription factor to increase the expression of anti-inflammatory genes, or acts inactivating inflammatory signalling pathways, for example the nuclear factor-kappa B (NF-kB) (Revollo & Cidlowski, 2009). In stress situations, however, reduction in GR function may result in GCs resistance, enhanced activation of inflammatory pathways contributing to depressive symptoms (Pace *et al.*, 2007). Recent evidence supports the hypothesis that activation of inflammatory immune system responses have important role in the pathophysiology of depression (Tagliari *et al.*, 2011). In fact, depressed patients present impaired GCs signaling and high levels of pro-inflammatory cytokines (Howren *et al.*, 2009) and elevated risk of inflammation (Miller *et al.*, 2002). It is relevant to point out that studies have shown that saturated fat diets promote inflammation in both, periphery and the brain (van Dijk *et al.*, 2009). In addition, social isolation in early life may also contribute to risk of inflammation (Danese *et al.*, 2007). In the present study, neither cytosolic GR nor nuclear NF-kB immunocontents were affected, as well as the contents of TNF-α and IL-1. Therefore, no inflammatory mechanisms related to depressive-like behavior in animals isolated during the pre-pubertal period or in animals receiving HFD were observed. Since most studies cited above concerning stress and inflammation used chronic exposure to a

stressor, differently from our study, when the stress exposure was conducted only during seven days in the prepubertal period, these differences can be attributed to the period in which stress and the initial access to a diet with high fat content was conducted.

5. Conclusion

In conclusion, chronic consumption of HFD from the prepubertal period until adulthood caused changes in some parameters which are considered risk factors for the development of metabolic syndrome and cardiovascular disease, such as caloric efficiency, leptin levels and abdominal fat deposition. These alterations were observed mainly in male rats, suggesting that males are more susceptible to diseases and comorbidities related to HFD consumption. Chronic consumption of HFD and stress (in the prepubertal period), both *per* se, had pro-depressive effects in adult rats. However, in the present model there was no involvement of any of the evaluated inflammatory markers.

Acknowledgments

Financial support: National Research Council of Brazil (CNPq), and

580 PRONEX, CAPES, FAPERGS/CNPq 10/0018.3.

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Table 1. Nutritional composition /100g of the food used in the studies performed.

HFD: high- fat diet.

Diet	Energy (Kcal)	Total Protein (g)	Total carbohydrate (g)	Total Fat (g)
Standard chow[a]	301.2	22	44.3 (from starch)	4 (0.62 from saturated and 3.4 from unsaturated fat)
HFD[b]	588	28	25 (12.5 from starch and 12.5 from sucrose)	42 (16 from saturated and 26 from unsaturated fat)

[a] Nuvilab®

[b] Adapted from Ziegler DR, et al. A ketogenic diet increases protein phosphorylation in brain slices of rats. J Nutr 2002, 132:483-487.

Table 2. Effects of isolation stress during the prepubertal period with chronic access to HFD on absolute weights of retroperitoneal fat, gonadal fat and relative weights of adrenal glands in adult male and female rats.

Sex		Group			
		Control		Stress	
		Chow	Chow+HFD	Chow	Chow+HFD
Male	Retroperitoneal Fat	3.78 ± 0.51	7.59 ± 0.78	3.94 ± 0.65	7.87 ± 0.70
	Gonadal Fat	2.68 ± 0.29	5.20 ± 0.65	2.83 ± 0.38	4.87 ± 0.28
	Adrenal Glands	0.23 ± 0.012	0.21 ± 0.023	0.24 ± 0.009	0.22 ± 0.025
Female	Retroperitoneal Fat	1.98 ± 0.18	3.64 ± 0.42	2.1 ± 0.18	3.43 ± 0.28
	Gonadal Fat	2.53 ± 0.19	5.2 ± 0.54	3.21 ± 0.19	5.63 ± 0.39
	Adrenal Glands	0.43 ± 0.05	0.38 ± 0.032	0.37 ± 0.025	0.44 ± 0.074

Adrenal weight is expressed in relation to the body weight of each rat (mg tissue/ g of body weight) and fat deposition is shown in grams. There was an interaction between diet and sex on retroperitoneal fat ($P=0.001$); effect of diet on gonadal fat ($P <0.001$) and effect of sex on adrenal glands ($P<0.001$). Data are expressed as mean ± S.E.M., N=7-12 / group.

Table 3. Effects of isolation stress during the prepuberal period with chronic access to HFD on plasma adiponectin (µg/mL) and leptin (pg/mL) levels in adult male and female rats.

Sex		Group			
		Contol		Stress	
		Chow	Chow+HFD	Chow	Chow+HFD
Male	Leptin	1420.10 ± 164.74	3670.17 ± 703.45	1435.35 ± 140.16	3996.87 ± 566.07
	Adiponectin	6.44 ± 0.63	7.21 ± 0.82	6.78 ± 0.51	7.6 ± 0.71
Female	Leptin	796.32 ± 90.67	2607.50 ± 503.81	960.15 ± 159.60	2001.93 ± 385.14
	Adiponectin	6.79 ± 0.73	9.60 ± 1.21	7.64 ± 1.14	8.17 ± 1.15

Leptin is expressed as pg/mL and Adiponectin is expressed as µg/mL. Three-way ANOVA showed an effect of diet ($P < 0.001$) and an effect of sex ($P = 0.002$) on leptin levels; an effect of diet ($P = 0.008$) and an effect of sex ($P = 0.033$) on adiponectin levels. Data are expressed as mean ± SEM, N = 5-7/ group to leptin and N=6-8/group for adiponectin.

Table 4. Effects of isolation stress during prepubertal period with chronic access to HFD on cytokines TNF-α (pg/mL) and IL-1(pg/mg).

Sex		Group			
		Control		Stress	
		Chow	Chow+HFD	Chow	Chow+HFD
Male	TNF-α	2.70 ± 0.71	1.27 ± 0.56	1.53 ± 0.82	0.95 ± 0.39
	IL-1	15.31 ± 2.11	19.06 ± 6.00	21.71 ± 6.96	15.50 ± 2.59
Female	TNF-α	1.85 ± 1.27	1.98 ± 0.54	1.85 ± 0.76	1.09 ± 0.78
	IL-1	22.18 ± 8.67	20.69 ± 5.99	15.36 ± 4.07	16.27 ± 3.53

There was no differences in the contents of TNF-α, and IL-1 ($P>0.05$). Data are expressed as mean ± SEM, N=4-5/group.

Legends to Figures

Figure 1 Effect of isolation stress during the prepubertal period with chronic access to HFD. **A.** Effect on body weight gain during the period of stress isolation (first week). Three-way ANOVA showed a main effect of sex (P= 0.006) and interaction between stress and diet (P = 0.048). Data are expressed as mean ± SEM, N= 13-18/group**. B.** Effect on caloric efficiency during the period of stress isolation (first week) [weight gained (grams)/kilocalorie ingested]. Three-way ANOVA showed a significant effect of diet (P= 0.026). Data are expressed as mean ± SEM, N=13-18/group. **C.** Effect on caloric consumption during the period of stress isolation (first week). During first week animals had increased caloric consumption over time (repeated measures ANOVA, P <0.001), with an interaction between time and diet (rats with access to HFD had more caloric consumption over time, P<0.001). Three-way ANOVA showed main effect of diet (P= 0.001) and of stress (P= 0. 01). Data are expressed as mean ± SEM, N= 13-18/group.

Figure 2 Effect of isolation stress during the prepubertal period with chronic access to HFD on body weight gain and on caloric efficiency after isolation (PND 28-60). **A.** Body weight gain (PND 28-60). Repeated measures ANOVA showed increased in body weight gain over the time (P <0.001), an interaction between time and HFD (P =0.002) and time and sex (P< 0.001). Three-way ANOVA showed an effect of sex (P<0.001), males gained more body weight than females. Data are expressed as mean ± SEM, N=13-18/group. **B**. Caloric efficiency. Caloric efficiency was calculated by [weight

gained (grams)/kilocalorie ingested]. Three-way ANOVA showed a significant effect of diet (P= 0.01) and an effect of sex (P< 0.001). Data are expressed as mean ± SEM, N= 13-18/group. **C**. Caloric consumption. Repeated measures ANOVA showed increased in the caloric consumption over the time (P <0.001), interaction between time and HFD (P= 0.001) and between time and sex (P < 0.001). Three-way ANOVA showed an effect of sex (P <0.001), males had greater increase in caloric consumption compared with females (P < 0.001). Data are expressed as mean ± SEM, N= 13-18 / group.

Figure 3A (male rats) and **3B** (female rats). Effect of isolation stress during the prepubertal period with chronic access to HFD on Immobility in Forced Swimming Test in adult male and female rats. Repeated measures ANOVA showed increased in the immobility over the time (P <0.001). Three-way ANOVA showed effect of stress (P= 0.047) and HFD (P= 0.014).). Data are expressed as mean ± SEM, N= 7-12/group.

Figure 4 Effect of isolation stress during the prepubertal period with chronic access to HFD on glucocorticoid receptors (GR) content. Data are expressed as mean ± S.E.M. N=4-5/group. **A-1** (males) and **A-2 (**females). Representative Western Blotting showing imunocontent to GR and β-actin. B. Quantification of GR imunocontent by β-actin imunocontent. No alterations in the immunocontent of citosolic GR as evaluated.

Figure 5 Effect of isolation stress during the prepubertal period with chronic access to HFD on nuclear factor-kappa B (NF-kB) content. Data are expressed as mean ± S.E.M.

N=3/group. **A-1** (males) and **A-2** (females). Representative Western Blotting showing imunocontent to NF-kB and Lamin B2. Quantification of NF-kB imunocontent by Lamin B2 imunocontent. No alterations in the immunocontent of citosolic GR as evaluated.

Fig. 1

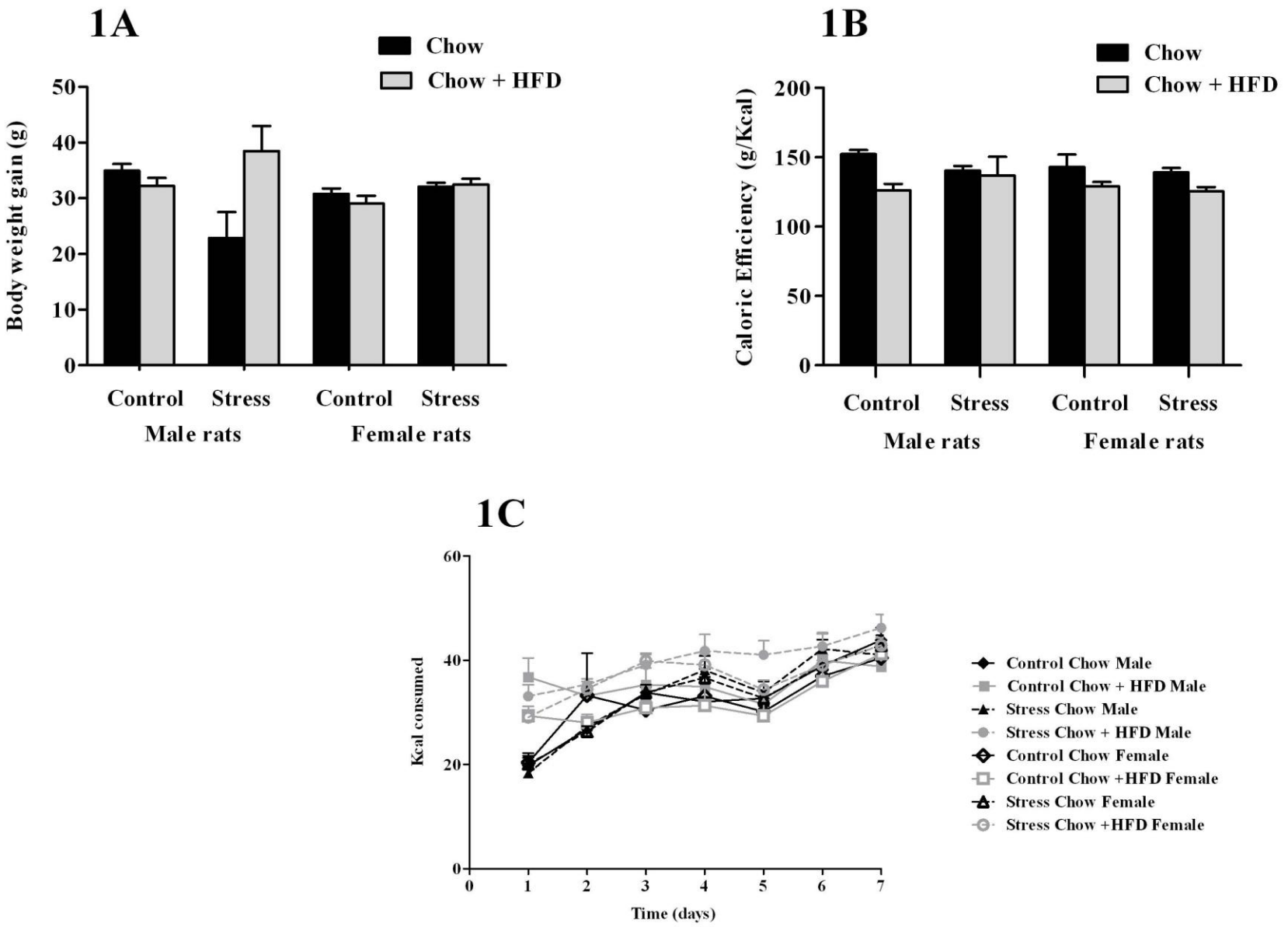
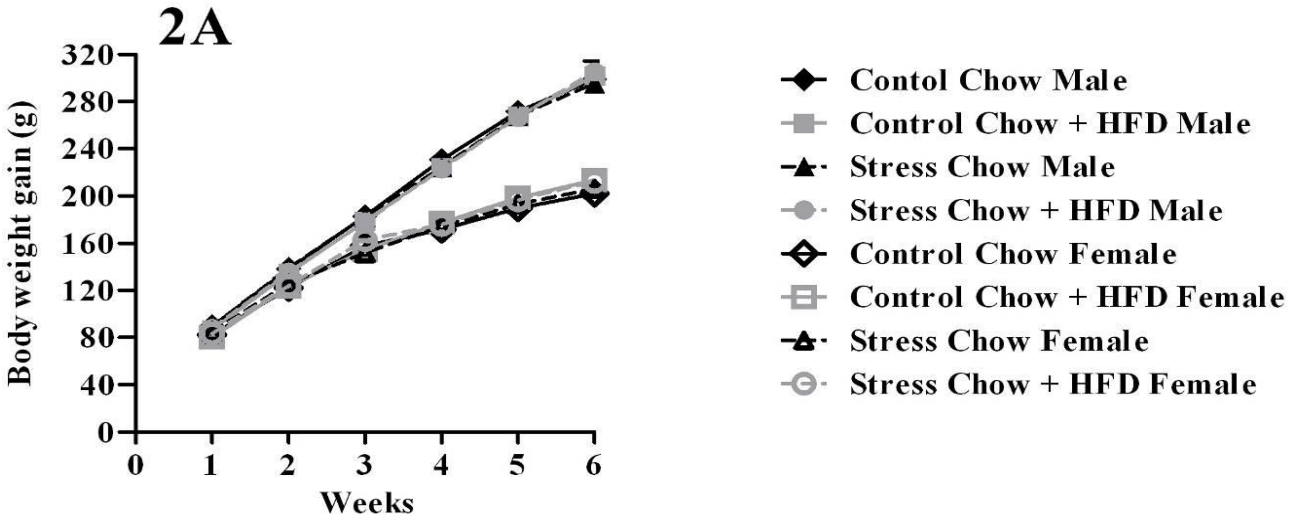
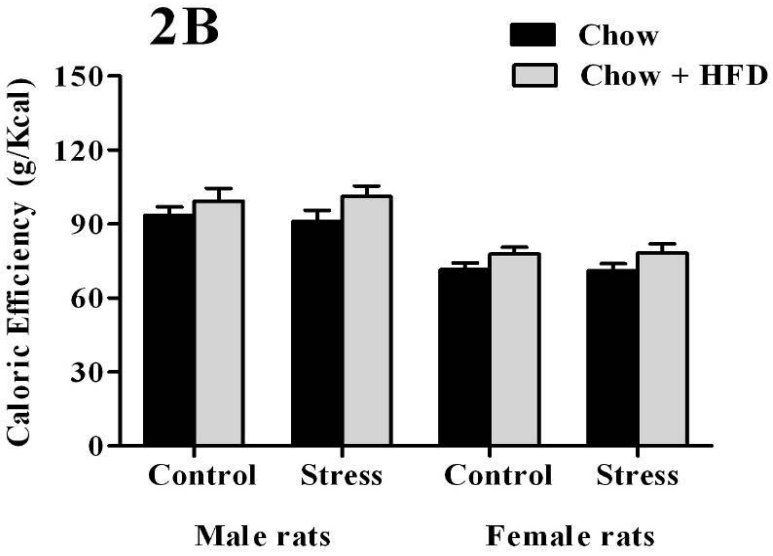

1A
Chow
Chow + HFD
Body weight gain (g)
50
40
30
20
10
0
Control
Stress
Control
Stress
Male rats
Female rats
1B
Chow
Chow + HFD
Caloric Efficiency (g/Kcal)
200
150
100
50
0
Control
Stress
Control
Stress
Male rats
Female rats
1C
Kcal consumed
60
40
20
0
0
1
2
3
4
5
6
7
Time (days)
Control Chow Male
Control Chow + HFD Male
Stress Chow Male
Stress Chow + HFD Male
Control Chow Female
Control Chow +HFD Female
Stress Chow Female
Stress Chow +HFD Female

Fig. 2


2A
Body weight gain (g)
320
280
240
200
160
120
80
40
0
0
1
2
3
4
5
6
Weeks
Contol Chow Male
Control Chow + HFD Male
Stress Chow Male
Stress Chow + HFD Male
Control Chow Female
Control Chow + HFD Female
Stress Chow Female
Stress Chow + HFD Female


2B
Chow
Chow + HFD
Caloric Efficiency (g/Kcal)
150
120
90
60
30
0
Control
Stress
Control
Stress
Male rats
Female rats

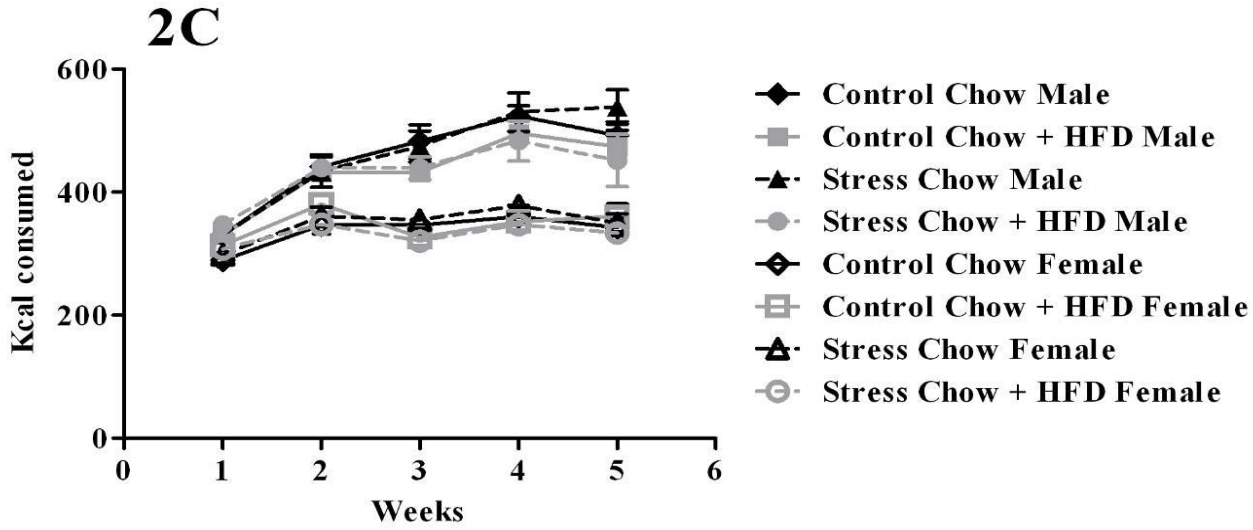

2C
Kcal consumed
600
400
200
0
0
1
2
3
4
5
6
Weeks
Control Chow Male
Control Chow + HFD Male
Stress Chow Male
Stress Chow + HFD Male
Control Chow Female
Control Chow + HFD Female
Stress Chow Female
Stress Chow + HFD Female

Fig. 3

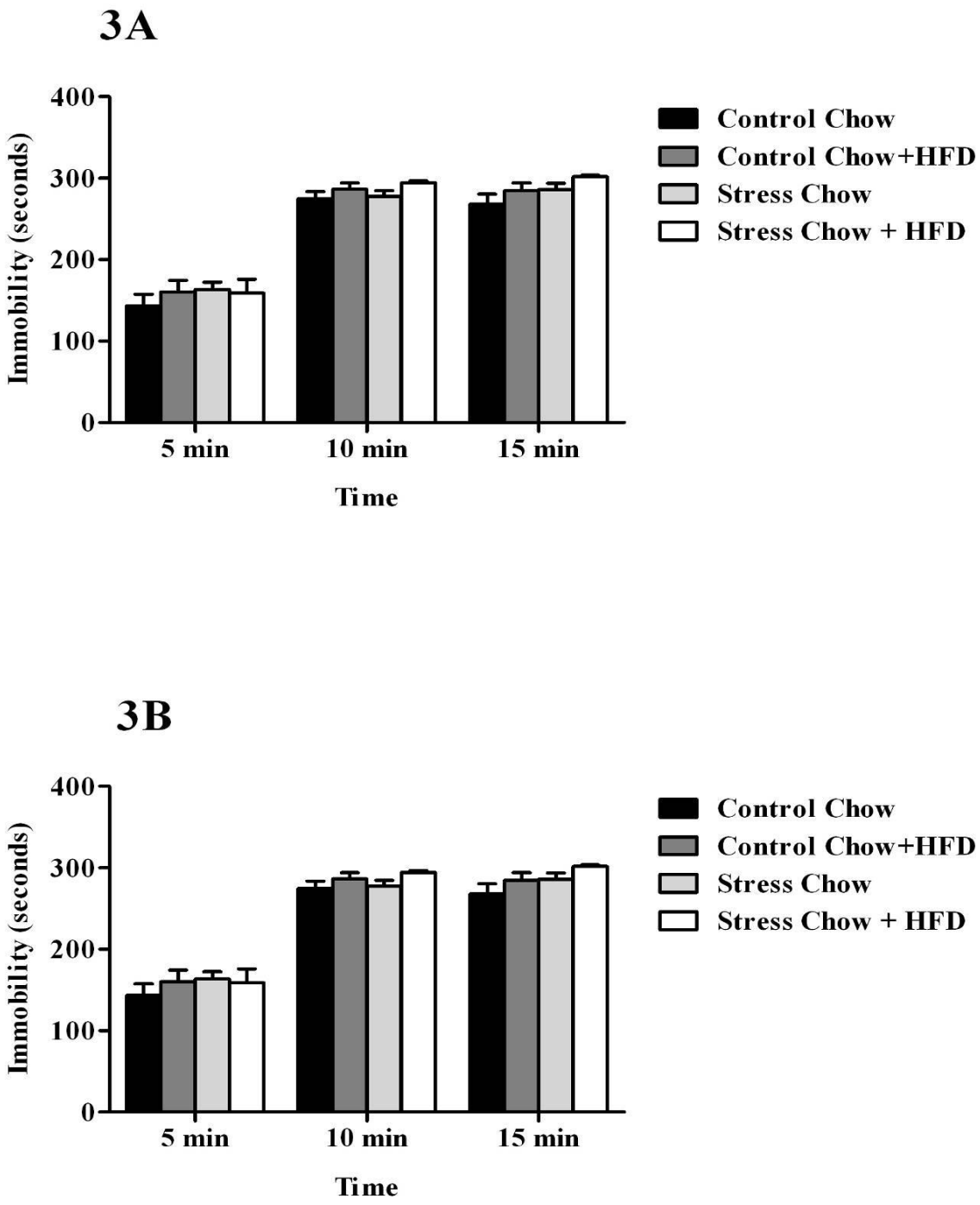

3A
400
300
200
100
0
Immobility (seconds)
5 min
10 min
15 min
Time
Control Chow
Control Chow+HFD
Stress Chow
Stress Chow + HFD
3B
400
300
200
100
0
Immobility (seconds)
5 min
10 min
15 min
Time
Control Chow
Control Chow+HFD
Stress Chow
Stress Chow + HFD

Fig. 4

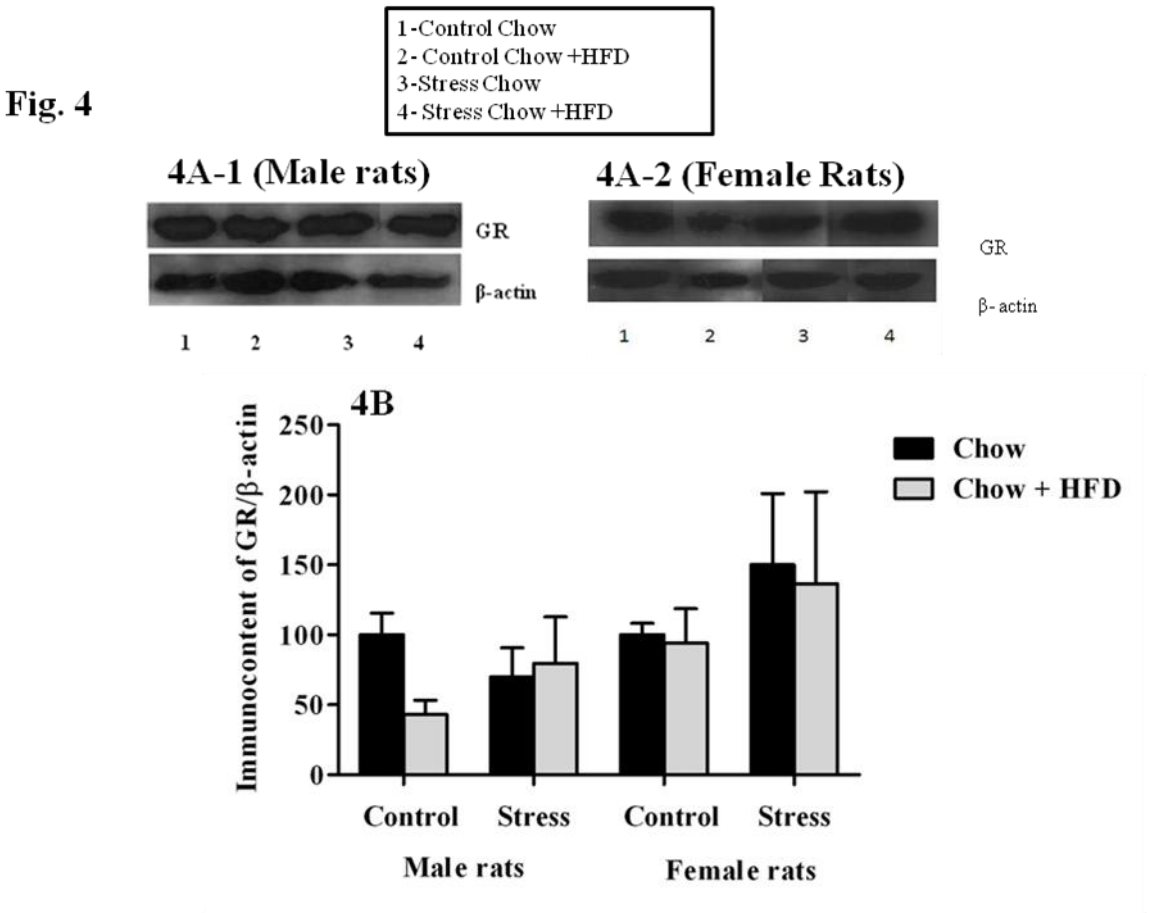
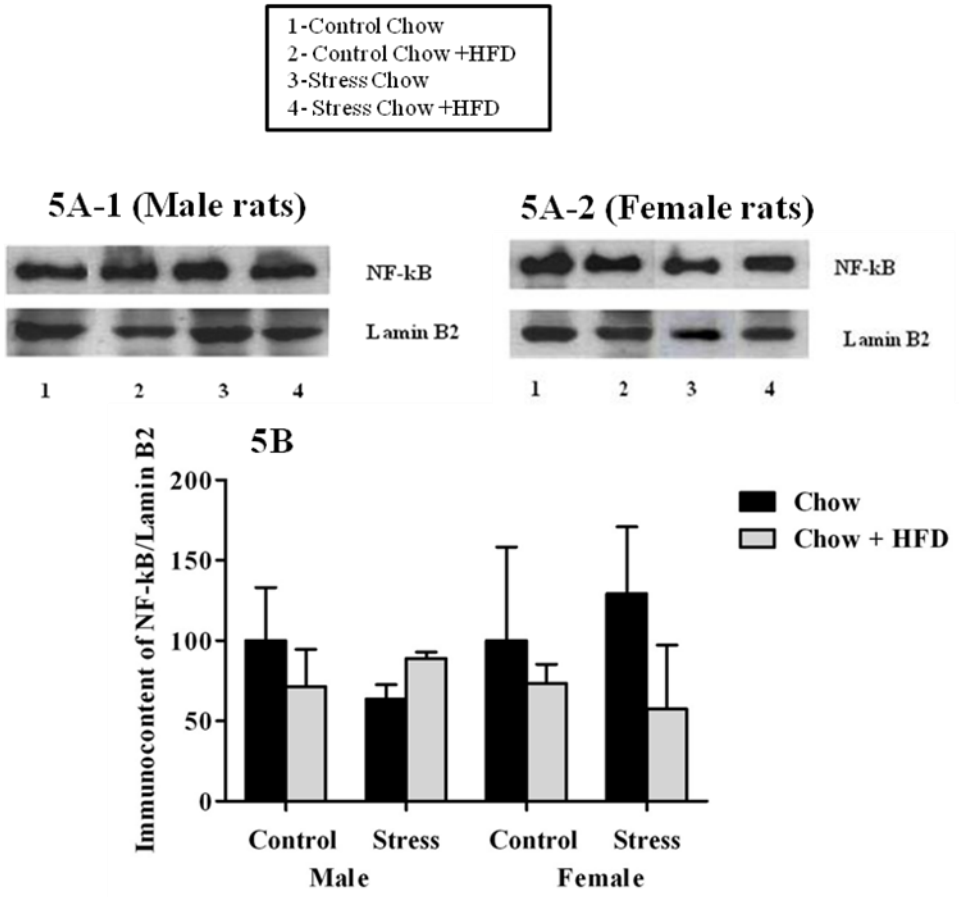

1-Control Chow
2- Control Chow +HFD
3-Stress Chow
4- Stress Chow +HFD
4A-1 (Male rats)
GR
β-actin
1 2 3 4
4A-2 (Female Rats)
GR
β-actin
1 2 3 4
4B
Immunocontent of GR/β-actin
250
200
150
100
50
0
Chow
Chow + HFD
Control
Stress
Control
Stress
Male rats
Female rats

Fig. 5


1-Control Chow
2- Control Chow +HFD
3-Stress Chow
4- Stress Chow +HFD
5A-1 (Male rats)
NF-kB
Lamin B2
1
2
3
4
5A-2 (Female rats)
NF-kB
Lamin B2
1
2
3
4
5B
Immunocontent of NF-kB/Lamin B2
200
150
100
50
0
Control
Stress
Control
Stress
Male
Female
Chow
Chow + HFD

4. DISCUSSÃO

O presente estudo fornece novos dados sobre as diferenças entre os sexos em relação à exposição a um estresse por isolamento no período da pré-puberdade e o acesso crônico a uma dieta rica em gordura. Os resultados mostraram que, durante o período do isolamento (21° ao 28° dias de vida pós-natal), os ratos machos estressados que receberam apenas ração padrão tiveram menor ganho de peso corporal comparado aos seus controles, efeito que foi revertido com o acesso à dieta rica em gordura. A exposição ao estresse aumentou o consumo de calorias total, efeito observado também nos animais que tiveram acesso a dieta rica em gordura. Contrário ao que aconteceu no período do isolamento, no período seguinte, os animais que receberam a dieta rica em gordura apresentaram aumento na eficiência calórica, sendo que esse aumento foi mais pronunciado nos machos. Além disso, o acesso livre à dieta rica em gordura aumentou os níveis de leptina e de adiponectina nos ratos adultos. Ambos os fatores, estresse por isolamento e a dieta rica em gordura induziram um comportamento do tipo depressivo nos animais adultos.

Está bem estabelecido que o estresse altera ingestão e o balanço energéticos (Harris *et al.*, 2006). A severidade, o tipo e a duração da exposição a um estressor podem influenciar de forma diferente no ganho de peso corporal (Marti *et al.*, 1994; Harris *et al.*, 2006). Conforme nossos resultados, ratos machos estressados e com acesso apenas a ração padrão apresentaram uma diminuição no ganho de peso corporal quando comparados aos seus controles, durante a semana do estresse. Estes dados estão de acordo com os encontrados na literatura que mostraram que mostram que a maioria dos estressores diminui o ganho de peso de ratos machos adultos quando lhes é oferecida apenas a ração padrão, incluindo o estresse por restrição (Harris *et al.*, 2006), estresse por derrota social (Tamashiro *et al.*, 2004; Pulliam *et al.*, 2010) e o estresse crônico variado (Ulrich-Lai *et al.*, 2007), provavelmente devido a um desequilíbrio entre a

energia ingerida e a energia perdida (Harris *et al.*, 2006). Por outro lado, durante o isolamento social, o consumo de dieta rica em gordura reverteu o efeito do estresse e aumentou o ganho de peso nos machos isolados. O aumento do consumo calórico provindo da dieta hiperlipídica, indica uma preferência por alimentos gordurosos durante o período do estresse, fato observado no modelo de estresse crônico variado (Teegarden & Bale, 2008). Esse resultado corrobora com achados que reforçam o fato de que, durante o estresse, animais preferem alimentos confortantes em detrimento da ração padrão (Pecoraro *et al.*, 2004).

O consumo crônico de dietas ricas em gordura aumenta a atividade basal e a atividade induzida pelo estresse do eixo HHA, exacerbando as respostas endócrinas ao estresse e resultando em aumento nos níveis de glicocorticoides circulantes, podendo ser considerada um estressor crônico (Tannenbaum *et al.*, 1997). Os GCs são os efetores finais do eixo HHA e participam do controle da homeostase de todo organismo, da regulação das respostas ao estresse e estão envolvidos no metabolismo energético (McCormick *et al.*, 2010). Além disso, os GCs influenciam na função da tireóide, afetando os níveis circulantes dos hormônios tireoidianos dependendo do estágio do desenvolvimento a que se foi exposto (Charmandari *et al.*, 2005; Van der Geyten & Darras, 2005). Durante o estresse, a ativação do eixo HHA inibe a função da tireóide (Charmandari *et al.*, 2005) podendo causar diminuição do metabolismo, aumento na deposição de gordura abdominal e um aumento na eficiência calórica, como pode ser observado no grupo dos ratos machos adultos recebendo dieta rica em gordura. A exposição ao estresse no presente estudo aconteceu durante a pré-puberdade, possivelmente ocorreram modificações durante esse período que, influenciadas pelo estresse que perduraram até a idade adulta.

Sabe-se que o gasto energético por quilo de gordura corporal durante períodos de repouso é maior em crianças do que em adultos (Goran *et al.*, 1995; Bitar *et al.*, 1999), provavelmente devido a vários fatores como: o crescimento, a puberdade, maior quantidade proporcional de órgãos internos em crianças, suas pernas curtas e menor massa muscular (Daniels *et al.*, 1978; Cooke *et al.*, 1991; Roemmich *et al.*, 2000). O aumento de massa muscular na puberdade é maior em meninos, por isso eles têm maior gasto energético que as meninas (Bitar *et al.*, 1999). Foi observada uma diminuição da eficiência calórica dos animais que receberam dieta rica em gordura em comparação com os que receberam ração padrão durante a primeira semana (isolamento), ou seja, não houve a conversão eficaz das calorias da dieta em massa corporal. A hiperfagia causa aumento da termogênese no tecido adiposo marrom, principalmente em ratos jovens, devido à maior proporção desse tecido em idades mais precoces (Nicholls, 1979; Brooks *et al.*, 1982). Estudos mostraram que a dieta de cafeteria, quando consumida em excesso, aumenta a termogênese de ratos jovens, como um mecanismo compensatório ao aumento calórico (Rothwell & Stock, 1979). Podemos especular que a dieta rica em gordura pode estar agindo de forma semelhante à dieta de cafeteria: o consumo em excesso da dieta rica em gordura pode estar ativando a termogênese desses animais que tem uma perda energética grande e, portanto uma menor eficiência calórica.

Entretanto, diferente do período pré-púbere, na idade adulta a dieta aumentou a eficiência calórica, sendo que os machos tiveram maior eficiência calórica que as fêmeas. Essas diferenças podem ser causadas por influência de hormônios sexuais (Richard, 1986) que, atuando nos tecidos periféricos, alteram os processos metabólicos e o balanço energético (Wade *et al.*, 1985). Sabe-se que as fêmeas são mais sensíveis aos efeitos da inibição do apetite causados pela leptina que os machos (Clegg *et al.*, 2006) e que o estrógeno está positivamente correlacionado com os níveis de leptina

reduzindo o consumo e o ganho de peso (Wabitsch *et al.*, 1997; Demerath *et al.*, 1999). Além disso, um estudo em que fêmeas receberam ração padrão mostrou que elas possuem um gasto energético basal maior que os machos, pois possuem uma maior proporção de órgãos metabolicamente ativos por unidade de massa corporal, além de apresentarem uma ativação maior da termogênese que os machos (Valle *et al.*, 2005). Em relação ao aumento da termogênese, estudos mostram que a leptina aumenta a energia perdida através da termogênese (Rahmouni & Morgan, 2007). Dessa forma, podemos sugerir que possivelmente as fêmeas sendo mais sensíveis aos efeitos da leptina que os machos, e apresentam maior perda energética na forma de calor, levando a uma menor eficiência calórica se comparadas aos machos.

Após a primeira semana, os animais que foram expostos ao isolamento retornaram aos seus grupos e continuaram recebendo dieta rica em gordura. O acesso crônico à dieta rica em gordura levou a um aumento na deposição de gordura abdominal (inguinal e perirrenal) que pode estar relacionado a um balanço energético positivo associado com aumento de energia armazenada como lipídeos (REF). Estes fatores contribuiem para o desenvolvimento da obesidade e da síndrome metabólica. A deposição de gordura abdominal ou visceral está associada a problemas cardiovasculares, diabetes tipo II e vários outros distúrbios relacionadas com a obesidade (Wajchenberg, 2000). O tecido adiposo abdominal tem características adipogênicas, pró-aterogênicas e pró-trombóticas (Trayhurn, 2005), importantes fatores de risco para o desenvolvimento da síndrome metabólica (Kyrou *et al.*, 2006; Lottenberg *et al.*, 2012). Adicionalmente, esse tecido libera citocinas pró-inflamatórias que podem atuar como um estímulo crônico adicional ao eixo HHA, formando um ciclo vicioso, pois a ativação do eixo libera mais cortisol que contribui para o aumento do acúmulo de gordura (Kyrou *et al.*, 2006). Por outro lado, o tecido adiposo secreta uma

variedade de adipocinas, entre as quais a leptina e a adiponectina, que controlam processos metabólicos, vasculares, endócrinos e imunes (Korner *et al.*, 2007; Wozniak *et al.*, 2009)

A leptina é secretada na mesma proporção da massa de tecido adiposo (Trayhurn & Bing, 2006; Badman & Flier, 2007). Animais e humanos obesos têm níveis aumentados de leptina (Considine *et al.*, 1996; Trayhurn & Bing, 2006). Ao interagir com seus receptores no hipotálamo a leptina regula a longo prazo a homeostase energética (Jeanrenaud & Rohner-Jeanrenaud, 2001) moderando a ingestão de alimentos e diminuindo o ganho de peso corporal (Klok *et al.*, 2007; Tomiyama *et al.*, 2012) além de sinalizar a composição dos macronutrientes da dieta (Havel, 2004). O consumo crônico de dieta rica em gordura aumentou os níveis plasmáticos de leptina em machos e fêmeas, porém de forma mais evidenciada nos machos, provavelmente relacionado a sua alta deposição de gordura abdominal e aumentada eficiência calórica (Arcego *et al.*, 2013). Porém, paralelo aos altos níveis de leptina o consumo calórico e o ganho de peso corporal também foram elevados nos machos. A baixa sensibilidade à leptina nas células do hipotálamo pode aumentar os níveis circulantes de leptina sem a redução da ingestão de alimentos, sugerindo uma resistência aos efeitos endógenos da leptina, fatores que caracterizam a obesidade e a síndrome metabólica (Zhang & Scarpace, 2006). Os hormônios sexuais influenciam na produção de leptina (Pinilla *et al.*, 1999) e como vimos anteriormente, estudos mostram que o estrógeno altera a sensibilidade à leptina em fêmeas (Clegg *et al.*, 2006), sugerindo que estas, por serem mais sensíveis à leptina, não são tão afetadas pela resistência a esse hormônio, de forma que apresentam um consumo menor e também menores níveis de leptina circulantes comparado aos machos.

Produzida pelo tecido adiposo maduro, a adiponectina é considerada uma adipocina benéfica. Estudos com roedores mostram que altos níveis de adiponectina melhoram alguns aspectos negativos da obesidade, enquanto que níveis baixos estão associados com a síndrome metabólica (Badman & Flier, 2007). Interessantemente, fêmeas com acesso crônico a dieta rica em gordura tiveram aumento dos níveis plasmáticos de adiponectina, adipocina com propriedades anti-inflamatórias, anti-aterogênicas e anti-diabéticas (Matsuzawa, 2006; Okamoto *et al.*, 2006). Outros estudos têm mostrado que intervenções durante o desenvolvimento podem aumentar os níveis de adiponectina em fêmeas, sugerindo um papel protetor dessa adipocina nestas (Krolow *et al.*, 2013).

Importante destacar que, apesar de contraditórios, estudos têm associado à leptina e a adiponectina com a depressão (Taylor & Macqueen, 2010). Estudos em animais e humanos têm relacionado baixos níveis de leptina (Lu, 2007) e de adiponectina (Leo *et al.*, 2006) circulantes com comportamentos depressivos. No presente estudo, foi observado que os animais expostos ao estresse na pré-puberdade e os animais que receberam dieta hiperlipídica cronicamente aumentaram o tempo de imobilidade no teste do nado forçado, podendo ser interpretado como um comportamento do tipo depressivo (Castagne *et al.*, 2009). Os aumentos nos níveis de leptina encontrados no nosso estudo estão associados com uma resistência à leptina, o que pode contribuir para a depressão. Além disto, importante ressaltar que os animais que foram estressados durante a pré-puberdade apresentaram comportamento do tipo depressivo na idade adulta, confirmando a importância desse período do desenvolvimento em programar os efeitos do estresse a longo prazo.

Pacientes com depressão maior tem uma hiperatividade do eixo HHA, prejuízo na retroalimentação negativa do eixo, resistência aos glicocorticoides e, portanto

aumento dos níveis circulantes de cortisol (Juruena *et al.*, 2004; Boyle *et al.*, 2005; Pariante, 2006). Estes sintomas que podem ser atribuídos a uma diminuição na quantidade ou na função dos receptores de glicocorticoides (Juruena *et al.*, 2004; Boyle *et al.*, 2005).

Em condições normais, os glicocorticoides são anti-inflamatórios, imunossupressores e imunomoduladores. Entretanto no sistema nervoso central altas concentrações de glicocorticoides podem causar reações inflamatórias (Sorrells & Sapolsky, 2007). Os GCs exercem seus efeitos através dos seus receptores RG no citosol, que após conjugados aos CGs translocam-se para o núcleo. No núcleo, o RG dimeriza-se e atua em fatores de transcrição para expressar genes anti-inflamatórios ou atua inativando rotas inflamatórias, como por exemplo, a do NF-kB (Zunszain *et al.*, 2013). Em condições de estresse, entretanto, a redução da função do RG pode resultar na resistência aos GCs, aumentando a ativação de rotas inflamatórias contribuindo para sintomas depressivos (Pace *et al.*, 2007). Evidências recentes mostram que a ativação das respostas inflamatórias do sistema imune tem importante papel no estabelecimento da depressão (Tagliari *et al.*, 2011). De fato, pacientes depressivos apresentam sinalização prejudicada dos GCs e altos níveis de citocinas pró-inflamatórias (Howren *et al.*, 2009) e elevado risco de inflamação (Miller *et al.*, 2002). É relevante pontuar que o consumo de dietas ricas em ácidos graxos saturados promove a inflamação tanto em órgãos da periferia quanto no cérebro (van Dijk *et al.*, 2009). Além disso, o isolamento social em períodos precoces pode contribuir para o risco de inflamação e um maior risco de doenças na idade adulta (Danese *et al.*, 2007). Contrário ao que a literatura têm sugerido, nem o RG citosólico nem o NF-kB nuclear foram afetados, assim como os conteúdos de TNF-α e IL-1. Assim sendo, nesse estudo, não observamos relação entre os mecanismos inflamatórios e o comportamento do tipo depressivo em animais

isolados durante a pré-puberdade e com acesso a dieta rica em gordura. Essas diferenças podem ser atribuídas ao período em que o estresse e o início do consumo de dieta rica em gordura foram conduzidos. No período da pré-puberdade vários sistemas estão em plena maturação, assim como o córtex pré-frontal, que tem uma maturação tardia e particularmente vulnerável a intervenções durante esse período (Gogtay *et al.*, 2004). Além disso, a maioria dos estudos já referidos sobre estresse e inflamação usaram a exposição crônica ao estresse, contrário ao nosso estudo, onde o estresse foi realizado apenas por sete dias (podendo ser considerado um estresse sub-agudo) no período pré-púbere. Assim, é importante que outros estudos sejam realizados para esclarecer que mecanismo pode estar relacionado ao comportamento do tipo depressivo nesses animais estressados durante a pré-puberdade e com acesso a dieta rica em gordura.

5. CONCLUSÕES

- O estresse durante a pré-puberdade e o acesso crônico a uma dieta rica em gordura induziram efeitos metabólicos sexo-específicos e tiveram influência sobre o comportamento do tipo depressivo observados durante a idade adulta.
- Os animais estressados que recebam ração padrão tiveram menor ganho de peso comparado ao controle, efeito revertido pelo acesso a dieta rica em gordura
- Na semana do estresse todos animais que receberam a dieta rica em gordura apresentaram uma diminuição na eficiência calórica, contrário ao que aconteceu na idade adulta em que o acesso a dieta aumentou a eficiência calórica dos animais, porém de forma mais expressiva nos machos.
- O acesso crônico a dieta rica em gordura modificou parâmetros considerados risco de síndrome metabólica e doenças cardiovasculares (como aumento de peso corporal, aumento na deposição de gordura abdominal e aumento nos níveis de leptina) parâmetros que foram mais destacados nos machos, sugerindo que estes teriam maior susceptibilidade a desenvolver patologias relacionadas a síndrome metabólica que as fêmeas.
- O aumento mais expressivo de adiponectina foi observado nas fêmeas com acesso a dieta rica em gordura, sugerindo que elas estão mais protegidas dos fatores que podem induzir a síndrome metabólica e suas comorbidades.
- Os animais expostos ao estresse durante o período da pré-puberdade ou os animais com acesso crônico a dieta rica em gordura tiveram um comportamento do tipo depressivo o qual não foi relacionado com a inflamação.

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