Universidade do Estado do Rio de Janeiro

Centro Biomédico

Faculdade de Ciências Médicas

Juliana Gastão Franco

Avaliação do estresse oxidativo em modelos de plasticidade ontogenética: papel do resveratrol

Rio de Janeiro

2012

Juliana Gastão Franco

Avaliação do estresse oxidativo em modelos de plasticidade ontogenética: papel do resveratrol

Tese apresentada, como requisito parcial para obtenção do título de Doutora ao Programa de Pós-graduação em Fisiopatologia Clínica e Experimental, da Universidade do Estado do Rio de Janeiro.

Orientador: Prof. Dr. Egberto Gaspar de Moura

Coorientadora: Prof[a]. Dra. Magna Cottini da Fonseca Passos

Rio de Janeiro

2012

Juliana Gastão Franco

Avaliação do estresse oxidativo em modelos de plasticidade ontogenética: papel do resveratrol

Tese apresentada, como requisito parcial para obtenção do título de Doutora, ao Programa de Pós-graduação em Fisiopatologia Clínica e Experimental, da Universidade do Estado do Rio de Janeiro.

Aprovada em 29 de fevereiro de 2012.

Coorientadora: Prof.ª Dra. Magna Cottini da Fonseca Passos

Banca Examinadora:

_____
Prof. Dr. Egberto Gaspar de Moura (Orientador)
Faculdade de Ciências Médicas - UERJ

_____
Prof.ª Dra . Ângela de Castro Resende
Universidade do Estado do Rio de Janeiro

_____
Prof.ª Dra. Patrícia Gama
Universidade de São Paulo

_____
Prof.ª Dra. Karen de Jesus Oliveira
Universidade Federal Fluminense

_____
Prof.ª Dra. Patricia Lisboa
Universidade Federal do Rio de Janeiro

Rio de Janeiro

2012

DEDICATÓRIA

Ao Pedro, meu amigo e meu amor.

AGRADECIMENTOS

A Deus, pela presença constante e por todas as bênçãos a mim concedidas.

Ao Professor Egberto Gaspar de Moura, pelo apoio, orientação e pelo constante incentivo à pesquisa.

À Professora Magna Cottini da Fonseca Passos, pela orientação, confiança e carinho.

As Professoras Patrícia Lisboa e Elaine de Oliveira, pela presença e atenção constantes e pela colaboração fundamental no desenvolvimento deste trabalho.

Aos pós-graduandos e alunos de iniciação científica do LFE pelo apoio durante o desenvolvimento deste trabalho. Obrigada equipe!

As minhas queridas amigas do LFE (e de todas as horas) Jéssica, Lígia, Natália, Aninha e Cíntia pela ajuda constante e por todos os momentos de descontração que fizeram destes anos no LFE muito especiais. Vou sentir muitas saudades!

À Isis, exemplo de excelente profissional e pessoa "rara", pela amizade, apoio, incentivo e orientação incansáveis.

À Professora Ângela de Castro Resende pela ajuda na realização deste trabalho.

À Taline, pela ajuda e paciência nas análises feitas neste trabalho.

À Nayara, pelo apoio nas análises morfológicas.

À Amélia, pelo constante e solícito atendimento na secretaria do FISCLINEX.

Ao Pedro, pela paciência, amor e companheirismo sempre.

Aos meus pais, irmãos e amigos pelo incentivo e amizade.

RESUMO

FRANCO, Juliana Gastão. *Avaliação do estresse oxidativo em modelos de plasticidade ontogenética: papel do resveratrol*. 2012. 102 f. Tese (Doutorado em Fisiopatologia Clínica e Experimental) – Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2012

A obesidade, cuja origem é multifatorial e a prevalência é crescente em diferentes regiões no mundo, está geralmente associada à produção desregulada de adipocinas, ao aumento do estresse oxidativo e à ocorrência de distúrbios metabólicos como dislipidemia, intolerância à glicose e hipertensão. A Programação Metabólica ou Plasticidade Ontogenética tem sido proposta como um importante fator na etiologia da obesidade. Este fenômeno sugere que alterações nutricionais, hormonais e ambientais durante períodos críticos do desenvolvimento, tais como gestação e lactação, podem alterar a fisiologia e o metabolismo de um organismo provocando o desenvolvimento de distúrbios metabólicos na vida adulta. Neste trabalho foram estudados dois modelos de plasticidade ontogenética que programam ratos Wistar para obesidade na vida adulta: a supernutrição na lactação e o desmame precoce. A supernutrição na lactação provocada pela redução da ninhada causou obesidade, hiperfagia, aumento do estresse oxidativo, resistência hepática à ação da insulina e esteatose nas proles na idade adulta. A desnutrição, provocada pelo desmame precoce, também se associou na idade adulta com obesidade visceral, aumento do estresse oxidativo e esteatose, assim como dislipidemia, resistência à insulina, hipertensão arterial e resistência central à leptina. No modelo de programação pelo desmame precoce, os animais adultos foram tratados com resveratrol (30mg/kg/MC), um polifenol encontrado nas uvas e conhecido por seus efeitos antioxidante e hipoglicemiante, por 30 dias. Os animais programados pelo desmame precoce que receberam resveratrol tiveram massa corporal, gordura visceral e morfologia hepática semelhantes aos animais controles. Ainda, o resveratrol normalizou a pressão arterial, a dislipidemia, a glicemia e as concentrações de adiponectina e leptina. A normalização da leptinemia esteve associada à correção da resistência central à leptina nestes animais, uma vez que o resveratrol normalizou além da ingestão, o conteúdo hipotalâmico de JAK2, pSTAT3 e NPY. Portanto, os animais programados pela supernutrição na lactação ou pelo desmame precoce apresentaram aumento do estresse oxidativo associado à obesidade e alterações metabólicas como esteatose. O tratamento com resveratrol nos animais programados pelo desmame precoce preveniu o aumento de estresse oxidativo, obesidade visceral, resistência à insulina, dislipidemia e esteatose. Além disso, o resveratrol causou normalização da leptinemia nestes animais, assim como da ação deste hormônio no hipotálamo, controlando a hiperfagia característica deste modelo.

Palavras-chave: Supernutrição. Desmame precoce. Programação Metabólica. Resveratrol. Etresse oxidativo.

ABSTRACT

Obesity, a multifactorial disease with increased prevalence in different regions in the world, is generally associated with a deregulated adipocytokines production, increased oxidative stress and the occurrence of metabolic disorders such as dyslipidemia, glucose intolerance and hypertension. The Metabolic Programming or Developmental Plasticity has been proposed as important factor in the etiology of obesity. This phenomenon suggests that dietary changes, hormonal and environmental factors during critical periods of development, such as pregnancy and lactation can alter the physiology and metabolism of an organism causing the development of metabolic disorders at adulthood. In this work, we studied two models of developmental plasticity in Wistar rats, which programmed for obesity at adulthood: overnutrition during lactation and early weaning. Overnutrition during lactation through litter size reduction caused obesity, hyperphagia, increased oxidative stress, liver insulin resistance and steatosis in offspring at adulthood. Malnutrition caused by early weaning was also associated with visceral obesity, increased oxidative stress and steatosis as well as dyslipidemia, insulin resistance, hypertension and central leptin resistance in adulthood. In this programming model by early weaning, adult animals were treated with resveratrol (30mg/kg/BW for 30 days), a polyphenol found in grapes and known for its antioxidant and hypoglycemic actions. Offspring programmed by early weaning that were treated with resveratrol had body mass, visceral fat and liver morphology similar to those found in control offspring. Also, resveratrol normalized blood pressure, dyslipidemia, and serum glucose, adiponectin and leptin. The normalization of leptin levels was associated with the correction of central leptin resistance in these animals, since resveratrol normalized food intake and hypothalamic content of JAK2, pSTAT3 and NPY. Therefore, offspring programmed by overnutrition during lactation or early weaning displayed increased oxidative stress, obesity and metabolic disorders such as steatosis. Treatment with resveratrol in early weaned offspring prevented the increase of oxidative stress, visceral obesity, insulin resistance, dyslipidemia and steatosis. Indeed, resveratrol normalized serum leptin as well as its action in the hypothalamus, controlling the hyperphagia characteristic of these offspring.

Keywords: Overnutrition. Early weaning. Programming. Resveratrol. Oxidative stress.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

AGL	Ácidos Graxos livres
AgRP	Peptídeo relacionado à proteína agouti
AKT	Proteína cinase B
ARC	Núcleo hipotalâmico arqueado
CART	Transcrito regulado pela cocaína e anfetamina
CoA	Coenzima A
Cox	Ciclooxigenase
CRH	Hormônio liberador de corticotrofina
DMN	Núcleo hipotalâmico dorsomedial
eNOS	Óxido Nítrico sintase endotelial
ERK	Cinase regulada por sinais extracelulares
ERO	Espécies reativas de oxigênio
ERN	Espécies reativas de nitrogênio
ET-1	Endotelina 1
FOXO	Fatores de transcrição “Forkhead” classe O (Forkhead box proteins)
GLUT-4	Transportador de Glicose Tipo 4
GSH	Glutationa
GSSG	Glutationa Reduzida
HAS	Hipertensão arterial sistêmica
HDL	Lipoproteína de alta densidade
IL-6	Interleucina 6
IRS	Substrato do receptor de insulina
IRβ	Receptor de insulina beta

JAK2	Janus cinase
LDL	Lipoproteína de baixa densidade
MAPK	Proteína cinase ativada por mitógenos
NADPH	Nicotinamida adenina dinucleotídeo fosfato reduzido
OBR	Receptor de leptina
p53	Proteína tumoral 53
PAI-1	Inibidor do ativador do plasminogênio tipo 1
PGC-1α	Receptor ativado por proliferador de peroxissoma
PI3K	Fosfatidilinositol-3 cinase
POMC	Pró opiomelanocortina
PPAR	Receptores ativados por proliferador de peroxissomo
PTP-1B	Proteína fosfatase 1B
PVN	Núcleo hipotalâmico paraventricular
SIRT1	Sirtuína1
SOCS3	Supressor da sinalização de citocinas, tipo 3
STAT3	Transdutores de sinal e ativadores da transcrição, tipo 3
TG	Triglicerídeos
TNF-α	Fator de necrose tumoral alfa
VLDL	Lipoproteína de muito baixa densidade
VMN	Núcleo hipotalâmico ventromedial
XO	Xantina oxidase
αMSH	Melanocortina

SUMÁRIO

INTRODUÇÃO

A obesidade é uma doença de origem multifatorial que se caracteriza pelo acúmulo de tecido adiposo associado à produção desregulada de adipocinas e aumento do estresse oxidativo (Otani, 2011; Galic et al., 2011). A prevalência da obesidade é crescente em diferentes regiões no mundo (WHO, 2011) e muitos estudos têm sido desenvolvidos com o objetivo de identificar estratégias de prevenção e tratamento desta epidemia. O aleitamento materno exclusivo até os seis meses de vida tem sido associado ao menor risco de obesidade e diabetes na vida adulta e, por esta razão, a amamentação tem sido considerada uma estratégia de prevenção da obesidade (Sichieri & Souza, 2008; WHO, 2010). A obesidade na infância, entretanto, se associou a permanência do excesso de peso e ao aumento do risco cardiovascular na vida adulta (Reilly et al., 2003; Must, 2003, Owen et al., 2009, Conde & Borges, 2011).

Estudos epidemiológicos e experimentais sugerem que a origem da obesidade e de doenças crônicas está relacionada com a ocorrência de eventos adversos em períodos críticos como a gestação e a lactação. Este fenômeno, conhecido como Programação Metabólia ou Plasticidade Ontogenética, propõe que, durante estes períodos, a fisiologia de órgãos e sistemas pode ser alterada em resposta a alterações ambientais, nutricionais ou hormonais (Barker et al., 1995; de Moura et al., 2008). Em nosso laboratório, desenvolvemos dois modelos de plasticidade ontogenética em ratos - desmame precoce e supernutrição neonatal - que se associaram ao desenvolvimento de obesidade, hiperfagia e resistência central à leptina na vida adulta (Rodrigues et al., 2009; Rodrigues et al., 2011; Conceição et al., 2011; Lima et al., 2011; Lopes Nobre et al., 2011).

O uso de compostos derivados de alimentos no tratamento da obesidade e de doenças cardiovasculares tem sido proposto nos últimos anos (Chachay et al., 2012). O resveratrol, um polifenól estilbeno encontrado principalmente nas uvas, tem sido bastante estudado devido as suas propriedades antioxidantes, hipoglicemiantes, antienvelhecimento e antimutagênicas (Pervaiz & Holme, 2009). Recentemente, devido a identificação de efeitos do resveratrol também na mobilização de lipídios (Baile et al., 2011), estudos têm investigado a utilização deste composto em modelos de obesidade (Tauriainen et al., 2010; Gómez-Zorita et al., 2012).

No presente trabalho estudamos o estresse oxidativo em dois modelos de plasticidade ontogenética relacionados com a obesidade na vida adulta - desmame precoce e supernutrição neonatal. Além disso, avaliamos os efeitos do resveratrol sobre o estresse oxidativo e outros distúrbios metabólicos característicos do modelo desmame precoce.

1 **REVISÃO DE LITERATURA**

1.1**Obesidade**

A prevalência da obesidade é crescente nos últimos anos e por isso ela é considerada um grave problema mundial de saúde pública. Estimativas feitas pela Organização Mundial de Saúde (OMS) em 2008 apontaram que 1,5 bilhões de pessoas no mundo apresentavam sobrepeso, sendo 500 milhões classificadas como obesas. Para o ano de 2015, a prevalência estimada é de 3,3 bilhões de sobrepeso e de 700 milhões de obesos (WHO, 2011).

A obesidade é o principal fator de risco para o desenvolvimento de complicações metabólicas como dislipidemia aterogênica, intolerância à glicose, resistência insulínica, hipertensão e esteatose hepática não alcóolica. Esta associação entre obesidade e tais complicações aumenta o risco cardiovascular. A síndrome metabólica (SM) relaciona-se diretamente com risco cardiovascular e é definida pela presença de aumento da circunferência da cintura abdominal e de pelo menos 3 dos seguintes componentes: HDLc diminuído, hipertrigliceridemia, resistência a insulina, hipertensão arterial. A prevalência de SM, assim como a de obesidade, está aumentando rapidamente em todo o mundo (Otani, 2011).

Embora os critérios da SM sejam diferentes em alguns países, a obesidade do tipo visceral é um componente indispensável para caracterização dessa síndrome. Acredita-se que na obesidade ocorram disfunções secretórias dos adipócitos, como o aumento da produção de adipocinas (leptina e resistina), citocinas inflamatórias (IL-6, IL-10 e TNF-α) e fatores prótrombóticos (PAI-1), principalmente nos adipócitos viscerais (Ahima & Lazar, 2008; Galic et al., 2010).

1.1.1 Obesidade e leptina

A leptina, derivada da palavra *leptos* cuja origem é grega e significa magro, é um dos principais hormônios envolvidos com as complicações clínicas da obesidade. É um hormônio

peptídico produzido pelo gene *ob*, expresso e secretado principalmente por adipócitos maduros. Outros tecidos como placenta, glândula mamária, músculo e cérebro também expressam o gene da leptina (Bjorbaek & Kahn, 2004; Ahima, 2005; Lappas et al., 2005).

O principal sítio de ação da leptina é o hipotálamo, onde este hormônio atua como fator de saciedade estimulando a liberação de neuropeptídios anorexigênicos (α-MSH, CART e CRH) e inibindo a produção de neuropeptídios orexigênicos (NPY, AgRP e orexinas). O NPY (Neuropeptídeo Y) é capaz de aumentar o consumo alimentar e inibir a termogênese, promovendo a adipogênese em ratos (Willians et al., 2004) e cuja expressão está geralmente co-localizada com o AgRP (peptídeo relacionado à proteína agouti). O AgRP age através de mecanismo competitivo pelo receptor de melanocortina (Valassi et al., 2008). O α-MSH (hormônio estimulante de melanócitos do tipo α ou melanocortina) e seu precursor POMC (peptídeo pró-opiomelanocortina) atuam via receptores de melanocortina. O neuropeptídeo transcrito regulado por cocaína e anfetamina (CART) encontra-se 90% co-localizado com POMC no núcleo arqueado hipotalâmico (Palou et al., 2009).

O receptor para a leptina (OBR) apresenta diversas isoformas em mamíferos e pelo menos seis já foram identificadas em roedores: OBRa, OBRb, OBRc, OBRd, OBRe e OBRf. A isoforma longa (OBRb) é considerada a mais importante para a ação da leptina em diversos tecidos por possuir maior domínio intracelular e desencadear mecanismos de sinalização que vão resultar efetivamente nas ações hormonais (Hileman et al., 2002). O hipotálamo, incluindo núcleos arqueado, ventromedial, paraventricular, dorsomedial e hipotálamo lateral, expressa a maior densidade de OBRb em mamíferos. Os efeitos da leptina são mediados pela ativação da via de sinalização janus quinase 2 - proteína sinalizadora e ativadora da transcrição, tipo 3 (JAK2-STAT3) (Figura 1) (Sahu, 2003; Ahima, 2005). As proteínas JAK2 são encontradas associadas ao domínio intracelular do OBRb. A ligação da leptina ao seu receptor OBRb resulta em autofosforilação de JAK2 em resíduos de tirosina presentes no domínio intracelular do receptor, com consequente ativação de STAT3 (proteína sinalizadora e ativadora da transcrição, tipo 3). A proteína STAT3 ativada sofre dimerização, translocando-se para o núcleo, onde regula a transcrição de diversos genes, entre eles o da proteína SOCS3 (supressor da sinalização de citocinas, tipo 3). SOCS 3 exerce efeito inibitório da via de sinalização através de feedback negativo intracelular e inibe tanto a fosforilação de JAK2 quanto a formação de STAT3 fosforilada (Bjorbaek et al., 1999; Sahu, 2003; Ahima, 2005).

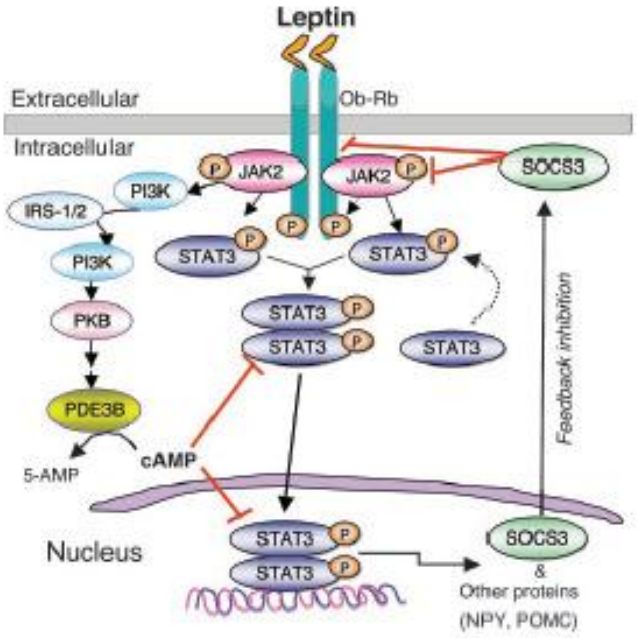


Figura 1. Via de sinalização da leptina no hipotálamo (adaptado de Sahu, 2003).

Os avanços na caracterização dos mecanismos de ação da leptina no hipotálamo mostraram que a transdução do sinal deste hormônio sofre importante controle por vias paralelas de sinalização celular e sugeriram um "*cross-talk*" entre as vias de sinalização da leptina e da insulina em determinadas regiões. A leptina é capaz de regular proteínas envolvidas na sinalização da insulina como IRS-1, MAPK, ERK, AKT e PI3K, que são co-expressas com proteínas da via JAK2-STAT3 em células de diversos tecidos (Carvalheira et al., 2005). Foi demonstrado que a inibição de PI3K e o aumento de SOCS3 bloqueiam tanto a resposta à leptina quanto à insulina, reforçando a existência de *cross-talk* entre as respectivas vias de sinalização (Niswender et al., 2004). Portanto, estados fisiopatológicos que alterem concentração sérica de leptina, como a obesidade, podem afetar as ações da insulina sobre o metabolismo.

As concentrações séricas de leptina se correlacionam positivamente com o índice de massa corporal e o percentual de gordura corporal em indivíduos obesos (Lustig, 2001, Morton & Schwartz, 2011). Entretanto, a hiperleptinemia na obesidade está geralmente relacionada com uma resistência ao efeito anorexigênico da leptina (Considine et al., 1996). A resistência à leptina pode estar associada à diminuição de receptores OBRb ou de outras proteínas da via de sinalização deste hormônio assim como ao aumento da expressão de inibidores da via, como

SOCS3 (Sahu et al., 2003). Outras proteínas, como a PTP-1B, que reconhece um sítio específico de JAK2 e diminui a sua fosforilação, também pode estar aumentada e inibir a via de sinalização intracelular da leptina (Zabolotny et al., 2002). A resistência à leptina também pode ser causada pela redução de receptores do tipo OBRa com consequente diminuição do transporte de leptina pela barreira hematoencefálica e de sua ação no hipotálamo (Bjorbaek et al., 1999; Kastin et al., 1999; Banks, 2001).

Alguns estudos demonstraram que a leptina pode promover estresse oxidativo, inflamação vascular, e proliferação e migração de células endoteliais e musculares lisas, favorecendo o desenvolvimento de aterosclerose. Em células vasculares, a leptina foi capaz de estimular a produção de endotelina 1 (ET-1) e promover calcificação vascular (Savoia & Schiffrin, 2004). Na inflamação, a leptina age diretamente sobre os macrófagos para aumentar a atividade fagocitária e a produção de citocinas pró-inflamatórias, além de exercer um efeito sobre as células T, monócitos, neutrófilos e células endoteliais (Fernández-Sánchez et al., 2011). Ainda, a redução dos níveis circulantes de leptina causada pela perda de peso em humanos esteve associada a redução de outros diferentes marcadores inflamatórios (Hukshorn et al., 2004, Steffes et al., 2006).

1.1.2 Obesidade e estresse oxidativo

Radicais livres são definidos como qualquer átomo, molécula ou fragmento de molécula contendo um ou mais elétrons desemparelhados na sua última camada de valência, o que faz com que essas espécies sejam altamente reativas, instáveis e de meia-vida muito curta. As espécies reativas de oxigênio (ERO) e de nitrogênio (ERN) são produzidas naturalmente ou por alguma disfunção biológica. Dentre os processos naturais de geração de ERO, destaca-se a respiração celular acoplada à fosforilação oxidativa para geração de ATP na mitocôndria. O oxigênio (O_2) sofre redução tetravalente pela enzima citocromo oxidase com aceitação de quatro elétrons, resultando na formação de água (H_2O). Durante esse processo são formados intermediários reativos, tais como o ânion superóxido (O_2^-), o peróxido de hidrogênio (H_2O_2) e o radical hidroxila (OH^-). Além das mitocôndrias, as ERO e outros tipos de radicais livres também são

produzidos por diferentes sítios celulares e vias metabólicas, tais como peroxisomos e os sistemas enzimáticos xantina oxidase (XO), nicotinamida dinucleotídeo fosfato reduzida (NADPH oxidase) e óxido nítrico sintase (NOs) (Halliwell, 1994; Halliwell, 2000).

O estresse oxidativo pode ocorrer como resultado do aumento da geração de ERO e/ou falha do sistema antioxidante (Sies, 1993; Roberts & Sindhu, 2009) (Figura 2). Recentemente, foi observado que os organismos possuem mecanismos para o uso vantajoso dos radicais livres, que estão envolvidos em importantes funções fisiológicas tais como a regulação do tônus vascular, controle da produção de eritropoetina e outras funções induzidas pela hipóxia e transdução de sinais envolvidos no sistema imunológico (Dröge, 2002). Por esta razão, a definição "desequilíbrio redox" também tem sido utilizada para se referir ao estresse oxidativo.

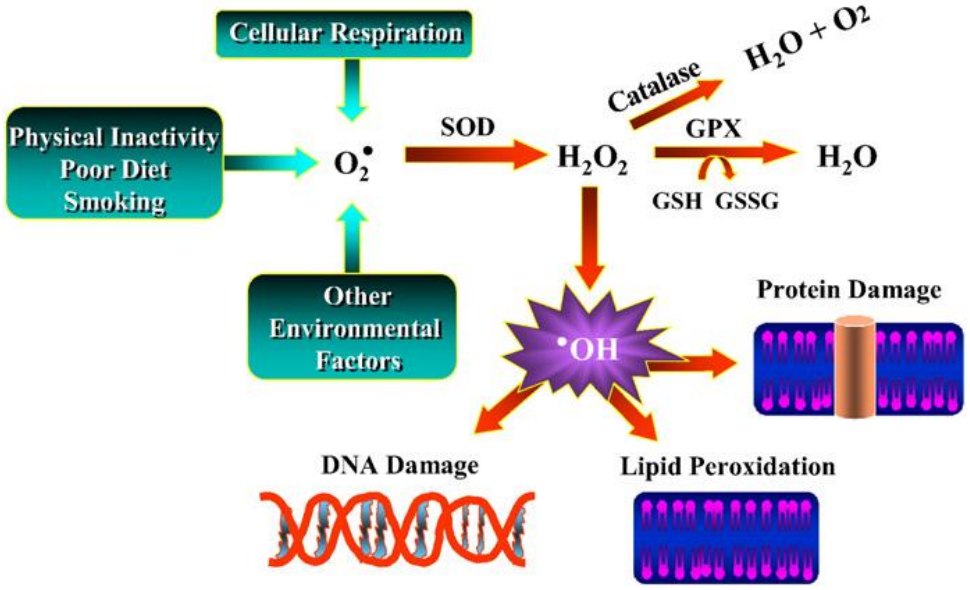


Figura 2. Visão geral da geração e da interceptação de EROs pelo sistema de defesa antioxidante enzimático (adaptado de Roberts & Sindhu, 2009).

O organismo dispõe de sistemas de defesa antioxidante enzimáticos e não enzimáticos ou dietéticos, os quais podem ter origem no próprio organismo ou são adquiridos através da dieta. Os antioxidantes são capazes de interceptar os radicais livres gerados pelo metabolismo celular ou por fontes exógenas, impedindo o ataque à dupla ligação dos ácidos graxos poliinsaturados, aos aminoácidos das proteínas e às bases de DNA, evitando assim a formação de lesões e perda

da integridade e estabilidade celular (Sies, 1993; Yu, 1994; Roberts & Sindhu, 2009). O sistema de defesa antioxidante enzimático é constituído principalmente pelas enzimas superóxido dismutase (SOD), catalase (CAT), glutationa peroxidase (GPx) e glutationa redutase (GR). A SOD é a principal enzima antioxidante contra os íons superóxido (O_2^-) produzidos por todo organismo. Esta enzima catalisa a dismutação do O_2^-, convertendo-o a peróxido de hidrogênio (H_2O_2) e oxigênio (Fridovich, 1995). Existem três formas principais de SOD em humanos: CuZnSOD citosólica, MnSOD mitocondrial, e SOD extracelular. O H_2O_2 é altamente reativo e, diferente do ânion superóxido, é capaz de difundir-se através das membranas celulares (Mates et al., 1999). A catalase, abundante em todas as células, atua sobre o H_2O_2 transformando-o em H_2O e oxigênio molecular. A GPx também é capaz de converter o H_2O_2 em O_2 e H_2O, utilizando a glutationa reduzida (GSH) e transformando-a em glutationa oxidada (GSSG) e água (Yu, 1994; Roberts & Sindhu, 2009). A GPx e a CAT possuem a mesma função sobre o H_2O_2, sendo a GPx mais eficiente em condições de elevada concentração e a CAT em baixas concentrações de espécies reativas de oxigênio (Finaud et al 2006). Ainda, o H_2O_2 pode ser convertido em água e O_2 por tiorredoxinas (Nordberg & Arnér, 2001).

As ERO são capazes de causar danos oxidativos em estruturas celulares através da oxidação de lipídeos de membrana, da carbonilação de proteínas e de danos estruturais ao DNA (Roberts & Sindhu, 2009). A oxidação resultante do ataque de radicais livres sobre ácidos graxos poliinsaturados presentes nas membranas celulares e em lipoproteínas é denominada lipoperoxidação. Durante esse processo são formados metabólitos secundários altamente citotóxicos como hidrocarbonetos de cadeia curta (etano, pentano), aldeídos (malondialdeído, 4-hidroxinonenal), epóxidos e outros. Como resultado da lipoperoxidação, as membranas sofrem alteração em sua estrutura com consequente alteração da permeabilidade, levando à perda da seletividade para entrada e/ou saída de nutrientes e substâncias tóxicas à célula (Nikki, 2009). Assim como os lipídeos, proteínas também são alvos de radicais livres. A oxidação de aminoácidos resulta na formação de carbonilas e tióis oxidados que alteram a função normal da proteína (Yan & Forster, 2011). O ataque de radicais livres ao DNA está associado com processos mutagênicos e carcinogênicos (Murtas et. al., 2010). A formação de radicais livres próximo ao DNA pode resultar na oxidação de bases de pirimidina e purina, formação de adutos e quebras na fita. Quando a cadeia do DNA é quebrada, pode ser “reconectada” em outra posição

alterando, assim, a ordem de suas bases, instalando-se um processo de mutação. O acúmulo de bases danificadas pode desencadear a oncogênese (Weinberg & Chandel, 2009).

Um importante mecanismo de patogenicidade das alterações metabólicas relacionadas à obesidade é o aumento do estresse oxidativo (Roberts & Sindhu, 2009; Otani, 2011). Estudos sugerem que o aumento de ERO nos adipócitos provoca a produção desregulada de adipocinas na obesidade e, consequentemente, o desenvolvimento de alterações da SM, como resistência à insulina, hipertensão e aterosclerose (Roberts & Sindhu, 2009; Otani, 2011). Embora a SM seja de origem multifatorial, a hipertensão, a intolerância à glicose e a dislipidemia podem ser causadas primariamente pelo mesmo mecanismo: a disfunção endotelial proveniente do aumento de estresse oxidativo (Maury & Brichard, 2010; Otani, 2011). A produção de ERO aumenta paralelamente ao acúmulo de gordura nos adipócitos. Foi demonstrado em ratos que o aumento dos níveis de ácidos graxos livres estimulou a produção de ERO em adipócitos através da ativação da NADPH oxidase e diminuição da expressão de enzimas antioxidantes (Furukawa et al., 2004). Ainda, ratos obesos tratados com inibidor de NADPH oxidase mostraram redução na produção de ERO no tecido adiposo, normalização da secreção de adipocinas e melhora do diabetes, da hiperlipidemia e da esteatose hepática (Fujita et al, 2006). Além disso, o estresse oxidativo diminuiu a secreção de adiponectina em adipócitos in vitro (Hattori et al., 2005; Soares et al., 2005) e aumentou a expressão de citocinas pró-inflamatórias in vivo (Furukawa et al., 2004; Chen et al., 2009; Sakurai et al., 2009). Portanto, é provável que o aumento na produção de ERO cause um aumento do estresse oxidativo no adipócito, gerando uma inflamação sistêmica, envolvida no desenvolvimento de complicações da SM (Figura 3).

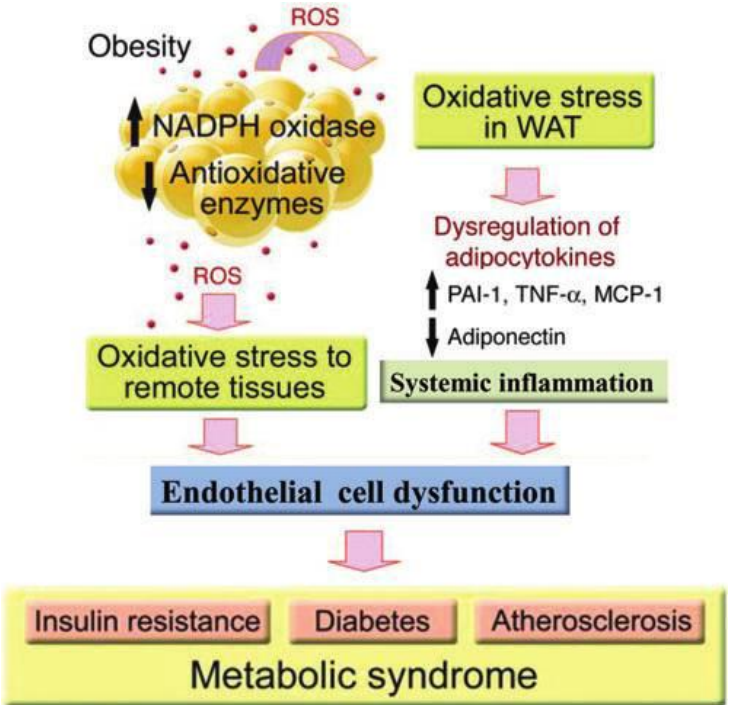


Figura 3. Mecanismos de gênese da síndrome metabólica induzida pela obesidade (adaptado de Otani, 2011).

1.2 **Programação Metabólica ou Plasticidade Ontogenética**

Alterações nutricionais, hormonais e ambientais durante estágios críticos do desenvolvimento, tais como gestação e lactação, podem alterar a fisiologia e o metabolismo de um organismo provocando o desenvolvimento de distúrbios metabólicos na vida adulta (de Moura et al., 2008, Fernandez-Twinn & Ozanne, 2010). Este fenômeno é conhecido como programação metabólica foi proposto por Barker, que sugeriu que o baixo peso ao nascer causado por um ambiente intrauterino adverso estava relacionado ao risco aumentado de desenvolvimento de doenças crônicas na idade adulta como diabetes tipo 2, hipertensão e doença cardiovascular (Barker, 1995; Barker, 2007). A programação metabólica, portanto, sugere que o feto é sensível a alterações nutricionais durante seu desenvolvimento. Do ponto de vista evolutivo, quando o fornecimento de nutrientes é precário, respostas adaptativas provocam prioridade no desenvolvimento de alguns órgãos em detrimento de outros. Tais alterações teriam como objetivo

preparar o metabolismo para a sobrevivência do indivíduo frente a condições adversas no futuro (Hales & Barker, 1992; Simmons, 2005). Entretanto, estas alterações adaptativas podem persistir mesmo em condições normais de fornecimento de nutrientes, fazendo com que a modificação de funções de órgãos e tecidos deixe de ser uma vantagem adaptativa, tornando-se deletéria para o organismo. Atualmente, o termo "plasticidade ontogenética" tem sido utilizado e propõe uma forma menos determinística e mais probabilística para explicar o surgimento de doenças em resposta a insultos durante um período crítico (Gluckman & Hanson, 2007).

Desde a primeira observação em humanos da relação entre o baixo peso ao nascer e o desenvolvimento de obesidade e doenças crônicas na vida adulta feita por Ravelli et al. (1976), estudos epidemiológicos em diferentes regiões no mundo têm reforçado esta hipótese (Gluckman & Hanson, 2004; Barker, 2006; Fan et al., 2010; Efstathiou et al., 2012). No Brasil, no município do Rio de Janeiro, evidenciou-se que a deposição abdominal de gordura e a hipertensão arterial em mulheres adultas estiveram associadas à desnutrição perinatal (Sichieri et al, 2000a, Sichieri et al, 2000b). Em São Paulo, a baixa estatura em crianças associada ao nível de pobreza ao nascer e, provavelmente, à má nutrição, indicou um maior risco para o desenvolvimento de obesidade abdominal e hipertensão arterial na vida adulta (Hoffman et al., 2000a; Hoffman et al., 2000b; Sawaya et al., 2003).

Nas últimas décadas, diversos estudos experimentais foram desenvolvidos para melhor compreender os mecanismos modulados pela plasticidade ontogenética. Diferentes estratégias como alteração da nutrição materna ou do crescimento fetal, exposição da mãe ou do filhote a hormônios, poluentes ou fatores ambientais, entre outros, têm sido utilizadas visando mimetizar alterações que pudessem ocorrer no ambiente pré ou pós-natal (Gluckman & Hanson, 2007). Em nosso laboratório desenvolvemos diferentes modelos de plasticidade ontogenética buscando compreender a programação da massa corporal e da adiposidade, do metabolismo energético, assim como de alterações hormonais em ratos na vida adulta. Animais cujas mães sofreram desnutrição calórica na lactação tiveram aumento do peso corporal e resistência ao efeito anorexigênico da leptina na vida adulta (Passos et al., 2000, Passos et al., 2004). Já a desnutrição proteica materna durante a lactação esteve associada com menores massas corporal e de gordura total e central, menores concentrações séricas de insulina e glicose e também resistência ao efeito anorexigênico da leptina na vida adulta (Passos et al., 2004; Fagundes et al., 2009). Além disso, estes animais apresentam aumento da capacidade antioxidante e maior sensibilidade ao efeito do

tratamento com o antioxidante resveratrol (Franco et al., 2010). Nestes dois modelos de alterações nutricionais na lactação, as mães apresentaram hipoprolactinemia (Lisboa et al., 2006) e suas proles apresentaram hiperleptinemia ao desmame (Teixeira et al., 2002) e esta alteração metabólica contribuiu para o desenvolvimento de modelos de exposição à leptina durante a lactação (administrada na rata lactante ou no filhote). As proles programadas pela hiperleptinemia neonatal apresentaram aumento da massa corporal e alterações endócrinas como hiperleptinemia, resistência ao efeito anorexigênico da leptina na vida adulta (Lins et al, 2005; Toste et al., 2006; Trevenzoli et al., 2007; Passos et al., 2009; Pereira-Toste et al., 2009; Trevenzoli et al, 2010). A inibição da produção da PRL (prolactina) por um agonista dopaminérgico - a bromocriptina causou um desmame precoce nas proles e programou para obesidade, dislipidemia, resistência à insulina e à leptina (Bonomo et al., 2007; de Moura et al., 2009). Estas mesmas alterações foram mostradas recentemente em proles adultas programadas por outro modelo de desmame precoce, sem uso de substância farmacológica ou separação materna (Lima et al., 2011; Lopes Nobre et al, 2011). Assim como a desnutrição, a supernutrição (SN) na lactação, mimetizada pelo modelo experimental de redução do tamanho da ninhada, também provocou obesidade do tipo visceral, hiperinsulinemia e resistência à insulina e à leptina nas proles adultas (Rodrigues et al. 2009, Conceição et al. 2011; Rodrigues et al., 2011).

1.2.1 Programação metabólica pelo desmame precoce

A amamentação exclusiva reduz a mortalidade infantil causada por doenças infecciosas gastrointestinais e respiratórias até o segundo ano de vida (WHO, 2000; Kramer et al., 2003; Melo et al., 2008). Muitos estudos epidemiológicos têm sugerido também que o aleitamento materno está associado à prevenção da obesidade. Entretanto, a avaliação dos achados sobre a relação entre aleitamento materno e risco futuro de obesidade torna-se difícil devido a curta duração dos estudos e a ocorrência de fatores de confusão (Kramer et al., 2007; Ryan, 2007; Karmer et al., 2009; Fewtrell, 2011). Uma análise de 14 estudos publicados entre 2003 e 2006 sobre relação entre amamentação e risco de sobrepeso e obesidade infantil mostrou que três estudos relataram um efeito protetor em crianças (ou seja, o aumento da duração da amamentação

foi associado a um menor risco de sobrepeso ou obesidade infantil), quatro relataram um efeito de proteção parcial (ou seja, apenas evidente em um subgrupo), seis relataram nenhum efeito protetor, e 1 relatou um efeito protetor em crianças, mas não em adultos (Ryan, 2007). Entretanto, Arenz et al. (2004) mostraram através de metanálise que incluiu nove estudos com mais de 69 mil participantes que a amamentação reduziu o risco de obesidade na infância. Assim como Harder et al. (2005), que mostraram uma associação inversa entre a duração da amamentação e o risco de sobrepeso. Mais recente, um estudo com 126 adolescentes identificou que o aleitamento materno até pelo menos os 3 meses de idade esteve associado com menor risco de obesidade e menor circunferência da cintura em crianças e adolescentes (de Armas et al., 2009). No Brasil, um estudo realizado na cidade de São Paulo, mostrou que crianças e adolescentes que nunca receberam aleitamento materno têm maior ocorrência de obesidade na idade escolar, porém não encontrou efeito dose-resposta entre duração da amamentação e risco de obesidade (Scanferla de Siqueira & Monteiro, 2007). Os achados controversos sobre a relação entre amamentação e o risco futuro de obesidade indicam a necessidade de mais estudos, em particular estudos longitudinais e experimentais. Em virtude dos inúmeros benefícios do leite materno à saúde da criança, a amamentação exclusiva até os seis meses de idade tem sido considerada estratégica em saúde pública e potencialmente útil na prevenção da obesidade (Sichieri & Souza, 2008).

A Organização Mundial de Saúde define amamentação exclusiva como o consumo exclusivo de leite materno, sem nenhum outro tipo de alimento, suco ou mesmo água durante os seis primeiros meses de vida (WHO 2010). A introdução de qualquer tipo de alimento na dieta de uma criança que, até então, se encontrava recebendo aleitamento materno exclusivo deve ser definida como desmame. Dessa forma, "desmame precoce" pode ser compreendido como a interrupção do aleitamento materno antes que a criança complete seis meses de idade (Candeia et al., 1983).

Recentemente, o Ministério da Saúde publicou um estudo sobre a prevalência da amamentação no Brasil (Brasil, 2009). Os dados deste estudo foram coletados no ano de 2008 e comparados aos resultados anteriores obtidos no ano de 1999. A prevalência de amamentação exclusiva em crianças menores de seis meses de idade foi de 41% nas capitais brasileiras, porém os resultados variaram de 27% em Cuiabá, até 56% em Belém. A duração média do aleitamento materno exclusivo foi de 1,8 meses e do aleitamento materno foi de 11,2 meses. A prevalência do

aleitamento materno exclusivo em crianças menores de quatro meses subiu de 35% em 1999 para 51% em 2008, principalmente nas regiões Sudeste, Norte e Centro-Oeste. Entretanto, o estudo evidenciou a introdução precoce de alimentação complementar: 21% das crianças com idade entre 3 e 6 meses já consumia alimentos sólidos salgados e 24% das crianças na mesma faixa etária já fazia o consumo de frutas.

No Brasil, em 8 de setembro de 2008, foi sancionada pelo então Presidente da República, Luis Inácio Lula da Silva, a lei número 11770, que prorroga o tempo da licença maternidade por mais 60 dias, somando ao todo 6 meses, que até o momento era de apenas 4 meses, conflitando com o período de amamentação exclusiva recomendado pela OMS (Lei 11770). Apesar dos avanços nas políticas públicas nacionais de incentivo ao aleitamento materno, o desmame precoce permanece um importante desafio para os órgãos de saúde.

Entre as razões para a interrupção do aleitamento materno antes dos seis meses de idade (desmame precoce), a falta de leite e necessidade de retorno ao trabalho foram frequentemente alegados pela mãe (Carrascoza et al., 2005; Volpini & Moura, 2005; Faleiros et al., 2006, Mascarenhas et al., 2006). A situação socioeconômica também é um importante fator que influencia o desmame precoce (Faleiros et al., 2006). Estudos indicaram que mães com maiores níveis de escolaridade amamentam mais tempo os seus filhos (Escobar et al., 2002; Volpini & Moura, 2005).

Em animais experimentais, a interrupção da amamentação e a introdução de alimentos sólidos antes do tempo natural são estratégias utilizadas para mimetizar o desmame precoce e estudar os efeitos a curto e em longo prazo da amamentação regular sobre diferentes parâmetros (Kikusui, et al., 2006; Osaki et al., 2011; dos Santos Oliveira et al., 2011). Ratas geralmente entram no cio e podem engravidar aproximadamente 21 a 22 dias pós-parto. Por essa razão, ratos utilizados na pesquisa experimental são geralmente desmamados aos 21 dias de idade (Kikusui, et al., 2006). Após cerca de duas semanas de vida, filhotes de ratos são capazes de comer, manter a temperatura corporal, e evacuar (Plaut e Davis, 1972).

Nos últimos anos, o desmame precoce tem sido relacionado ao desenvolvimento de distúrbios comportamentais em roedores na vida adulta. Ono et al. (2008) observaram que o desmame precoce em camundongos machos aumentou os níveis de ansiedade nas 3º a 5º semanas de idade. A privação materna aos 14 dias da lactação levou a um substancial aumento nos níveis séricos de corticosterona dos filhotes, além de aumentar a resposta ao estresse, associada à

diminuição de receptores para glicocorticoides no hipocampo (Kikusui et al., 2006). Além disso, o desmame precoce em ratos machos está associado ao aumento da agressividade na idade adulta (Kikusui & Mori, 2009). Estes estudos ressaltam a importância da interação física entre mãe-filhote, uma vez que utilizam a separação materna para induzir o desmame precoce (Liu, et al. 2000, Uriarte, et al. 2007).

Em nosso laboratório foi desenvolvido um modelo experimental de desmame precoce com intuito de compreender sua relação com a programação da massa corporal, da adiposidade e de disfunções hormonais, em virtude da escassez de trabalhos na literatura. Primeiramente, foi realizado um estudo onde ratas Wistar lactantes receberam injeção de bromocriptina, um agonista dopaminérgico, inibidor da produção de PRL (prolactina), nos últimos três dias da lactação (Bonomo et al., 2005). Na idade adulta, as proles programadas apresentaram obesidade, hiperleptinemia e resistência ao efeito anorexígeno da leptina, porém sem alteração do consumo alimentar (Bonomo et al., 2007) e hipotireoidismo central, sugerindo um hipometabolismo nestes animais (Bonomo et al., 2008). Ainda, mostramos o desenvolvimento de dislipidemia, resistência a insulina e hipoadiponectinemia (de Moura et al, 2009) e disfunção renal (Passos et al., 2011) na idade adulta neste mesmo modelo experimental. Fraga et al. (2011) evidenciou que estes animais apresentaram distúrbios comportamentais como diminuição de memória e aprendizado e aumento de ansiedade. Em outro modelo de administração de bromocriptina a ratas lactantes (dias 7, 8 e 9 da lactação), as proles desenvolveram menor adiposidade visceral e hipotireoidismo na idade adulta (Lisboa et al., 2010).

No entanto, não se sabia se as alterações encontradas na vida adulta de filhotes cujas mães receberam bromocriptina ocorreram pelo uso deste fármaco ou somente pela menor oferta de leite materno às proles. Desta, maneira objetivando isolar os fatores de confusão, foi desenvolvido um novo modelo de desmame precoce sem utilização de fármaco e sem a necessidade de separação materna (DP). Neste modelo, ao fim do 17º dia da lactação, as mães foram anestesiadas com dose não letal de tiopental (0,06mg/100g de peso corporal) e enfaixadas com fita adesiva do tipo esparadrapo que impediu o acesso dos filhotes ao leite materno nos últimos 3 dias da lactação. Lima et al. (2011) mostraram que o desmame precoce, provocado então pelo bloqueio mecânico da amamentação, causou desnutrição nas proles ao desmame e provocou o desenvolvimento de sobrepeso, hiperfagia, aumento da adiposidade visceral, hiperglicemia, resistência à insulina, hipoadiponectinemia, aumento de triglicerídeos séricos, hiperleptinemia e resistência central à

leptina evidenciada pelo menor conteúdo hipotalâmico das proteínas JAK2 e pSTAT3 na vida adulta. Recentemente, evidenciamos que estes animais também apresentaram aumento da expressão de NPY e diminuição de CART especificamente no PVN (Younes-Rapozo et al., 2012).

1.2.2 Programação metabólica pela supernutrição na lactação

A prevalência de obesidade infantil nas últimas décadas aumentou no mundo todo (de Onis et al., 2010). Nos Estados Unidos, o número de crianças obesas triplicou nos últimos 30 anos. O percentual de crianças obesas com idade entre 6 e 11 anos aumentou de 7% em 1980 para quase 20% em 2008, e o percentual de adolescentes obesos entre 12 e 19 anos de idade aumentou de 5% para 18% no mesmo período. Em 2008, evidenciou-se mais de 1/3 de crianças e adolescentes com sobrepeso ou obesidade (Flegal et al., 2012; Ogden et al., 2010). No Brasil, um estudo recente revelou que 22% de crianças e adolescentes com idade entre 5 e 19 anos apresentavam obesidade (Gupta et al., 2012). A Pesquisa de Orçamentos Familiares (POF 2008-2009) realizada pelo Instituto Brasileiro de Geografia e Estatística (IBGE), em parceria com o Ministério da Saúde, mostrou um aumento importante no número de crianças com sobrepeso no país, principalmente na faixa etária entre 5 e 9 anos de idade. O número de meninos com sobrepeso aumentou de 15% em 1989 para 34,8% em 2009, assim como o número de obesos nesse mesmo grupo etário mais que triplicou, passando de 4,1% para 16,6%. Esse aumento também foi visto em meninas entre 5 e 9 anos de idade: a prevalência de obesidade aumentou de 2,4% em 1989 para 11,8% em 2009 (IBGE, 2010).

Estudos epidemiológicos sugerem que jovens com sobrepeso e obesidade apresentam maiores chances de se tornarem adultos com sobrepeso e maior risco cardiovascular (Reilly et al., 2003; Must, 2003, Owen et al., 2009, Conde & Borges, 2011). Esta associação tem sido reforçada através de estudos experimentais de excesso de nutrientes no período neonatal (Davidowa & Plagemann, 2007; Rodrigues et al., 2009; Rodrigues et al., 2011; Conceição et al., 2011).

A redução do número de filhotes da ninhada é um modelo experimental proposto para induzir a supernutrição na infância em roedores (Plagemann et al., 1992). Em condições de

grande oferta de leite, os filhotes ingerem um volume próximo da capacidade do trato gastrointestinal (Houpt & Epstein, 1973). A ingestão abundante de leite pode levar o animal à supernutrição, pois o controle hipotalâmico da ingestão de alimentos no início da vida pós-natal não está totalmente estruturado. O desenvolvimento da rede hipotalâmica reguladora do apetite em roedores ocorre predominantemente após o nascimento. Somente em torno de 20 dias pós-nascimento, os neurônios do núcleo ventromedial (VMN) adquirem aparência citológica semelhante à de um animal adulto (Pozzo Miller & Aoki, 1992). Enquanto o NPY está presente no núcleo arqueado (ARC) do feto próximo já ao 14° dia de gestação, projeções de NPY/AgRP entre o ARC e o núcleo dorso medial (DMN) não estão completas até aproximadamente 10-11 dias após o nascimento, assim como projeções de NPY para o núcleo paraventricular (PVN) hipotalâmico não estão completamente desenvolvidas até o 15°-16° dia de vida (McMillen et al., 2005). Durante a primeira semana de vida, parece haver uma dominância relativa de inervações NPY e αMSH no PVN derivadas do tronco cerebral sobre inervações derivadas do ARC, sugerindo, portanto, que a informação sensorial vagal no intestino relativa à presença do alimento pode ser importante na regulação do comportamento alimentar em filhotes de ratos neste período (Grove & Smith, 2003).

A supernutrição perinatal tem sido relacionada à permanência de excesso de peso, hiperfagia, hipertensão e hiperinsulinemia na vida adulta (You et al., 1990; Plagemann et al., 1992; Boullu-Ciocca et al., 2005; Davidowa & Plagemann, 2007; Rodrigues et al., 2007; Lopez et al., 2007). Mais recente, foi observado que a supernutrição na lactação esteve associada à hipermetilação das regiões promotoras do receptor de insulina e de POMC (Plagemann et al., 2009; Plagemann et al., 2010). A metilação, um fenômeno epigenético bastante estudado atualmente, consiste na ligação covalente de um radical metil à posição 5 do anel de citosina de um dinucleotídio CpG (uma base citosina unida a uma base guanina) localizado em uma seqüência de DNA. Como a maioria dos genes dos mamíferos possuem ilhas CpG contidas na região promotora, o acúmulo de metilações nessa região pode impedir a ligação dos fatores de transcrição a seus respectivos domínios específicos, inibindo, dessa forma, o processo de transcrição de um determinado gene (Tate et al., 1993; Robertson, 2001). A metilação (hiper ou hipometilação) do DNA, juntamente com a desacetilação de histonas, provocando alteração da atividade de um determinando gene, é um mecanismo epigenético sugerido para explicar como

eventos ambientais no início da vida causam modificações nos padrões de expressão gênica fenotípica tardiamente (Pinney & Simmons, 2012).

Em nosso laboratório, evidenciamos que a supernutrição na lactação, induzida pela redução do número de filhotes ao 3° dia da lactação para 3 filhotes por ninhada, causou obesidade total e visceral, diminuição de HDL-c e resistência central à leptina, evidenciada pelo menor conteúdo de pSTAT3 e maior de SOCS3 no hipotálamo (Rodrigues et al, 2011), hipotireoidismo (Rodrigues et al, 2009) e aumento da área dos adipócitos viscerais associada a uma menor produção de leptina em ratos aos 180 dias de idade (Conceição et al., 2011).

1.3 **Resveratrol**

O resveratrol (Res, 3, 5, 4'-trihidroxi-trans-estilbeno) é um polifenol estilbeno presente em mais de 70 espécies de plantas das quais fazem parte da dieta humana uvas, amoras, amendoim, entre outras. Em 1963, o resveratrol foi identificado em raízes de *Polygonum cuspidatum*, utilizada na medicina chinesa e japonesa. Porém, o interesse pelo resveratrol foi despertado na década de 90, quando quantidades significativas foram identificadas no vinho tinto (Gu et al., 1999) e este polifenol foi considerado um fator importante para o Paradoxo Francês, um termo cunhado para descrever a observação de que apesar de uma dieta rica em gorduras saturadas, a população francesa tem uma incidência muito baixa de doenças cardiovasculares (Renaud & de Lorgeril, 1992).

As mais importantes uvas que contém resveratrol são *Vitis vinifera*, *Vitis labrusca* e *Vitis moscadine*, nas quais esse composto é encontrado pincipalmente na casca em quantidades que podem variar de 50 a 100 μg/g. (Ector et al., 1996). No vinho tinto, a concentração de resveratrol pode variar de 1,5 a 3,0 mg/L (Dong, 2003), podendo chegar até 7 mg/L (Bertelli et al., 1996). Um estudo revelou que o vinho brasileiro pode ser considerado uma boa fonte de resveratrol, uma vez que foi estimado um consumo total de 5,3 mg/dia de compostos estilbenos em pessoas com um consumo regular de 160 ml de vinho tinto (Vitrac et al., 2005).

Nos últimos anos, o resveratrol tem recebido bastante atenção devido a suas propriedades anti-inflamatórias, anti-carcinogênicas e antioxidantes, bem como sua capacidade de aumentar a

longevidade em pequenos organismos e melhorar a saúde geral em mamíferos (Pervaiz & Holme, 2009). Os efeitos biológicos do resveratrol incluem: supressão da proliferação celular via inibição de etapas chave em vias de transdução de sinais, promoção de diferenciação celular, varredura de ERO e intermediários, indução da morte celular por apoptose através da ativação de vias dependentes ou não da mitocôndria e atividade antiinflamatória via subsensibilização de citocinas pró inflamatórias (Pervaiz, 2004; Markus & Morris; 2008; Pervaiz & Holme, 2009). Estudos mostraram que o resveratrol aumentou a captação de glicose pelo músculo, fígado e adipócito (Su et al., 2006); aumentou a síntese e liberação de óxido nítrico pela eNOS (Baur & Sinclair, 2006); diminui o triglicerídeo e o colesterol, através da redução da síntese "de novo" de ácidos graxos, da incorporação de acetato em triglicerídeos e da atividade da acetil-CoA carboxilase (Gnoni & Paglialonga, 2009) e inibiu a secreção de hormônios tais como a leptina em adipócitos (Szkudelska et al., 2009) e a insulina em ilhotas pancreáticas (Szkudelski, 2006). Dessa maneira, seus efeitos sobre alterações metabólicas como diabetes tipo 2, dislipidemia e hipertensão arterial, têm sido bastante estudados nos últimos anos.

A atuação do resveratrol como antioxidante se dá tanto de maneira direta através da varredura de espécies reativas como o radical hidroxila e o ânion superóxido ou por quelar-se a metais pesados, e indireta, através da modulação de sistemas pró ou antioxidante (Bradamante et al., 2004). Porém, a atividade antioxidante do resveratrol parece ser mais eficiente através da modulação de sistemas pró ou antioxidante (Spanier et al., 2009). O resveratrol atua inibindo genes pró-oxidantes, como a NADPH oxidase, e induzindo a atividade e expressão de enzimas oxidantes. A capacidade do resveratrol em aumentar a atividade e/ou expressão de enzimas antioxidantes tem sido demonstrada tanto em estudos *in vitro* (Tatlidede et al., 2009; Zheng et al., 2010) quanto *in vivo* (Robb et al., 2008; Bhatt et al., 2011; Schmatz et al., 2012).

Estudos demonstraram que o resveratrol protegeu camundongos contra a obesidade induzida por dieta (Lagouge et al., 2006) e, *in vitro*, inibiu a diferenciação de pré-adipóctos e induziu a apoptose de adipócitos maduros (Yang et al., 2008). Desde então, modelos de obesidade em roedores têm sido desenvolvidos para melhor compreender os mecanismos pelos quais o resveratrol promove resistência contra o acúmulo de tecido adiposo (Rivéra et al., 2009; Louis et al., 2011; Goméz-Zorita et al., 2012).

Outro mecanismo conhecido de ação do resveratrol é a ativação de vias que também são estimuladas pela restrição calórica crônica, uma condição conhecida por aumentar o período de

vida e retardar a incidência de doenças em muitas espécies, desde leveduras até mamíferos (Koubova & Guarente, 2003). Howitz et al. (2003) demonstraram, em leveduras, que e mimetização dos efeitos benéficos da restrição calórica crônica estavam associados a ativação da sirtuína 1 (SIRT1), uma histona desacetilase. Recentemente, outros estudos confirmaram a capacidade do resveratrol em ativar a SIRT1 em roedores (Franco et al., 2010; Yar et al., 2011). Além de utilizar histonas como substratos, a SIRT1 atua sobre proteínas não-histonas, principalmente sobre fatores de transcrição, tais como: a subfamília ForkHead O (FOXO), o receptor ativado por proliferadores do peroxissoma (PPAR γ), o coativador de transcrição gênica 1α (PGC-1 α), o gene supressor tumoral p53 e acetil-CoA sintetase (Yamamoto et al., 2007; Yu & Auwerx, 2009). Dessa maneira, a ativação da SIRT 1 pelo resveratrol pode estar envolvida em muitas de suas ações, principalmente aquelas envolvidas no metabolismo energético.

2 **OBJETIVOS**

Em ratos adultos programados pela supernutrição na lactação: avaliar o estresse oxidativo, a morfologia hepática e a via de sinalização da insulina.

Em ratos adultos programados pelo desmame precoce: avaliar o estresse oxidativo, a pressão arterial e a morfologia hepática e os efeitos do resveratrol sobre estes parâmetros bem como sobre a sinalização da leptina e o NPY no hipotálamo.

3 **ARTIGOS**

3.1 **Artigo1-** Oxidative stress programming in a rat model of postnatal early overnutrition – role of insulin resistance

Conceição EPS; **Franco JG,** Oliveira E, Resende AC, Amaral TAS, Passos MCF, Moura EG, Lisboa PC. Oxidative stress programming in a rat model of postnatal early overnutrition – role of insulin resistance. Artigo submetido ao *The Journal of Nutritional Biochemistry.*

Oxidative Stress Programming In a Rat Model of Postnatal Early Overnutrition – Role of Insulin Resistance

Ellen PS Conceição[1*], Juliana G Franco[1*], Elaine Oliveira[1], Angela C Resende[2], Taline AS Amaral[2], Nayara Peixoto-Silva[1], Magna C F Passos[1], Egberto G Moura[1], Patrícia C Lisboa[1]

[1]Department of Physiological Sciences, Roberto Alcântara Gomes Biology Institute, State University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.
[2]Department of Pharmacology and Psychobiology, State University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Running Title: Early overfeeding and oxidative stress
Key words: small litter; reactive oxygen species; insulin signaling.

Corresponding Author:
Dr Patrícia Cristina Lisboa – PhD
Departamento de Ciências Fisiológicas – 5º andar
Instituto de Biologia - Universidade do Estado do Rio de Janeiro
Av. 28 de Setembro, 87- Rio de Janeiro, RJ, 20550-030 - Brazil
Phone #: (+55.21) 25876434, FAX #: (+55.21) 25876129

E-mail: pclisboa@uerj.br / patricialisboa@pq.cnpq.br

*EPSC and JGF contributed equally to this study.

Funding: This research was supported by the "National Council for Scientific and Technological Development" (Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq), the "Carlos Chagas Filho Research Foundation of the State of Rio de Janeiro" (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro-FAPERJ) and Coordination for the Enhancement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES).

ABSTRACT

Postnatal early overfeeding (EO) is related to later development of overweight and other metabolic disorders. As oxidative stress is implicated in most human diseases, as obesity and diabetes, we decided to study some parameters related to oxidative stress and insulin signaling in liver from EO animals in adult life. To induce EO, litter size was reduced to 3 pups/litter (SL: small litter) and groups with normal litter size (NL: 10 pups/litter) were used as control. After weaning, rats had free access to standard diet and water. Body weight and food intake were monitored daily and offspring were killed at 180 days-old. Significant differences had $p<0.05$ or less. As expected, SL rats had hyperphagia, higher body weight and higher visceral fat mass at weaning and adulthood. In liver, postnatal EO programmed for lower catalase (-42%), superoxide dismutase (-45%) and glutathione peroxidase (-65%) activities. The evaluation of liver injury in adult SL group showed lower nitrite content (-10%), higher liver and plasma malondialdehyde content (+25% and 1.1 fold-increase, respectively). No changes of total protein bound carbonyl or Cu/Zn SOD protein expression in liver were detected between the groups. Regarding insulin signaling pathway in liver, SL offspring showed lower IRβ (-66%), IRS1 (-50%), phospho-IRS1 (-73%), PI3-K (-30%) and Akt1 (-58%). Indeed, morphological analysis showed that SL rats presented focal areas of inflammatory cell infiltrate and lipid drops in their cytoplasm characterizing a microsteatosis. Thus, we evidenced that postnatal EO can program the oxidative stress in liver, maybe contributing for impairment of the insulin signaling.

INTRODUCTION

In recent decades, the prevalence of childhood obesity has greatly increased worldwide [1]. It is known that nutritional, environmental and/or hormonal influences during critical periods early in life can permanently change the structure and function of body tissues and systems; this association is denominated metabolic programming [2] and it was confirmed by epidemiological and experimental data [3,4]. Studies in animal models have shown that excess of nutrition in perinatal life represents a risk factor for obesity and associated metabolic disturbances in adulthood [5,6,7]. Recently, in a systematic review and meta-analysis, Risnes *et al*. [8] showed a strong association between higher birthweight and increased risk of cancer deaths.

Rats raised in 'small litters' (SL) are an established animal model to study short- and long-term consequences of childhood obesity. This model of postnatal early overnutrition (EO) was associated with hyperphagia, obesity, hypertension and hyperinsulinemia in adult life [10, 11, 12,13,14]. Other studies have suggested that oxidative stress, the imbalance between cellular production of reactive oxygen species (ROS) and antioxidant defenses in cells, could be an early event in the development of obesity-related chronic diseases, such as cardiovascular diseases, diabetes mellitus and cancer [15, 16]. Nutrient overload and obesity increase ROS generation and oxidative stress. Excessive nutrient in the metabolic pathways leads to an increased electron flux through mitochondrial electron transfer chain. The consequent electron leak from respiratory complex I and III of electron transfer chain leads to an increased production of ROS from the mitochondria, such as superoxide and hydrogen peroxide [15].

Previously, we have shown in adult SL rats, the programming for overweight, higher total and visceral fat mass, lower HDL-C, hyperphagia, central leptin resistance and thyroid hypofuntion in adult life [6, 7]. At weaning, SL rats have insulin resistance characterized by an increase in fasting glucose levels and hyperinsulinemia, while at 6 months old, these animals showed a slight impairment in glucose tolerance test, 60 and 120 minutes after glucose load, suggesting insulin resistance, despite basal normoglycemia and normoinsulinemia [7]. Other reports showed that older (8 months old) SL rats present insulin resistance, suggesting that insulin resistance in this experimental model seem to be age dependent [10, 11].

Since ROS have been proposed as a unifying mechanism linking nutrient excess and obesity-associated disturbances, in the present study we evaluated some parameters related to oxidative stress in adult rats programmed by EO. In addition, considering that there are two-way

association between excessive ROS and insulin resistance, we studied the insulin signaling in liver.

METHODS AND MATERIALS

The use of the animals according to our experimental design was approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro (CEUA/184/2007; CEUA/006/2009), which based their analysis on the principles adopted and promulgated by the Brazilian Law issued on November 8, 2008 [17,18]. Wistar rats were housed in a room with controlled temperature (25± 1°C) and artificial dark–light cycles (lights on 07:00h, lights off 19:00h). Adult virgin female rats were caged with male rats (3:1) and after mating, each female was placed in an individual cage with free access to water and food until delivery.

Experimental Model of Postnatal EO

To induce EO during lactation, 3 days after birth, the litter size was adjusted to three male rats per litter (SL) [11,6]. Litter containing 10 pups per mother was used as control (NL). The rats analyzed were randomly chosen from 16 different litters (8 SL litters and 8 NL litters). After postnatal day 21 (PN21) that corresponds to weaning period, both groups have free access to water and standard diet. During lactation, body weight (BW) gain was daily monitored and from weaning until PN180, body weight and food intake (g/100g BW) were monitored every 4 days.

At PN180, rats were killed after to be anaesthetized with pentobarbital (0.06 g/kg BW) in order to collect blood, liver and visceral fat mass (VFM). The blood was collected by cardiac puncture and poured in a tube containing EDTA. The VFM (mesenteric, epididymal and retroperitoneal white adipose tissue) was excised and immediately weighed for evaluation of central adiposity. Plasma and liver samples were frozen at -80°C until analysis.

Determination of antioxidant enzyme activities in liver

Liver samples of 200 mg were homogenized in potassium phosphate buffer with EDTA in mechanical homogenizer (CT – 136 model from Cientec - laboratory equipment, Campinas, SP, Brazil). After centrifugation, homogenates were stored at -80°C until analysis. The total protein content was determined by the Bradford method [19].

Total superoxide dismutase (SOD) activity was assayed by measuring the inhibition of adrenaline auto-oxidation as absorbance at 480 nm [20]. Catalase (CAT) activity was measured

by the rate of decrease in H_2O_2 at 240 nm according to the method of Aebi [21]. Glutathione peroxidase (GPx) activity was evaluated according to Flohé & Günzler [22] by measuring the oxidation of NADPH at 340 nm in the presence of H_2O_2.

Nitrite assay

The yield of radical nitric oxide (NO) an indirect measurement of nitric oxide content was evaluated by Griss reaction through quantification of nitrite (NO_2^-) in liver at 540 nm [23].

Thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation was measured by malondialdehyde (MDA) concentration using the thiobarbituric acid reactive substances (TBARS) method as previously described [24, 25]. Briefly, plasma and liver homogenates were mixed with 1mL of 10% trichloroacetic acid and 1mL of 0.67% thiobarbituric acid (Sigma Chemical Co., St. Louis, MO, USA); subsequently they were heated in a boiling water bath for 30 min. The absorbance of the organic phase containing the pink chromogen was measured spectrophotometrically at 532 nm. MDA equivalents were expressed in nMol/mg protein.

Protein oxidation

Protein oxidation was evaluated in liver accordingly Levine *et al*. [26] as carbonyl groups reacting with 2,4-dinitrophenyl-hydrazine (DNPH; Sigma, St. Louis, MO, USA). Values of absorbance were obtained at 380 nm and expressed in nmol of carbonyl by 0,5 mg of protein.

Western blotting analysis

Liver samples were homogenized in cold lysis buffer (50 mM Hepes, pH 6.4, 1 mM MgCl2, 10 mM EDTA and 1% Triton X-100) containing protease inhibitors (10 µg/µl aprotinin, 10 µg/µl leupeptin, 2 µg/µ l pepstatin and 1 mM phenylmethylsuphonic fluoride - Sigma-Aldrich, St Louis, MO, USA) using a Ultra-Turrax® homogenizer (IKA Werke GmbH & Co. KG, Staufen, Germany). After centrifugation, homogenates were stored at -20 ºC. The total protein content was determined by the BCA™ protein assay kit (Pierce, Rockford, IL, USA).

Samples (30 µg total protein) were electrophoresed in 10-12% tris-glycine sodium dodecyl sulfate (SDS) polyacrylamide gels. Proteins were transferred for polyvinylidene fluoride membranes (Hybond ECL; Amersham Pharmacia Biotech, London, UK), blocked in 5% dry milk in T-TBS (0.02 M Tris/0.15 M NaCl, pH 7.5 containing 0.1% Tween 20) at room temperature for 1 hour, washed 3x with T-TBS and incubated with the primary antibodies (Cu/Zn SOD, IR β, IRS1, phospho-IRS1, PI3-K, Akt1 and phospho-Akt1 at 1:500 concentration) overnight at 4°C.

Primary antibodies were purchased from Santa Cruz Biotechnology Inc. (San Francisco, CA, USA). After washing 3x with T-TBS, blots were incubated with appropriate secondary antibodies at 1:5000 concentration (Santa Cruz Biotechnology, CA, USA) for 1 hour and then, incubated with streptavidin (Zymed, CA, USA) in the same dilution of the secondary antibody for 1 hour. Blots were developed with diaminobenzamidine (DAB; Sigma Chemical Co., St. Louis, MO) as chromogenic substrate or with enhanced chemiluminescence (ECL; Amersham Biosciences Inc., Piscataway, NJ).

Liver histology

Liver samples were fixed in formalin (freshly prepared 1.27 mol/L formaldehyde, 0.1 M phosphate-buffered saline, pH 7.2) and embedded in paraffin to non-serial sections of 5 µm. Sections were placed in glass slides to stain in hematoxylin/eosin. The morphological study was performed utilizing digital images, acquired at random (TIFF format, 36-bit color, 1360x1024 pixels) with an Olympus DP71 camera and an Olympus BX40 epifluorescence microscope (Olympus, Tokyo, Japan).

Statistical analysis

Data are reported as mean±S.E.M. The *GraphPad Prism 4* program (GraphPad softwares, Inc., La Jolla, CA, USA) was used for statistical analyses and graphics. Two-way ANOVA and Bonferroni post test were used to analyze body weight and food intake changes. Cu/Zn SOD expression and insulin signaling were analyzed by the non-parametric Mann-Whitney *U* test. The other experimental observations were analyzed by unpaired Student's t test, with significance level set at $P<0.05$.

RESULTS

Body weight, food intake and visceral fat mass

Body weight and food intake from weaning (PN21) to the sacrifice (PN180) are shown in Figure 1. Offspring overfed during lactation (SL) had higher body weight than NL rats from PN7 until the end of lactation (+10%, $p<0.0001$, Fig. 1A). SL rats remained overweight until PN180 (+15%, $p<0.05$, Fig. 1B). SL group presented a higher relative food intake from weaning until adulthood (PN180: +7%, $p<0.05$, Fig. 1C). Also VFM was higher (+92%, $p<0.0001$, Fig. 1D) in SL rats compared to NL rats.

Evaluation of oxidative stress parameters

As shown in Figure 2, adult SL offspring showed lower CAT (-42%, $p<0.0001$; Fig. 2A), SOD (-45%, $p<0.0001$, Fig. 2B) and GPx activities (-65%, $P<0.0001$, Fig. 2C) than the NL group. Despite the lower SOD activity, western blot analysis showed that Cu/Zn SOD content was not different between the groups (NL:101.49 ± 6.36 *vs* SL:86.60 ± 5.43).

As depicted in Figure 3, liver nitrite bioavailability was lower in SL than NL group (-10%, $p<0.0001$, Fig. 3A). Oxidative damage assessed by MDA quantification was higher in SL group both in liver (+ 25%, $p<0.05$; Fig. 3B) and in plasma (1.1 fold-increase; $p<0.05$; Fig. 3C).

No significant difference in liver total protein bound carbonyl was observed between groups (Fig. 3D).

Insulin signaling

Liver content of insulin signaling molecules IRβ, IRS1, phospho-IRS1, PI3-K, Akt1 and phospho-Akt1 are shown in Figure 4. The content of IRβ, phospho-IRS1, IRS1, PI3-K, Akt1 were lower in SL compared to NL group: IRβ (-66%; $p<0.05$); Fig. 4A; phospho-IRS1 (-73%; Fig 4B); IRS1 (-50%; $p<0.05$; Fig. 4C), PI3-K (-30%; $p<0.05$; Fig. 4D) and Akt1 (-58%; $p<0.05$; Fig. 4E). We did not found differences in the content of phospho-Akt1 between the groups (Fig 4F).

Liver histology

The morphological analysis showed a dysfunction in the hepatic tissue of adult SL offspring. As demonstrated in Figure 5, SL rats presented focal areas of inflammatory cell infiltrate and drops of lipids in their cytoplasm characterizing a microsteatosis, differently of the NL rats that demonstrated a liver with preserved architecture

DISCUSSION

In the present study we observed that EO induced by small litter size causes an increase in body weight gain during lactation and programs for hyperphagia and overweight in adult life, confirming previous reports [11, 27, 28, 14] and also our previous results showing that SL rats presented higher central adiposity as well as central leptin resistance at 180 days old [6, 7]. Since nutrient overload and obesity were associated with increased ROS generation the main focus of this study was to evaluate the oxidative stress in rats programmed by EO during lactation.

Obesity is associated with an unbalance of both lipid and carbohydrate metabolisms. These nutrients in excess also increase the demand on the mitochondria and the utilization of the

electron transport chain leading to an increased generation of ROS [29, 25]. Oxidative stress can occur as a result of increased ROS generation and/or failure of antioxidant system. The antioxidant system involves several nonenzymatic compounds and antioxidant enzymes such as SOD, CAT and GPx. SOD is the first line of antioxidant defense system. The two main isoforms of SOD, manganese superoxide dismutase (MnSOD) in mitochondria and copper-zinc superoxide dismutase (Cu/Zn SOD) in cytosol converts superoxide radical into H_2O_2. H_2O_2, in turn, is converted to oxygen and H_2O by CAT or GPX [30]. Our present findings reveal that SOD, CAT and GPx activities are significantly decreased in adult SL rats, suggesting a reduced antioxidant defense, although differences in Cu/ZnSOD content was not observed. Rector *et al*. [31] have demonstrated a reduced hepatic activity of the free radical scavenger SOD and increased oxidized glutathione in obese rodent model of nonalcoholic fatty liver disease. This negative imbalance between reduced antioxidant defense and increased oxidative damage likely predisposes hepatocytes and hepatic mitochondria to progressive injury.

In this study, the lipid oxidative damage assessed by plasma and liver levels of MDA, one of the key end products of lipid peroxidation was increased in SL rats. To our knowledge, this is the first evidence showing an increase of MDA levels associated with a deficient antioxidant defense in adult rats programmed by postnatal early overnutrition. The underlying causes for increased MDA levels in this model probably associated with increased ROS production such as O_2^- are not yet established, but a decreased activity of the enzymatic antioxidant defense system represented by SOD, CAT and GPx enzymes can be implicated.

The ROS or peroxynitrite are powerful oxidizing agents that might cause depletion of sulfhydryl groups and oxidation of many molecules causing damage [32]. They can also cause DNA damage such as breaks, protein oxidation, and nitration of aromatic amino acid residues in proteins [33]. Measurement of NO content through quantification of nitrite in the liver showed a decreased nitrite bioavailability in SL rats. One of the most important reactions under physiological conditions is that O_2^- and NO radicals result in peroxynitrite. It is well known that O_2^- is important in the breakdown of NO to peroxynitrite, thereby depleting NO [34]. Therefore, this decrease in nitrite levels presumably represents enhanced NO degradation by O_2^- in the presence of a deficient antioxidant mechanism of defense. On the other hand, insulin resistance is associated with lower NO generation [35].

The deficiency of activity of antioxidant enzymes and the higher plasma and liver MDA concentrations in SL rats can indicate a higher oxidative stress in these animals. Some studies have associated oxidative stress and its role in the development of insulin resistance. ROS have been shown to activate the stress-sensitive serine/threonine kinase c-jun N-terminal kinase (JNK), which in turn phosphorylates IRS at serine residues and thus attenuate insulin signaling [36]. In the present study, SL rats presented an impairment of the insulin signaling in the liver, confirmed by reduction of IR, IRS1, p-IRS1, PI-3K and Akt1 content. Previously, Rodrigues *et al.* [14] have demonstrated, in the 90 days-old SL rats, lower IRS1, PI-3K and GLUT-4 expressions and lower Akt activity in adipocytes. Also, Martins *et al.* [37] have found that 150 days-old SL swiss mice had decreased insulin sensitivity in the heart. However, in 1-year-old SL rats, no changes in liver and heart insulin pathway signaling were observed [38].

It is possible that the excessive fat tissue or the inabilities of fat storage, common on obesity, links nutrient excess to insulin resistance. The food intake reduction found in both C and SL groups at 180 days old compared with 120 days old is probably due to ageing, since orexigenic hypothalamic peptides are reduced during ageing [39]. Also, the higher body weight compared to the lower food intake could be explained by the lower rest metabolic rate (RMR) associated with ageing. We know that even in humans, ageing is associated with a higher visceral fat mass gain compared to total body mass, especially in men [40]. The increased free fat acids (FFA) flux into circulation causes ectopic accumulation of fat in tissues such as muscle and liver [41, 42]. Besides, adipose tissue not only releases FFA but also produces several inflammatory molecules including TNF-α and IL-6 which may have local effects on adipose metabolism and also systemic effects on other tissues [43, 44]. In liver, TNF-α inhibits insulin signaling by mechanisms including the activation of serine kinases such as JNK-1 and induction of SOCS proteins [45]. Likewise, IL-6 impairs insulin signaling in liver through serine phosphorylation of IRS-1 and activating SOCS proteins [43]. Furthermore, IL-6 induces VLDL secretion and hypertriglyceridemia and it could directly affect liver lipid metabolism [46, 47, 48].

The higher oxidative stress evidenced by the lower activity of antioxidant enzymes CAT, SOD and GPx and the higher MDA liver and plasma content could be responsible for the impairment in liver insulin signaling in the SL group. Kathirvel *et al*. [49] demonstrated the relation between the higher liver oxidative stress and impairment of insulin signaling in transgenic mouse model of nonalcoholic fatty liver disease. In vitro oxidative stress in

mammalian skeletal muscle leads to loss of IRS-1and IRS-2 proteins, increased relative IRS-1Ser^{307}phosphorylation, and decreased phosphorylation of Akt Ser ^{473} [16].

The oxidative stress is recognized as a promoter of important hepatic injury [50]. This damage is associated to an inflammatory response and microsteatosis (nonalcoholic steatosis) as demonstrated in postnatal EO offspring. Additionally, hepatic injury could be suggested in SL offspring through reduction of serum albumin and increased serum globulin demonstrated in our previous report [7]. Several studies in different experimental models have shown that the diminished ratio between albumin and globulin (A/G) could be considered as marker of hepatic tissue lesion [51, 52, 53].

In general terms, epigenetic mechanisms, such as DNA methylation or hystone acethylation/deacethylation, induced by neonatal imprinting factors (diets, hormones, pollutants) may lead to an increased risk of metabolic disorders in the adult offspring [4]. Studies correlate the visceral obesity to DNA hypermethylation of important enzymes involved in mitochondrial fatty acid oxidation, gluconeogenesis, and lipogenesis in the liver causing a silencing of your expression and contributing to obesity-induced liver insulin resistance [54, 55]. Thus, this explanation may help to understand the mechanism involved in the permanent changes of oxidative stress parameters and insulin signaling induced by overnutrition during lactation. Whether this alteration can turn overfed children more susceptible to cell damage caused by higher ROS generation in adult life, which deserves epidemiological and prospective studies.

In conclusion our present findings evidenced that postnatal EO can program the oxidative stress in liver, maybe contributing for impairment of the insulin signaling.

Declarations of interest: Authors declare no conflict of interest.

Acknowledgments: Authors are grateful to Ms Mônica Moura, Ulisses Siqueira and Carlos Alberto Guimarães for animal care and technical assistance.

REFERENCES

[1] de Onis M, Blössner M, Borghi E. Global prevalence and trends of overweight and obesity among preschool children. Am J Clin Nutr. 2010;92:1257-64.

[2] Lucas A. Role of nutritional programming in determining adult morbidity. Arch Dis Child. 1994;71:288-90.

[3] Barker DJ. The developmental origins of adult disease. J Am Coll Nutr. 2004;23:588-95.

[4] de Moura EG, Lisboa PC, Passos MC. Neonatal programming of neuroimmunomodulation - role of adipocytokines and neuropeptides. Neuroimmunomodulation. 2008;15:176-88.

[5] Dörner G, Hagen N & Witthuhn W. Early postnatal overfeeding as an etiopathogenetic factor in adult obesity. Acta Biol Med Ger. 1976;35:799-803.

[6] Rodrigues AL, de Moura EG, Passos MC, Dutra SC, Lisboa PC. Postnatal early overnutrition changes the leptin signaling pathway in the hypothalamic-pituitary-thyroid axis of young and adult rats. J Physiol. 2009;587:2647-61.

[7] Rodrigues AL, de Moura EG, Passos MC, Trevenzoli IH, da Conceição EP, Bonono IT, Neto JF, Lisboa PC. Postnatal early overfeeding induces hypothalamic higher SOCS3 expression and lower STAT3 activity in adult rats. J Nutr Biochem. 2011;22:109-17.

[8] Risnes KR, Vatten LJ, Baker JL, Jameson K, Sovio U, Kajantie E, Osler M, Morley R, Jokela M, Painter RC, Sundh V, Jacobsen GW, Eriksson JG, Sørensen TI, Bracken MB. Birthweight and mortality in adulthood: a systematic review and meta-analysis. Int J Epidemiol. 2011;DOI: 10.1093/ije/dyq267.

[9] Plagemann A, Roepke K, Harder T, Brunn M, Harder A, Wittrock-Staar M, Ziska T, Schellong K, Rodekamp E, Melchior K, Dudenhausen JW. Epigenetic malprogramming of the insulin receptor promoter due to developmental overfeeding. J Perinat Med. 2010;38:393-400.

[10] You S, Gotz F, RohdeW, Dorner G. Early postnatal overfeeding and diabetes susceptibility. Exp Clin Endocrinol. 1990;96:301-6.

[11] Plagemann A, Heidrich I, Götz F, Rohde W, Dörner G. Obesity and enhanced diabetes and cardiovascular risk in adult rats due to early postnatal overfeeding. Exp Clin Endocrinol. 1992;99:154-8.

[12] Boullu-Ciocca S, Dutour A, Guillaume V, Achard V, Oliver C, Grino M. Postnatal diet-induced obesity in rats upregulates systemic and adipose tissue glucocorticoid metabolism during development and in adulthood: its relationship with the metabolic syndrome. Diabetes. 2005;54:197-203.

[13] Davidowa H, Plagemann A. Hypothalamic neurons of postnatally overfed, overweight rats respond differentially to corticotropin-releasing hormones. Neurosci Lett. 2004;371:64-8.

[14] Rodrigues AL, De Souza EP, da Silva SV, Rodrigues DS, Nascimento AB, Barja-Fidalgo C, de Freitas MS. Low expression of insulin signaling molecules impairs glucose uptake in adipocytes after early overnutrition. J Endocrinol. 2007;195:485-94.

[15] Chang YC, Chuang LM. The role of oxidative stress in the pathogenesis of type 2 diabetes: from molecular mechanism to clinical implication. Am J Transl Res. 2010;2:316-31.

[16] Archuleta TL, Lemieux AM, Saengsirisuwan V, Teachey MK, Lindborg KA, Kim JS, Henriksen EJ. Oxidant stress-induced loss of IRS-1 and IRS-2 proteins in rat skeletal muscle: role of p38 MAPK. Free Radic Biol Med. 2009;47:1486-93.

[17] Marques RG, Morales MM, Petroianu A. Brazilian law for scientific use of animals. Acta Cir Bras. 2009;24:69-74.

[18] Drummond GB. Reporting ethical matters in The Journal of Physiology: standards and advice. J Physiol. 2009;587:713-19.

[19] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54.

[20] Bannister JV, Calabrese L. Assays for superoxide dismutase. Methods Biochem Anal. 1987; 32:279-312.

[21] Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-6.

[22] Flohé L, Günzler WA. Assays of glutathione peroxidase. Methods Enzymol. 1984;105:114-21.

[23] Valença SS, Pimenta WA, Rueff-Barroso CR, Ferreira TS, Resende AC, Moura RS, Porto LC. Involvement of nitric oxide in acute lung inflammation induced by cigarette smoke in the mouse. Nitric Oxide. 2009;20:175-81.

[24] Franco JG, de Moura EG, Koury JC, Trotta PA, Cordeiro A, Souza LL, Almeida NA, Lima N da S, Pazos-Moura CC, Lisboa PC, Passos MC. Resveratrol reduces lipid peroxidation and increases sirtuin 1 expression in adult animals programmed by neonatal protein restriction. J Endocrinol. 2010;207:319-28.

[25] de Oliveira PR, da Costa CA, de Bem GF, Marins de Cavalho LC, de Sousa MA, de Lemos Neto M, da Cunha Sousa PJ, de Moura RS, Resende AC. Effects of an extract obtained from fruits of Euterpe Oleracea Mart in the components of metabolic syndrome induced in C57BL/6J mice fed a high-fat diet. J Cardiovasc Pharmacol. 2010;56:619-26.

[26] Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 1990;186:464-78.

[27] Velkoska E, Cole TJ, Morris MJ. Early dietary intervention: long-term effects on blood pressure, brain neuropeptide Y, and adiposity markers. Am J Physiol Endocrinol Metab. 2005;288:1236-43.

[28] Davidowa H, Plagemann A. Insulin resistance of hypothalamic arcuate neurons in neonatally overfed rats. Neuroreport. 2007;18:521-4.

[29] Rudich A, Kanety H, Bashan N. Adipose stress-sensing kinases: linking obesity to malfunction. Trends Endocrinol Metab. 2007;18:291-9.

[30] Paravicini TM, Touyz RM. NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities. Diabetes Care. 2008;31:170-80.

[31] Rector RS, Thyfault JP, Uptergrove GM, Morris EM, Naples SP, Borengasser SJ, Mikus CR, Laye MJ, Laughlin MH, Booth FW, Ibdah JA. Mitochondrial dysfunction precedes insulin resistance and hepatic steatosis and contributes to the natural history of non-alcoholic fatty liver disease in an obese rodent model. J Hepatol. 2010;52:727-36.

[32] Goldstein S, Merényi G. The chemistry of peroxynitrite: implications for biological activity. Methods Enzymol. 2008;436:49-61.

[33] Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol. 2002;30:620-50.

[34] Gryglewski RJ, Palmer RM, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. Nature. 1986;320:454-6.

[35] Tabit CE, Chung WB, Hamburg NM, Vita JA. Endothelial dysfunction in diabetes mellitus: molecular mechanisms and clinical implications. Rev Endocr Metab Disord. 2010;11:61-74.

[36] Hirosumi J, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. Nature. 2002;420:333-6.

[37] Martins MR, Vieira AKG, de Souza EPG, Moura AS. Early overnutrition impaired insulin signaling in the heart of adult Swiss mice. J Endocrinol. 2008;198:591-8.

[38] Cunha AC, Pereira RO, Pereira MJ, Soares Vde M, Martins MR, Teixeira MT, Souza EP, Moura AS. Long-term effects of overfeeding during lactation on insulin secretion - the role of GLUT-2. J Nutr Biochem. 2009;20:435-42.

[39] Kmiec Z. Aging and peptide control of food intake. Curr Protein Pept Sci. 2011;12(4):271-9.

[40] Rossi AP, Watson NL, Newman AB, Harris TB, Kritchevsky SB, Bauer DC, Satterfield S, Goodpaster BH, Zamboni M. Effects of body composition and adipose tissue distribution on respiratory function in elderly men and women: the health, aging, and body composition study. J Gerontol A Biol Sci Med Sci. 2011;66(7):801-8.

[41] Garg A. Regional adiposity and insulin resistance. J Clin Endocrinol Metab. 2004;89:4206-10.

[42] Savage DB, Petersen KF, Shulman GI. Disordered lipid metabolism and the pathogenesis of insulin resistance. Physiol Rev. 2007;87:507-20.

[43] Meshkani R, Adeli K. Hepatic insulin resistance, metabolic syndrome and cardiovascular disease Clin Biochem. 2009;42:1331-46.

[44] Fan JG, Farrell GC. VAT fat is bad for the liver, SAT fat is not! J Gastroenterol Hepatol. 2008;23:829-32.

[45] Popa C, Riel PLCM, Meer WM, Stalenhoef AFH. The role of TNF-α in chronic inflammatory conditions, intermediary metabolism, and cardiovascular risk. J Lipid Res. 2007;48:751-62.

[46] Nonogaki K, Fuller GM, Fuentes NL, Moser AH, Staprans I, Grunfeld C, Feingold KR. Interleukin-6 stimulates hepatic triglyceride secretion in rats. Endocrinology. 1995;136:2143-9.

[47] Kroder G, Bossenmaier B, Kellerer M, Capp E, Stoyanov B, Mühlhöfer A, Berti L, Horikoshi H, Ullrich A, Häring H. Tumor necrosis factor-alpha- and hyperglycemia-induced insulin resistance. Evidence for different mechanisms and different effects on insulin signalling. J Clin Invest. 1996;97:1471-7.

[48] Mooney RA, Senn J, Cameron S, Inamdar N, Boivin LM, Shang Y, Furlanetto RW. Suppressors of cytokine signalling-1 and 6 associate with and inhibit the insulin receptor. A potential mechanism for cytokine-mediated insulin resistance. J Biol Chem. 2001;276:25889-93.

[49] Kathirvel E, Morgan K, French SW, Morgan TR. Overexpression of liver-specific cytochrome P4502E1 impairs hepatic insulin signaling in a transgenic mouse model of nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol. 2009;21:973-83.

[50] Rolo AP, Teodoro JS, Palmeira CM. Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. Free Radic Biol Med. 2011 [Epub ahead of print]

[51] Wong MC, Portmann B, Sherwood R, Niemela O, Koivisto H, Parkkila S, Trick K, L'abbe MR, Wilson J, Dash PR, Srirajaskanthan R, Preedy VR, Wiseman H. The cytoprotective effect of a-tocopherol and daidzein against d-galactosamine induced oxidative damage in the rat liver. Metabolism Clinical and Experimental. 2007;56:865-75.

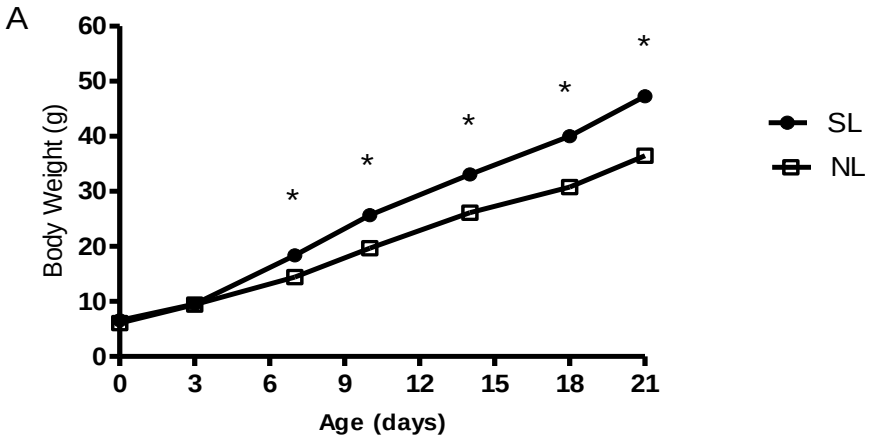
[52] Pooranaperundevi M, Sumiyabanu MS, Viswanathan P, Sundarapandiyan R, Anuradha CV. Insulin resistance induced by a high- fructose diet potentiates thioacetamide hepatotoxicity. Singapore Med J. 2010;51(5):389-98.

[53] Huo HZ, Wang B, Liang YK, Bao YY, Gu Y. Hepatoprotective and Antioxidant Effects of Licorice Extract against CCl4-Induced Oxidative Damage in Rats. Int. J. Mol. Sci. 2011;12:6529-43.

[54] Jiang M, Zhang Y, Liu M, Lan MS, Fei J, Fan W, Gao X, Lu D. Hypermethylation of hepatic glucokinase and L-type pyruvate kinase promoters in high-fat diet-induced obese rats. Endocrinology. 2011;152(4):1284-9.

[55] Sookoian S, Rosselli MS, Gemma C, Burgueño AL, Fernández Gianotti T, Castaño GO, Pirola CJ. Epigenetic regulation of insulin resistance in nonalcoholic fatty liver disease: impact of liver methylation of the peroxisome proliferator-activated receptor γ coactivator 1α promoter.Hepatology. 2010;52(6):1992-2000.

Figure 1



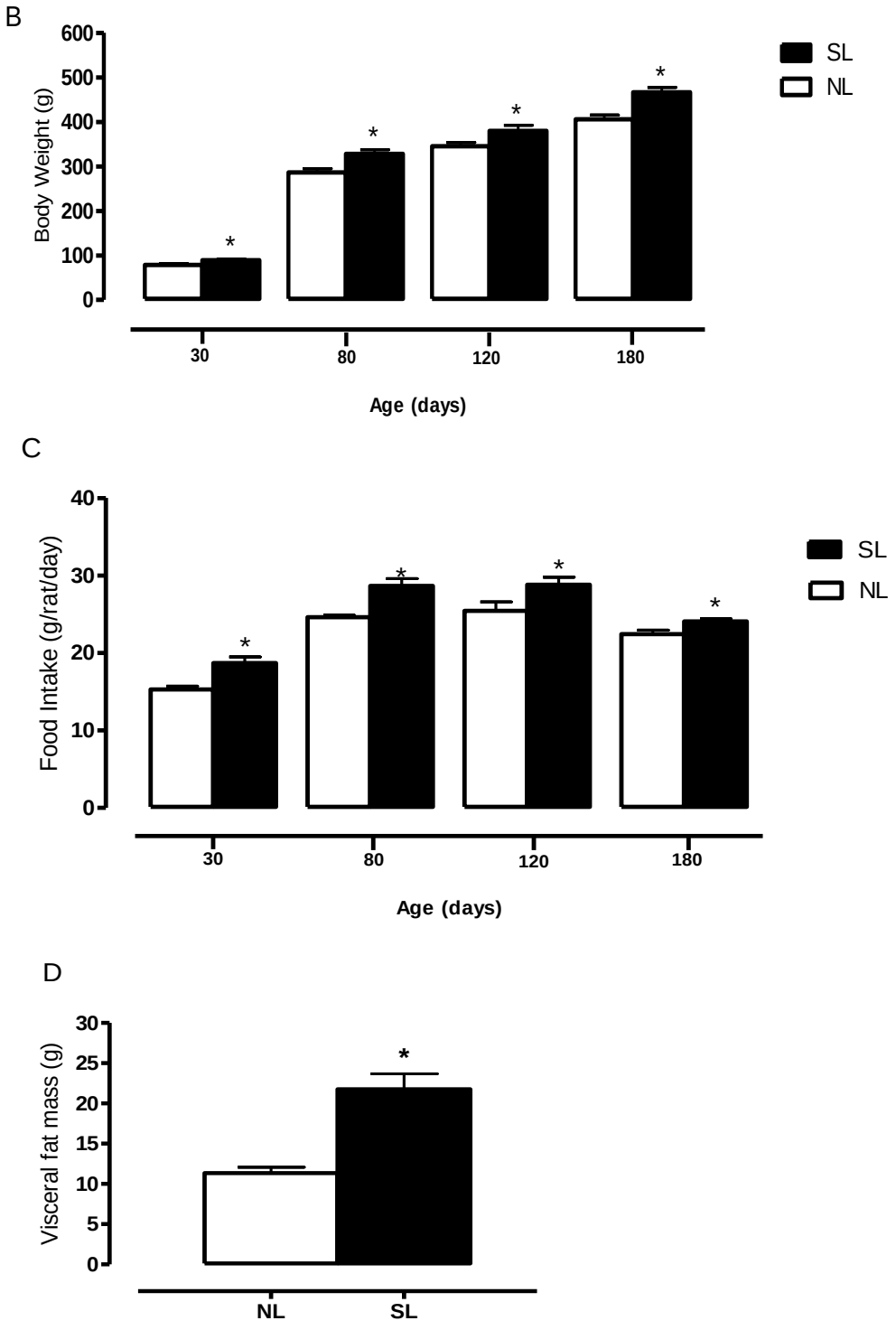


Figure 1: Body weight evolution of SL (●) and NL (□) rats during lactation (A) and after weaning (B) until 180 days old. Food intake at 30, 80, 120 and 180 days of NL and SL rats (C).

Visceral fat mass of NL and SL rats (D). Values are reported as mean ± SEM. *p<0.05; n=8 animals/group

Figure 2

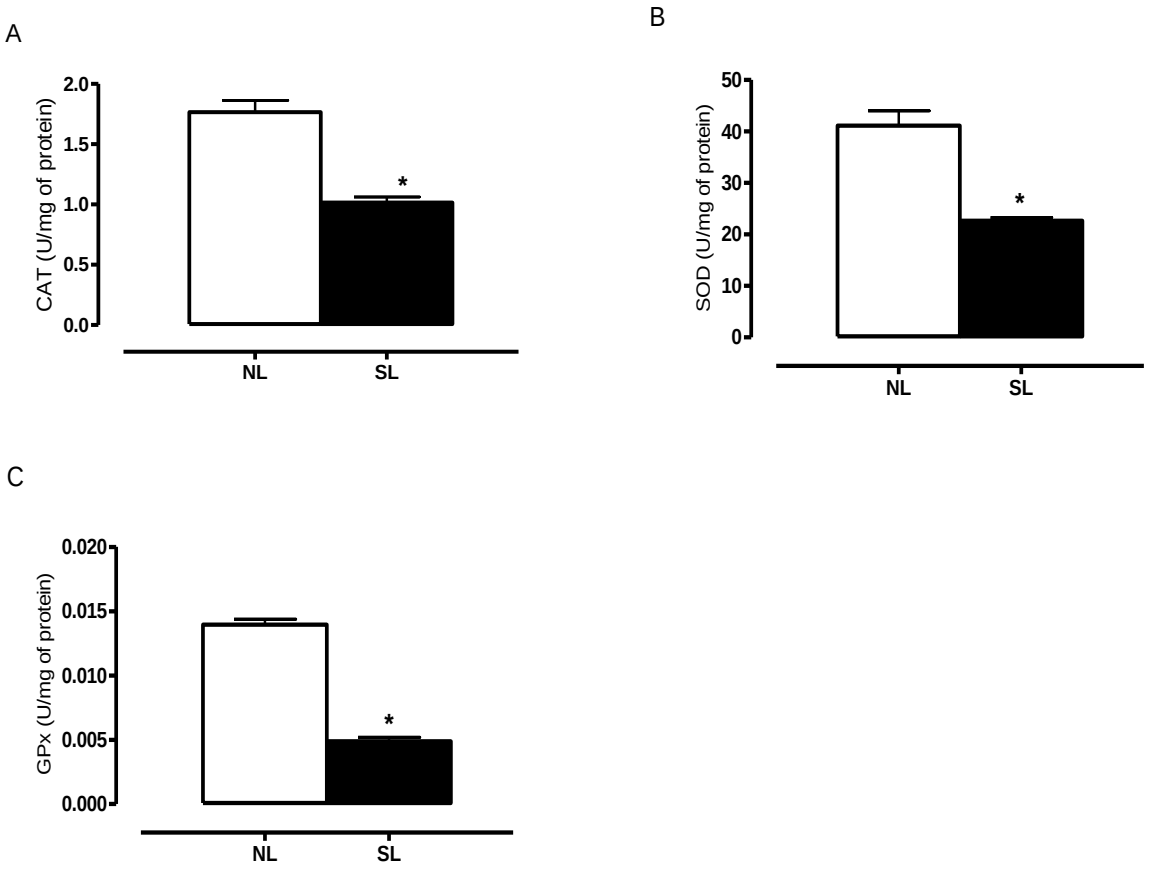


Figure 2: Liver catalase activity (A), superoxide dismutase activity (B) and glutathione peroxidase activity (C) in adult SL (black) and NL (white) rats. Values are reported as mean ±SEM. *p<0.001, n=8 animals/group.

Figure 3

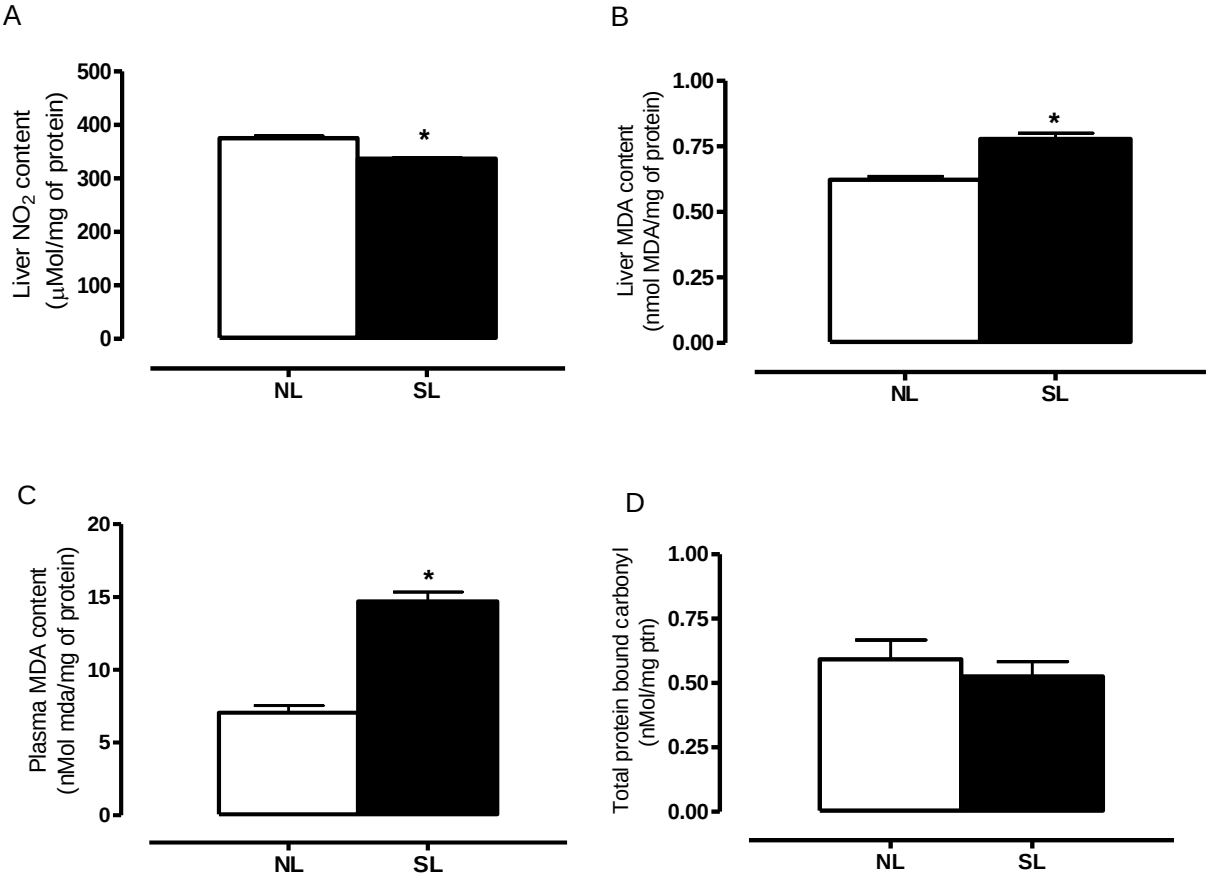
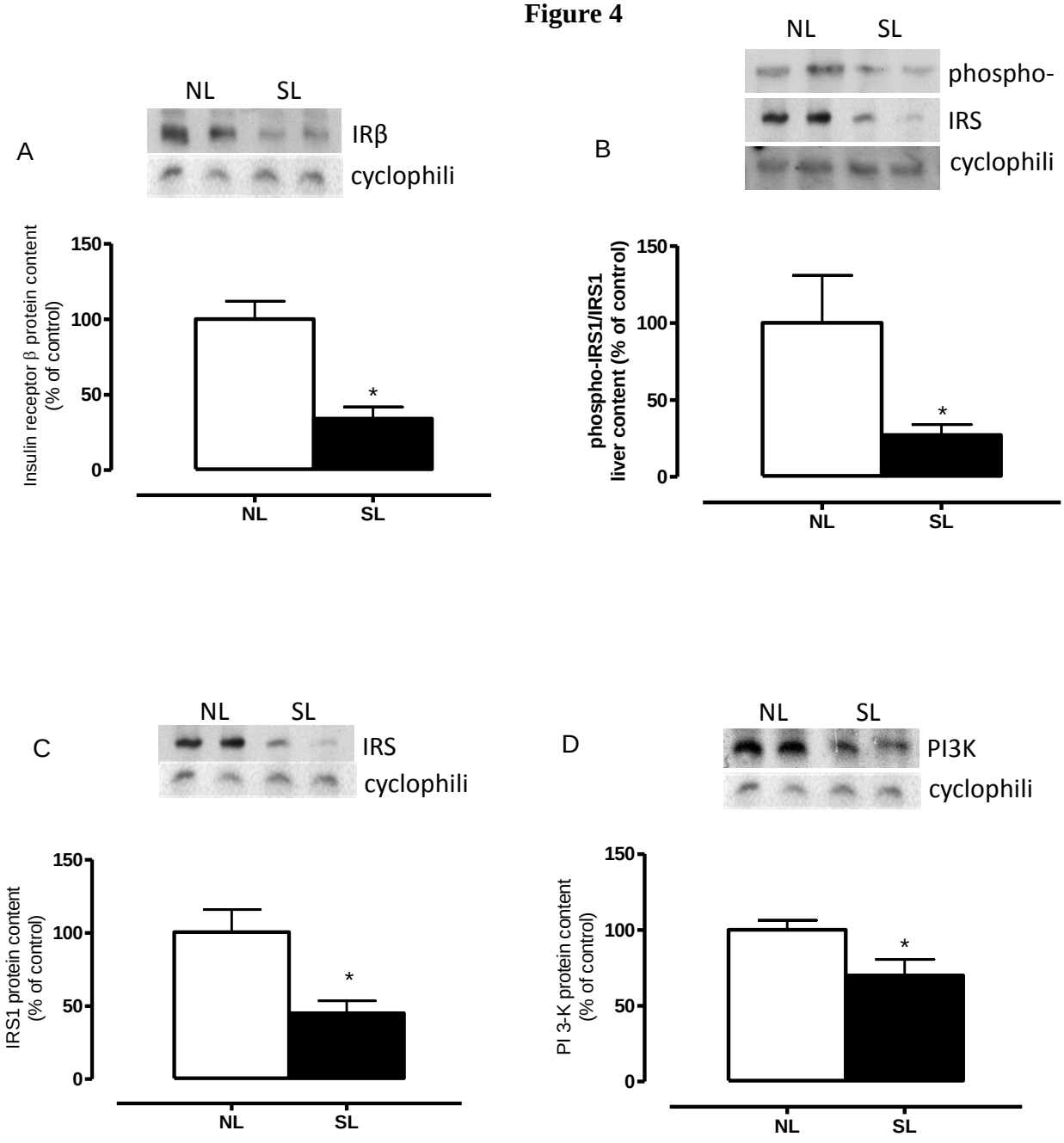


Figure 3: Liver nitrite content (A), liver TBARS (B), plasma TBARS (C) and liver total protein bound carbonyl content (D) in adult SL (black) and NL (white) rats. Values are reported as mean ± SEM *. $p<0.05$; n=8 animals/group.

Figure 4


A
NL
SL
IRβ
cyclophili
Insulin receptor β protein content (% of control)
150
100
50
0
*
NL
SL
B
NL
SL
phospho-
IRS
cyclophili
phospho-IRS1/IRS1 liver content (% of control)
150
100
50
0
*
NL
SL
C
NL
SL
IRS
cyclophili
IRS1 protein content (% of control)
150
100
50
0
*
NL
SL
D
NL
SL
PI3K
cyclophili
PI 3-K protein content (% of control)
150
100
50
0
*
NL
SL

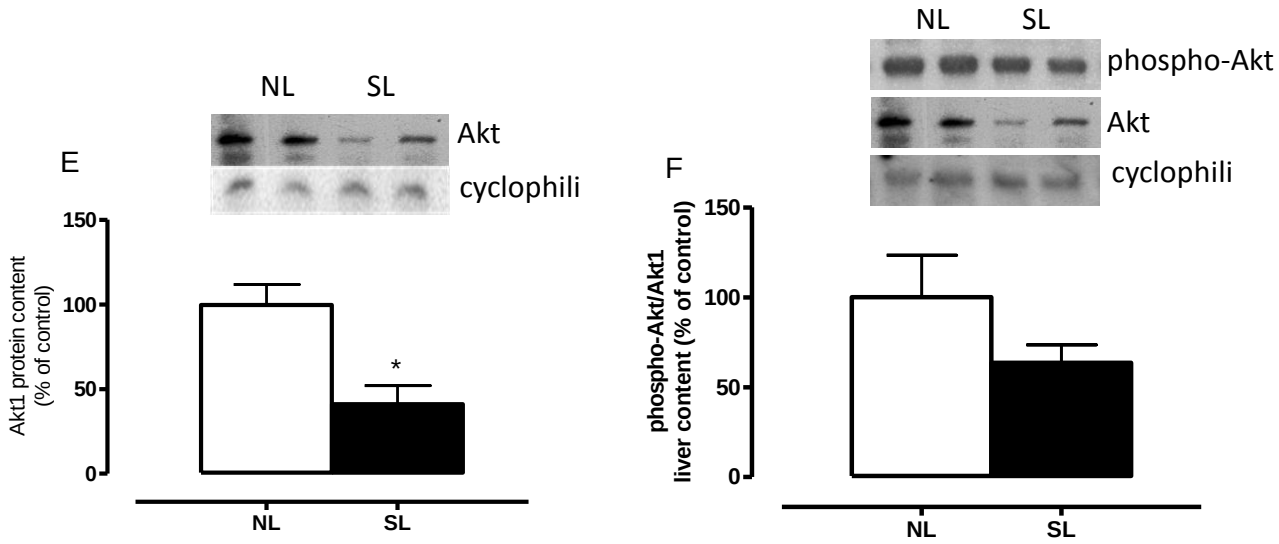


Figure 4: Liver IRβ (A), phospho-IRS-1 (B), IRS-1 (C), PI3K (D), Akt (E) and phospho-Akt1 (F) protein content in adult SL (black) and NL (white) rats**.** Values are reported as mean ± SEM. *p<0.05; n=8 animals/group.

Figure 5

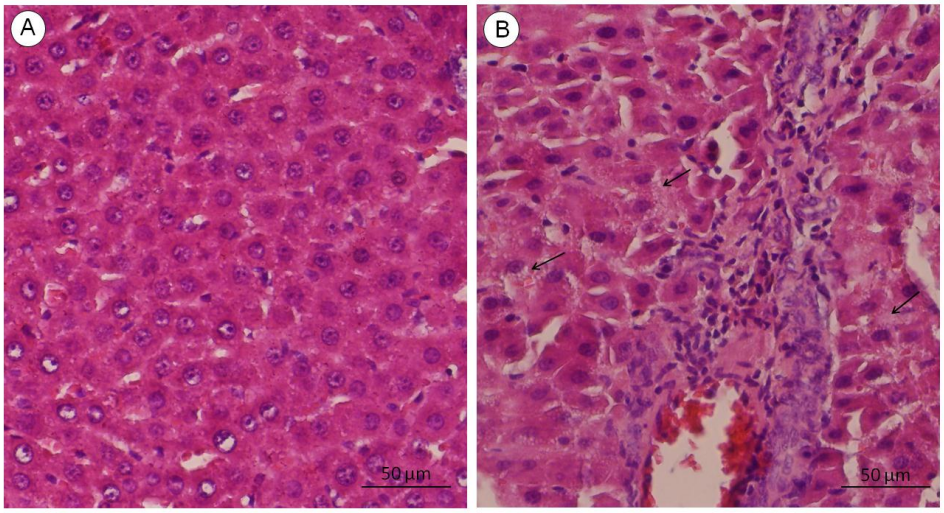


Figure 5: Liver histology. Photomicrographs of the liver with same magnification (x40) and stained with hematoxylin-eosin (H&E): (A) typical architecture of a NL offspring; (B) liver of SL offspring with microsteatosis (arrow) and inflammatory cell infiltrate.

3.2 **Artigo2 -** Resveratrol attenuates oxidative stress and prevents steatosis and hypertension in obese rats programmed by early weaning

Franco JG, Lisboa PC, Lima NS, Amaral TAS, Peixoto-Silva N, Resende AC, OliveiraE, Passos MCF, Moura EG. Resveratrol attenuates oxidative stress and prevents steatosis and hypertension in obese rats programmed by early weaning. Artigo submentido ao *Journal of Nutrition and Biochemistry*.

Resveratrol attenuates oxidative stress and prevents steatosis and hypertension in obese rats programmed by early weaning

Juliana G Franco[1], Patrícia C Lisboa[1]*, Natália S Lima[1], Taline AS Amaral[2], Nayara Peixoto-Silva[1], Angela C Resende[2], Elaine Oliveira[1], Magna C F Passos[1], Egberto G Moura[1]

[1]Department of Physiological Sciences and [2]Department of Pharmacology and Psychobiology, Roberto Alcantara Gomes Biology Institute State University of Rio de Janeiro, Rio de Janeiro, RJ, 20550-030, Brazil.

**** Corresponding author:***

Professor Patricia Cristina Lisboa – PhD

Departamento de Ciências Fisiológicas – 5º andar
Instituto de Biologia - Universidade do Estado do Rio de Janeiro
Av. 28 de Setembro, 87- Rio de Janeiro, RJ, 20550-030 - Brazil
Phone #: (+55.21) 25876434, FAX #: (+55.21) 25876129
E-mail: pclisboa@uerj.br

RUNNING TITLE: Resveratrol effects in rats programmed by early weaning
KEY WORDS: early weaning; resveratrol; programming; oxidative stress

ABSTRACT

We hypothesized that resveratrol, a natural phytoalexin found in grapes, can prevent oxidative stress, obesity and its related disturbances in obese rats programmed by early weaning. Lactating Wistar rats were separated into two groups: EW – dams were wrapped with a bandage to interrupt the lactation in the last 3 d of lactation; control – dams whose pups had free access to milk during all lactation. At the 150th day, EW offspring were randomly subdivided into: EW + resveratrol (EW+Res) - resveratrol (30 mg/kg/day); EW + vehicle (EW) rats received 0.5% (w/v) aqueous methylcellulose. The control group received vehicle. Rats were treated by gavage daily for 30 days. EW offspring developed hyperphagia, higher body weight, visceral obesity, higher SBP and DBP (+15% and +20%, respectively, $p<0.05$) and higher serum TG e LDL-c but lower HDL-c (+55%, +33% and -13%, respectively, $p<0.05$). Resveratrol normalized food intake, SBP and DBP and prevented obesity and dyslipidemia in EW+Res. EW rats had higher plasma and liver TBARS and lower plasma SOD and liver GPx activities (+51%, +18%, -58%, -31%, respectively, $p<0.05$) and resveratrol normalized both plasma and liver TBARS and increased the activity of SOD and CAT in plasma. EW rats presented liver steatosis and higher liver TG and resveratrol prevented these hepatic alterations in EW+Res rats. In conclusion, this study demonstrated a potential therapeutic use of resveratrol preventing obesity and oxidative stress and reducing the risk of hypertension, dyslipidemia and steatosis in adult rats programmed by early weaning.

INTRODUCTION

Adverse conditions in critical periods (gestation and lactation) as malnutrition could affect permanently the progeny both in humans and animals [1, 2]. This association is termed metabolic programming and was proposed by Barker that suggested an association between low size at birth of the newborn and an increased risk of type 2 diabetes, hypertension and cardiovascular disease at adulthood [3]. More recently, the term programming has been reviewed and renamed as developmental plasticity, which suggest a more probabilistic than deterministic phenomenon [4].

Obesity is increasing at an alarming rate throughout the world. Today it is estimated that there are more than 1.5 billion overweight adults and nearly 43 million overweight children under five years old worldwide [5]. Studies have focused on identifying obesity early determinants, especially during infancy and childhood when central and peripheral

systems that regulate energy balance could be programming [6, 7]. Breast-feeding duration and exclusivity have been associated with obesity prevention in humans [8, 9]. The World Health Organization (WHO) defines exclusive breast-feeding by the consumption of breast milk until 6 months without any other type of food intake, juice or even water [10]. However, only 35% of children worldwide are exclusively breast-fed during the first postnatal 4 months [11]. We previously showed that early weaning in rats caused by prolactin blocking with bromocriptine [12, 13] as well as through a non-pharmacological model [14, 15] caused neonatal malnutrition for a short period and programmed the adult offspring for higher adiposity, insulin resistance, dyslipidemia and central leptin resistance.

Growing evidence suggest that increased oxidative stress is involved in the pathogenesis of cardiovascular disease in metabolic syndrome [16]. Oxidative stress, which may occurs in consequence of the imbalance between free radical production and the capacity of cellular antioxidant systems, induces cell damage and the deregulated production of adipocytokines that contribute to obesity-associated insulin resistance, hypertension, dyslipidemia and liver steatosis [17]. Therefore, in the programming model of early weaning the higher adiposity and metabolic disturbances [14, 15, 12, 13] could be at least in part due to a higher oxidative stress. Thus, we hypothesized that treating oxidative stress is an important target to prevent and treat obesity and its associated comorbidities in these animals.

The use of bioactive food compounds at pharmacological doses is a recent therapeutic approach to prevent obesity-related chronic diseases [18, 19, 20]. Resveratrol (trans-3, 40, 5-trihydroxystilbene) is a natural phytoalexin mainly found in grapes, red wine, peanuts and other plants [21, 22] that has been recognized to have beneficial properties including anti-inflammatory, antioxidant, anti-tumor actions and anti-ageing effects [23, 24]. Early studies of our laboratory showed that resveratrol decreased oxidative stress in a model of programming by protein restriction during lactation [25]. In this present study, we evaluated the oxidative stress in adult rats programmed by early weaning without maternal separation or pharmacological approach as well as the effects of resveratrol in the treatment of metabolic disturbances developed as a consequence of obesity in this experimental model.

METHODS AND MATERIALS

Experimental model of early weaning

Wistar rats were kept in a temperature controlled room (25±1ºC) with artificial dark-light cycles (lights on 07:00 h, lights off 19:00 h). Virgin female rats, 3 months old, were caged with male rats (3:1), and after mating, each female was placed in an individual cage

with free access to food and water until delivery. Our experimental design was approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro (CEA/017/2009), according to the Brazilian Law issued on November 8, 2008, which concerns the rearing and use of animals in teaching and research activities in Brazil [26].

At birth, 20 lactating rats were randomly assigned to each one of the groups: early weaning (EW, n=10) - dams were lightly anaesthetized with thiopental (0.06 mg/ml per 100 g) and wrapped with a bandage to interrupt the lactation in the last 3 days of lactation; control (n=10) - dams whose pups had free access to milk throughout lactation (21 days). All litters were adjusted to six males to each dam at birth to maximize lactation performance.

EW and C groups received food directly into the cage and had free access to drinking water. During lactation, offspring body weight (BW) was daily monitored. Two pups from each litter were randomly chosen and followed during the experimental period. From postnatal day (PN) 21 to PN 180, BW and food intake (g) of offspring were monitored every 4 days.

Oral treatment with resveratrol

In PN 150, two EW offspring of each litter were randomly subdivided into two groups: EW + resveratrol (EW+Res, n=10) - rats received resveratrol (30 mg/kg/day); EW + vehicle (EW, n=10) rats received 0.5% (w/v) aqueous methylcellulose (vehicle). Rats received resveratrol or vehicle once a day by gavage during 30 days. The control group received vehicle solution. Because of its low solubility in water, resveratrol was suspended into carboxymethylcellulose solution [27, 25] and this suspension was prepared daily.

In PN 180, animals were euthanized with a non-lethal dose of thiopental (0.06 g/Kg/BW) to collected blood, carcass, visceral fat mass and liver samples. The blood was collected by cardiac puncture and poured in a tube containing heparin. Plasma and tissue samples were frozen at -80°C until analysis.

Body composition evaluation

Visceral fat-pads from 3 different regions (epididymal, mesenteric, and retroperitoneal) were removed and weighed. Total body fat and protein contents were determined by carcass analysis. The offspring eviscerated carcass was weighed, autoclaved for 1 h and homogenized on distilled water (1:1). Fat content was measured in the homogenates by a gravimetrical method described previously [14] and protein content was determined by the Lowry method [28].

Blood pressure

Systolic (SBP) and diastolic blood pressure (DBP) were measured in conscious rats by in the PN 178 use of tail-cuff plethysmography (LE 5000, LETICA Scientific Instruments, Barcelona, Spain). The first measurement of SBP and DBP was discarded and the mean of three subsequent measurements was recorded.

Lipid profile

Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL-c), serum levels were analyzed using Biosystem (Barcelona, Spain) commercial test kits. LDL-c and VLDL-c were obtained using Friedewald calculations:

1) LDL-c (mg / dl) = total cholesterol – (triglycerides / 5) – HDL-c

2) VLDL-c (mg / dl) = triglycerides / 5

Determination of antioxidant enzyme activities

Plasma and liver homogenates were used to determine superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities. SOD activity was evaluated by measuring the inhibition of adrenaline auto-oxidation as absorbance at 480 nm [29]. CAT activity was assayed by the rate of decrease in H2O2 at 240 nm [30]. GPx activity was evaluated by measuring the oxidation of NADPH at 340 nm in the presence of H2O2 [31]. The total protein content in each sample was determined by the Bradford method [32].

Thiobarbituric acid-reactive substances (TBARS)

Lipid peroxidation was measured by malondialdehyde (MDA) concentration using the thiobarbituric acid reactive substances (TBARS) method as previously described [33, 25]. Briefly, plasma and liver homogenates were mixed with 1mL of 10% trichloroacetic acid and 1mL of 0.67% thiobarbituric acid (Sigma Chemical Co., St. Louis, MO, USA) and heated in a boiling water bath for 30 minutes. The absorbance of the organic phase containing the pink chromogen was measured in a spectrophotometer at 532 nm. MDA equivalents were expressed in nMol/mg protein.

Western Blotting analysis

Liver samples were homogenized in cold lysis buffer (50 mM Hepes, pH 6.4, 1 mM MgCl2, 10 mM EDTA and 1% Triton X-100) containing Complete Protease Inhibitor Cocktail Tablets™ (Roche®, Basel, Switzerland) using a Ultra-Turrax® homogenizer (IKA

Werke GmbH & Co. KG, Staufen, Germany). The total protein content was determined by the BCA™ protein assay kit (Pierce, Rockford, IL, USA).

Samples (30 µg total protein) were electrophoresed in 12% tris-glycine sodium dodecyl sulfate (SDS) polyacrylamide gels. Proteins were transferred for polyvinylidene fluoride membranes (Hybond ECL; Amersham Pharmacia Biotech, London, UK), blocked in 2% bovine albumin (Sigma-Aldrich Co., St. Louis, MO, United States) in T-TBS (0.02 M Tris/0.15 M NaCl, pH 7.5 containing 0.1% Tween 20) at room temperature for 1 hour, washed 3x with T-TBS and incubated with the primary antibodies (CuZnSOD, catalase and glutathione peroxidase at 1:500 concentration) overnight at 4°C. CuZnSOD and GPx antibodies were purchased from Santa Cruz Biotechnology Inc. (San Francisco, CA, USA) and catalase from Sigma-Aldrich Co. (Sigma-Aldrich Co., St. Louis, MO, United States). After washing 3x with T-TBS, blots were incubated with corresponding secondary antibodies at 1:5000 concentration (Santa Cruz Biotechnology, CA, USA) for 1 hour and then, incubated with streptavidin (Zymed, CA, USA) in the same dilution of the secondary antibody for 1 hour. Blots were developed with enhanced chemiluminescence (ECL; Amersham Biosciences Inc., Piscataway, NJ).

Liver histology

Liver samples were fixed in formalin (freshly prepared 1.27 mol/L formaldehyde, 0.1 M phosphate-buffered saline, pH 7.2) and embedded in paraffin to non-serial sections of 5 µm. Sections were placed in glass slides to stain in hematoxylin/eosin. The morphological study was performed using digital images, acquired at random (TIFF format, 36-bit color, 1360x1024 pixels) with an Olympus DP71 camera and an Olympus BX40 epifluorescence microscope (Olympus, Tokyo, Japan).

Liver TG content

Total lipids were extracted from liver according the method described by Folch et al. [34]. Liver samples (50mg) were homogenized in 1 ml isopropanol and centrifuged for 10 minutes, at 4°C. TG content was measured by using commercial kits (Quibasa, Minas Gerais, Brazil).

Statistical analysis

Results are reported as mean ± SEM. Differences among the groups were analyzed by One-way ANOVA followed by Newman Keuls post-test. Differences were considered significant at $p<0.05$.

RESULTS

Table 1 showed that EW offspring developed hyperphagia (+18%, $p<0.05$) compared with C group and the treatment with resveratrol for 30 days normalized food intake in EW+Res. As expected, EW offspring had higher body weight (+9%, $p<0.05$), total body fat (+67%, $p<0.05$) but lower total body protein (-28%, $p<0.05$) compared with controls. In addition, EW rats had higher epididymal (+42% *vs* C, $p<0.05$) and retroperitoneal (+33% *vs* C, $p<0.05$) fat depots, without changes in mesenteric fat. EW+Res group had lower body weight gain (-66% *vs* EW, $p<0.05$), total body fat content (-37% *vs* EW, $p<0.05$), epididymal (-44% *vs* EW, $p<0.05$) and retroperitoneal (-33% *vs* EW, $p<0.05$) fat depots.

As depicted in table 2, EW offspring presented higher SBP and DBP (+15% and +20%, respectively, $p<0.05$), serum TG (+55%, $p<0.05$), LDL-c (+33%, $p<0.05$) but lower HDL-c (-13%, $p<0.05$) compared with controls. Resveratrol treatment normalized SBP (-15% *vs* EW, $p<0.05$) and DBP (-19% *vs* EW, $p<0.05$), serum TG (-35% *vs* EW, $p<0.05$), LDL-c (-37% *vs* EW, $p<0.05$) and HDL-c to close that those found in C group.

As demonstrated in Figure 1, EW rats had higher plasma and liver TBARS (+51 and +18%, respectively; $p<0.05$, fig 1A and 1B) compared with C group. In addition these animals had lower plasma SOD (-58%, $p<0.05$, fig 2A) and liver GPx (-31%, $p<0.05$, fig 2F) activity with no changes in plasma catalase and GPx activities (figs 2C and 2E) and liver SOD and catalase activities (figs 2B and 2D) compared with C group. Resveratrol treatment normalize both plasma and liver TBARS to concentrations close to those found in C group (+45 and +13% *vs* EW, respectively; $p<0.05$, figs 1A and 1B) and increased the activity of SOD (2 fold-increase *vs* EW, $p<0.05$, fig 2A) and catalase (1.1 fold-increase and +94% *vs* EW and C, respectively, $p<0.05$, fig 2C) in plasma. We did not found differences in plasma GPx as well as in SOD, catalase and GPx activities in liver of EW+Res group. No significant changes in liver content of were observed among the groups (Figure 3).

The morphological analysis showed a dysfunctional hepatic tissue of adult EW offspring. As demonstrated in Figure 4, EW rats presented drops of lipids in their cytoplasm characterizing a microsteatosis, differently of the C and EW+Res rats which demonstrated a liver with preserved architecture. Liver TG content was higher in EW group (+47% *vs* C, $p<0.05$, fig4B) and resveratrol normalized it in EW+Res group (-40% *vs* EW, $p<0.05$, fig4B).

DISCUSSION

Early weaning without maternal separation or pharmacological approach programmed adult rats for visceral obesity, hyperphagia and dyslipidemia reinforcing our previous

findings [14, 15]. Here, for the first time, we showed that these animals had higher oxidative stress, higher blood pressure and liver steatosis, confirming our hypothesis of an association among these parameters in the early weaning model. Also we reported the effects of resveratrol attenuating the oxidative stress and concomitantly reducing serum lipids, blood pressure, visceral fat accumulation, hepatic triglyceride and liver steatosis in rats programmed by early weaning.

In humans exclusive breastfeeding has been considered an important strategy to prevent the later development of obesity [8, 35]. Experimental studies in rodents showed that milk is an important source of nutrients and hormones such as leptin [36]. It is known that leptin is required for a normal postnatal development of hypothalamic pathways in the arcuate nucleus responsible for the future control of energy homeostasis [37]. These findings suggest that obesity and hyperphagia in adult EW rats could be related to the decreased leptin action in the hypothalamus of pups in the last 3 days of lactation. Previously we showed that different models of neonatal malnutrition - maternal energy malnutrition during lactation and maternal hypoprolactinaemia caused by bromocriptine administration in the end of lactation - also programmed the development of obesity and hyperphagia at adulthood [38, 12, 13] reinforcing the importance of adequate supply of milk throughout lactation for the future control of adipogenesis and food intake.

EW programmed rats treated with resveratrol had lower body weight gain, food intake and epididymal and retroperitoneal fat pad mass compared with EW rats. These results suggested that resveratrol seems to prevent or treat visceral obesity in these animals and reduce food intake. The effects of resveratrol on body weight and food intake are unclear. Obese Zucker rats treated with resveratrol at both doses of 15 mg/kg or 45 mg/kg body weight per day for 6 weeks showed reduced final body weight and visceral fat pads weight without significant changes in food intake [39]. Other findings in high-fat-fed mice showed that resveratrol (about 20 mg/kg) did not reduce final body weight [24] and had no effects on energy intake or body fat percentage [40]. However, Kim et al. [41] demonstrated that one-time intraperitoneal injection of resveratrol (100 mg/kg) suppressed food intake during 24 and 48 h in C57BL/6J mice. Thus, it seems that the effect of resveratrol is dose and time-dependent and further studies are necessary to understand its mechanism on body weight and food intake.

Increased oxidative stress is generally involved in the pathogenesis of hypertension, dyslipidemia and steatosis [16, 17]. Oxidative stress may occur as a result of increased ROS production and/or failure of antioxidant system. The antioxidant system is composed by

nonenzymatic molecules and antioxidant enzymes such as SOD, CAT and GPx. The higher oxidative stress in EW rats could be evidenced by the higher plasma and liver TBARS concentration and lower activities of SOD in plasma and GPx in both plasma and liver. Resveratrol prevented this higher plasma and liver TBARS in EW+Res rats and it was probably associated with enhanced antioxidant defense in this animals, suggested by the normalization of plasma SOD activity close to that found in C rats and the increasing in plasma CAT activity. The role of oxidative stress in the pathogenesis of hypertension has been reported in several studies and the main mechanism proposed is the reduced bioavailability of nitric oxide (NO) promoting endothelial dysfunction [42, 16, 17]. The superoxide anion (O_2^-) in the presence of oxidative stress reacts with NO stimulating the production of peroxynitrite and reducing the NO bioactivity [43]. Peroxynitrite in turn causes uncoupling of endothelial nitric oxide synthase (eNOS) producing superoxide [42]. Resveratrol prevented the higher blood pressure and the higher lipid peroxidation in EW+Res. Bhatt et al. [44] demonstrated that resveratrol attenuated the development of hypertension in spontaneously hypertensive rats by increasing SOD activity and prevented the eNOS uncoupling. In addition resveratrol was also reported to increase NO synthesis [45, 46]. Although we did not measure the NO content in this present study, the improvement of antioxidant defense in plasma and the lower lipid peroxidation caused by resveratrol in EW+Res rats suggest that scavenging of superoxide is occurring in these animals.

Steatosis is generally caused by an increased flux of free fatty acids and glycerol to the liver contributing to TG synthesis [47]. EW programmed rats had microvesicular steatosis compared with controls and this alteration was did not found in EW+Res. Since this is a semi-quantitative evaluation, we measured the liver TG content and the results confirmed that resveratrol reduced liver lipid accumulation in EW+Res animals. Oxidative stress is recognized as a promoter of hepatic injury and steatosis [48]. The higher TBARS and lower GPx activity in liver of EW offspring indicated oxidative damage in these animals. Although we did not find significant increased activity or content of antioxidant enzymes SOD, CAT or GPx in liver of EW+Res group, resveratrol reduced liver TBARS. These effects of resveratrol reducing the availability of liver triglycerides and oxidative stress and thus preventing the progression of steatosis are supported by several models of steatosis in rodents [49, 50, 39]. Studies have shown that resveratrol directly activates enzymes involved in both mitochondrial and peroxisome fat oxidation [39] and may indirectly improve mitochondrial activity and function by activation of the histone deacetilase SIRT1 [51] and adiponectin [52]. In fact, previously we showed that resveratrol increased SIRT1 in a programming model

of protein restriction [25]. Resveratrol seems to avoid the dyslipidemia progression in EW treated rats since they did not developed higher serum TG and LDL-c and lower HDL-c. These changes could be associated with the effects of resveratrol on preventing visceral and liver fat accumulation. In addition, it was reported that resveratrol reduces cholesterol synthesis by downregulation of HMG-CoA reductase and inhibits the activity of CETP, and consequently, decreases the transfer of cholesteryl esters from the atheroprotective HDL to the proatherogenic LDL and VLDL [53, 54].

In conclusion, we showed higher oxidative stress, liver steatosis and hypertension in adult rats that were early weaned pointing out the importance of exclusive breastfeeding to prevent the risk of future development of metabolic syndrome. Also we demonstrated that resveratrol preventing obesity and oxidative stress and, thus, reducing the risk of hypertension, dyslipidemia and steatosis. The use of resveratrol as a therapeutic approach to treat obesity and chronic diseases is an important issue to be investigated in future studies.

Declarations of interest: Authors declare no conflict of interest.

Acknowledgement

This research was supported by National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq), Coordination for the Enhancement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES) and State of Rio de Janeiro Carlos Chagas Filho Research Foundation (Fundacão Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro –FAPERJ). J.G.F. and N.S.L. are recipients of CNPq fellowships; E.O., N.P.S. and T.A.S are recipients of CAPES fellowship. All authors are grateful to José Firmino Nogueira Neto, Ulisses Risso Siqueira and Monica Moura for excellent technical assistance. The authors declare no conflict of interest.

REFERENCES

[1] Barker DJ. The developmental origins of chronic adult disease. Acta Paediatr Suppl. 2004; 93:26-33.

[2] Fernandez-Twinn DS, Ozanne SE. Early life nutrition and metabolic programming. Ann N Y Acad Sci. 2010; 1212: 78-96.

[3] Barker DJ. The fetal and infant origins of disease. Eur J Clin Invest. 1995; 25:457-63.

[4] Gluckman PD, Hanson MA. Developmental plasticity and human disease: research directions. J Intern Med. 2007; 261:461-71.

[5] Obesity and Overweight. 2011. World Health Organization.

[6] de Moura EG, Lisboa PC, Passos MC. Neonatal programming of neuroimmunomodulation: role of adipocytokines and neuropeptides. Neuroimmunomodulation. 2008; 15:176-88.

[7] Sullivan EL, Grove KL. Metabolic imprinting in obesity. Forum Nutr. 2010; 63:186-94.

[8] Arenz S, Rückerl R, Koletzko B, von Kries R. Breast-feeding and childhood obesity – a systematic review. Int J Obes Relat Metab Disord. 2004; 28:1247-56.

[9] Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG. Does breastfeeding influence risk of type 2 diabetes in later life? A quantitative analysis of published evidence. Am J Clin Nutr. 2006; 84:1043-54.

[10] Breastfeeding: A Vital Emergency Response. Are you ready? 2009. World Health Organization.

[11] Global Strategy for Infant and Young Child Feeding. 2003. World Health Organization.

[12] Bonomo IT, Lisboa PC, Pereira AR, Passos MC, de Moura EG. Prolactin inhibition in dams during lactation programs for overweight and leptin resistance in adult offspring. J Endocrinol. 2007; 192:339-44.

[13] Moura EG, Bonomo IT, Nogueira-Neto JF, de Oliveira E, Trevenzoli IH, Reis AM, Passos MC, Lisboa PC. Maternal prolactin inhibition during lactation programs for metabolic syndrome in adult progeny. J Physiol. 2009; 15:4919-29.

[14] Lima Nda S, de Moura EG, Passos MC, Nogueira Neto FJ, Reis AM, de Oliveira E, Lisboa PC. Early weaning causes undernutrition for a short period and programmes some metabolic syndrome components and leptin resistance in adult rat offspring. Br J Nutr. 2011; 105:1405-13.

[15] Lopes Nobre J, Lisboa PC, da Silva Lima N, Franco JG, Firmino Nogueira Neto J, de Moura EG, de Oliveira E. Calcium supplementation prevents obesity, hyperleptinaemia and hyperglycaemia in adult rats programmed by early weaning. Br J Nutr. 2011. DOI: 10.1017/S0007114511003928.

[16] Roberts CK, Sindhu KK. Oxidative stress and metabolic syndrome. Life Sci. 2009; 84:705-12.

[17] Otani H. Oxidative stress as pathogenesis of cardiovascular risk associated with metabolic syndrome. Antioxid Redox Signal. 2011; 15:1911-26.

[18] Xia X, Weng J. Targeting metabolic syndrome: candidate natural agents. J Diabetes. 2010; 2:243-9.

[19] Prasad S, Phromnoi K, Yadav VR, Chaturvedi MM, Aggarwal BB. Targeting inflammatory pathways by flavonoids for prevention and treatment of cancer. Planta Med. 2010; 76:1044-63.

[20] Chachay VS, Kirkpatrick CM, Hickman IJ, Ferguson M, Prins JB , Martin JH Resveratrol--pills to replace a healthy diet? Br J Clin Pharmacol. 2011; 72:27-38.

[21] Marques FZ, Markus MA, Morris BJ. Resveratrol: cellular actions of a potent natural chemical that confers a diversity of health benefits. Int J Biochem Cell Biol. 2009; 41:2125-8.

[22] Fernández AF, Fraga MF. The effects of the dietary polyphenol resveratrol on human healthy aging and lifespan. Epigenetics. 2011; 6:870-4.

[23] Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang LL, Scherer B, Sinclair DA. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature. 2003; 425:191-6.

[24] Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. Nature. 2006; 444:337-42.

[25] Franco JG, de Moura EG, Koury JC, Trotta PA, Cordeiro A, Souza LL, Almeida NA, Lima Nda S, Pazos-Moura CC, Lisboa PC, Passos MC. Resveratrol reduces lipid peroxidation and increases sirtuin 1 expression in adult animals programmed by neonatal protein restriction. J Endocrinol. 2010; 207: 319-28.

[26] Marques RG, Morales MM, Petroianu A. Brazilian law for scientific use of animals. Acta Cir Bras. 2009; 24:69-74.

[27] Das S, Lin HS, Ho PC, Ng KY. The impact of aqueous solubility and dose on the pharmacokinetic profiles of resveratrol. Pharm Res. 2008; 25:2593-600.

[28] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193:265-75.

[29] Bannister JV, Calabrese L. Assays for superoxide dismutase. Methods Biochem Anal. 1987; 32:279-312.

[30] Aebi H. Catalase in vitro. Methods Enzymol. 1984; 105:121-6.

[31] Flohé L, Günzler WA. Assays of glutathione peroxidase. Methods Enzymol. 1984; 105:114-21.

[32] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976; 72, 248-54.

[33] de Oliveira PR, da Costa CA, de Bem GF, de Cavalho LC, de Souza MA, de Lemos Neto M, da Cunha Sousa PJ, de Moura RS, Resende AC. Effects of an extract obtained from fruits of Euterpe oleracea Mart. in the components of metabolic syndrome induced in C57BL/6J mice fed a high-fat diet. J Cardiovasc Pharmacol. 2010; 56, 619-26.

[34] Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957; 226:497-509.

[35] Bartz S, Freemark M. Pathogenesis and prevention of type 2 diabetes: parental determinants, breastfeeding, and early childhood nutrition. Curr Diab Rep. 2012; 12:82-7.

[36] Picó C, Sánchez J, Oliver P, Miralles O, Ceresi E, Palou A. Role of leptin present in maternal milk in the control of energy balance during the post-natal period. Genes Nutr. 2007; 2:139-41.

[37] Grove KL, Smith MS. Ontogeny of the hypothalamic neuropeptide Y system. Physiol Behav. 2003; 79:47-63.

[38] Passos M, Ramos CF, Moura EG. Short and long term effects of malnutrition in rats during lactation on the body weight of offspring. Nutr Res. 2000; 20:1603-12.

[39] Gómez-Zorita S, Fernández-Quintela A, Macarulla MT, Aguirre L, Hijona E, Bujanda L, Milagro F, Martínez JA, Portillo MP. Resveratrol attenuates steatosis in obese Zucker rats by decreasing fatty acid availability and reducing oxidative stress. Br J Nutr. 2012; 107:202-10.

[40] Tauriainen E, Luostarinen M, Martonen E, Finckenberg P, Kovalainen M, Huotari A, Herzig KH, Lecklin A, Mervaala E. Distinct effects of calorie restriction and resveratrol on diet-induced obesity and Fatty liver formation. J Nutr Metab. 2011; 201, 525094.

[41] Kim SJ, Lee YH, Han MD, Mar W, Kim WK, Nam KW. Resveratrol, purified from the stem of Vitis coignetiae Pulliat, inhibits food intake in C57BL/6J Mice. Arch Pharm Res. 2010; 33:775-80.

[42] Schulz E, Jansen T, Wenzel P, Daiber A, Munzel T. Nitric oxide, tetrahydrobiopterin, oxidative stress, and endothelial dysfunction in hypertension. Antioxid Redox Signal. 2008; 10:1115-26.

[43] Gryglewski RJ, Palmer RM, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. Nature. 1986; 320:454-6.

[44] Bhatt SR, Lokhandwala MF, Banday AA. Resveratrol prevents endothelial nitric oxide synthase uncoupling and attenuates development of hypertension in spontaneously hypertensive rats. Eur J Pharmacol. 2011; 667:258-64.

[45] Wallerath T, Deckert G, Ternes T, Anderson H, Li H, Witte K, Förstermann U. Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase. Circulation. 2002; 106, 1652-8.

[46] Frombaum M, Le Clanche S, Bonnefont-Rousselot D, Borderie D. Antioxidant effects of resveratrol and other stilbene derivatives on oxidative stress and NO bioavailability: Potential benefits to cardiovascular diseases. Biochimie. 2012; 94:269-76.

[47] Cave M, Deaciuc I, Mendez C, Song Z, Joshi-Barve S, Barve S, McClain C. Nonalcoholic fatty liver disease: predisposing factors and the role of nutrition. J Nutr Biochem. 2007; 18:184-95.

[48] Rolo AP, Teodoro JS, Palmeira CM. Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. Free Radic Biol Med. 2012; 52:59-69.

[49] Macarulla MT, Alberdi G, Gómez S, Tueros I, Bald C, Rodríguez VM, Martínez JA, Portillo MP. Effects of different doses of resveratrol on body fat and serum parameters in rats fed a hypercaloric diet. J Physiol Biochem. 2009; 65:369-76.

[50] Rivera L, Morón R, Zarzuelo A, Galisteo M. Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats. Biochem Pharmacol. 2009; 77:1053-63.

[51] Szkudelska K, Szkudelski T. Resveratrol, obesity and diabetes. Eur J Pharmacol. 2010; 635:1-8.

[52] Wang A, Liu M, Liu X, Dong LQ, Glickman RD, Slaga TJ, Zhou Z, Liu F. Up-regulation of adiponectin by resveratrol: the essential roles of the Akt/FOXO1 and AMP-activated protein kinase signaling pathways and DsbA-L. J Biol Chem. 2010; 286:60-6.

[53] Weber O, Bischoff H, Schmeck C, Böttcher MF. Cholesteryl ester transfer protein and its inhibition. Cell Mol Life Sci. 2010; 67:3139-49.

[54] Wang H, Yang YJ, Qian HY, Zhang Q, Xu H, Li JJ. Resveratrol in cardiovascular disease: what is known from current research? Heart Fail Rev. 2011. DOI: 10.1007/s10741-011-9260-4.

Table 1: Anthropometric parameters of adult control (C) and early weaned offspring treated (EW+Res) or no with resveratrol (EW) for 30 days

	C		EW		EW+Res	
	Mean	SEM	Mean	SEM	Mean	SEM
Body weight (g)	391,4	7,46	421,0*	9,62	400,0	14,97
Body weight gain in 30 days (g)	11,4	3,63	15,5	2,85	5,2#	1,45
Food Intake in 30 days (g/day)	19,6	0,32	23,2*	0,37	19,2	0,37
Total body fat (g/100g carcass)	5,8	1,00	9,7*	1,15	6,1#	0,79
Total body protein (g/100g carcass)	18,8	1,21	13,6*	0,79	15,7	0,59
Epididymal fat weight (g/100gBW)	0,8	0,06	1,2*	0,16	0,8	0,09
Retroperitoneal fat weight (g/100gBW)	1,2	0,11	1,7*	0,15	1,2	0,10
Mesenteric fat weight (g/100gBW)	0,9	0.07	1,2	0,12	1,1	0,10

(Mean values with their standard errors, n=10)

Significant different at $p<0.05$; * vs C; # vs EW

Table 2: Serum lipid profile, liver TG content and blood pressure of adult control (C) and early weaned offspring treated (EW+Res) or no with resveratrol (EW) for 30 days.

	C		EW		EW+Res	
	Mean	SEM	Mean	SEM	Mean	SEM
SBP (mmHg)	139,3	3,82	160,7*	6,07	137,0	5,16
DBP (mmHg)	105,0	5,71	126,2*	6,15	101,3	4,35
TG (mg/dl)	30,9	2,86	48,0*	4,79	31,1#	3,88
Total cholesterol (mg/dl)	53,9	4,61	60,7	2,95	51,7	2,28
LDL-cholesterol (mg/dl)	19,7	3,19	26,3*	1,93	16,4#	1,39
HDL-cholesterol (mg/dl)	30,9	1,17	26,8*	0,99	28,0	0,49
VLDL-cholesterol (mg/dl)	6,5	0,73	8,0	0,93	6,2	0,77
TG (mg/g hepatic tissue)	18,5	1,86	27,2	1,71*	19,4#	1,92

Mean values with their standard errors, n=10)

Significant diferent at p<0.05; * vs C; # vs EW

Figure 1

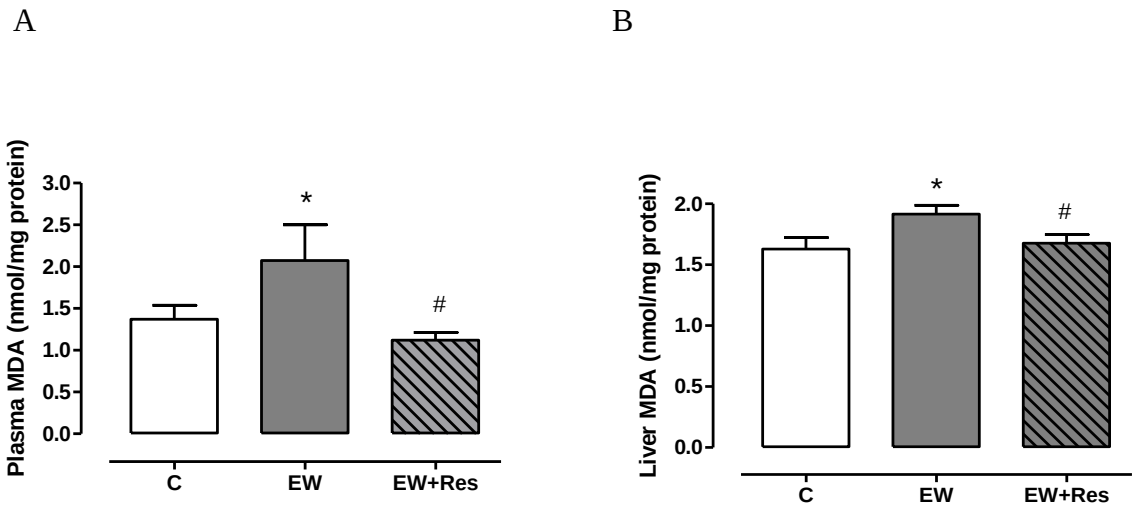


Figure 1. Plasma (A) and liver (B) TBARS concentration of adult control (C) and early weaned offspring treated (EW+Res) or no with resveratrol (EW) for 30 days. Values are means, with their standard errors represented by vertical bars, n=10. Mean values were significantly diferent at p<0.05; * vs C; # vs EW.

Figure 2

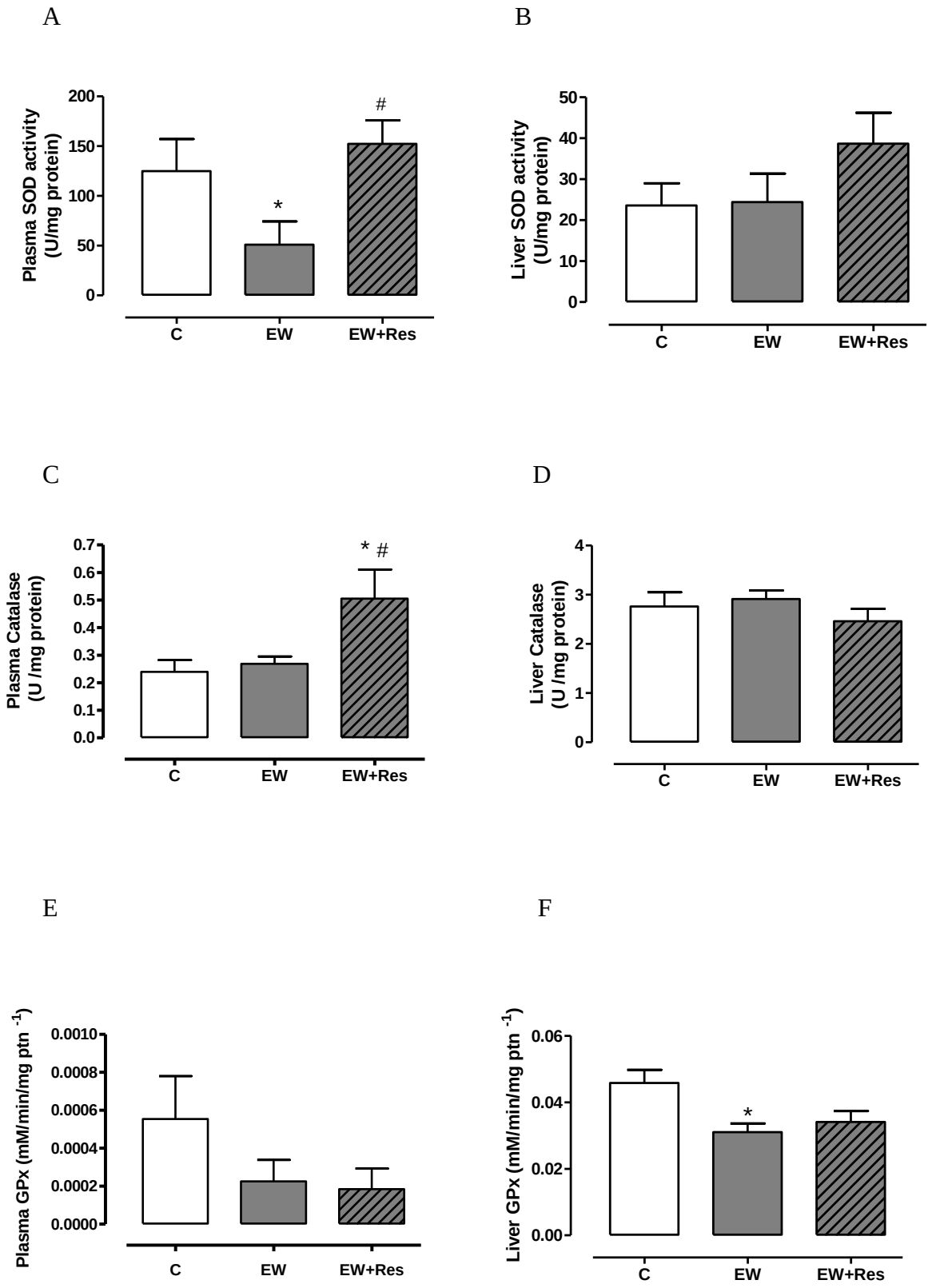


Figure 2. Plasma (A) and liver SOD (B) activity, plasma (C) and liver GPx activity (D) and plasma (E) and liver (F) catalase activity of adult control (C) and early weaning offspring

treated (EW+Res) or no with resveratrol (EW) for 30 days. Values are means, with their standard errors represented by vertical bars, n=10. Mean values were significantly diferent at p<0,05; * vs C; # vs EW.

Figure 3

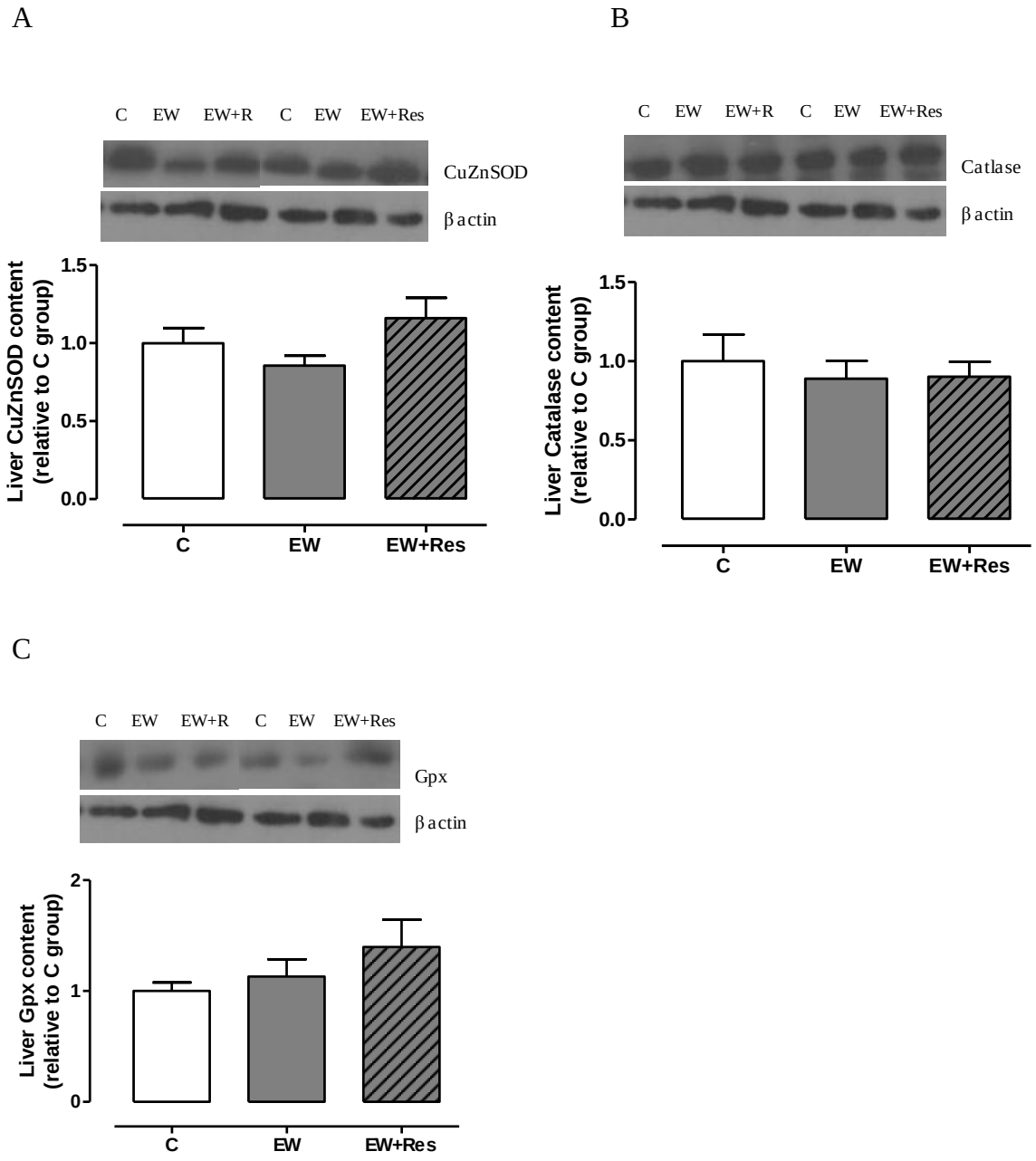


Figure 3. Liver content of CuZn-SOD (A), catalase (B) and GPx (C) of adult control (C) and early weaning offspring treated (EW+Res) or no with resveratrol (EW) for 30 days. Values

are means, with their standard errors represented by vertical bars, n=10. Mean values were significantly diferent at p<0,05; * vs C; # vs EW.

Figure 4

A B

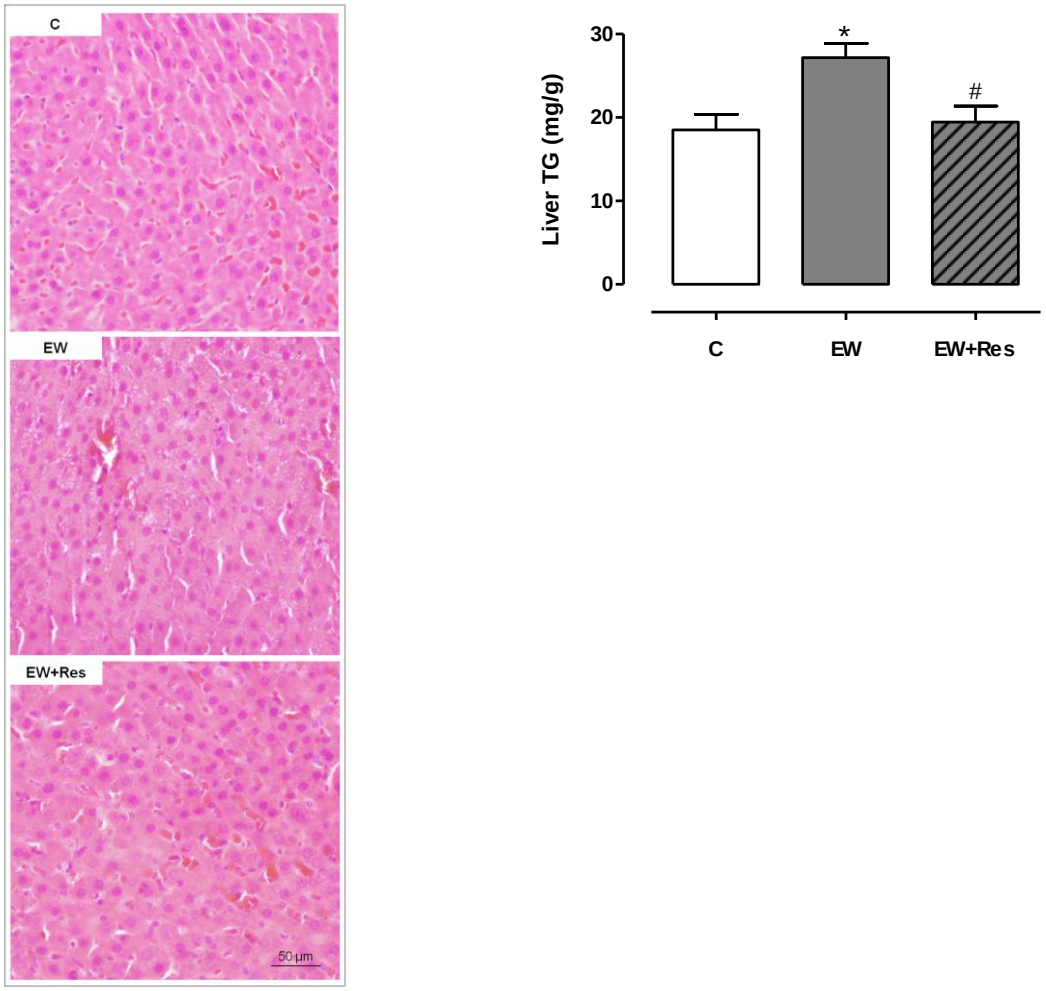


Figure 4. Liver histology (A) and TG content (B). Photomicrographs of the liver with same magnification (x40) and stained with hematoxylin-eosin (H&E). The normal appearance in control offspring (C), microvesicular steatosis in early weaning offspring (EW) and liver with architecture similar to control group in early weaning offspring treated with resveratrol (EW+Res). Values are means, with their standard errors represented by vertical bars, n=10. Mean values were significantly diferent at p<0,05; * vs C; # vs EW

3.3 **Artigo 3** - Resveratrol prevents hyperleptinemia and central leptin resistance in adult rats programmed by early weaning

Franco JG, Lisboa PC, Lima NS, Peixoto-Silva N, Maia LA, Oliveira E, Passos MCF Moura EG. Resveratrol prevents hyperleptinemia and central leptin resistance in adult rats programmed by early weaning. Artigo submetido ao *Journal of Endocrinology*.

Resveratrol prevents hyperleptinemia and central leptin resistance in adult rats programmed by early weaning

Juliana G Franco[1], Patrícia C Lisboa[1], Natália S Lima[1], Nayara Peixoto-Silva[1], Lígia A Maia[1], Elaine Oliveira[1], Magna C F Passos[1], Egberto G Moura[1]*

[1]Department of Physiological Sciences, Roberto Alcantara Gomes Biology Institute, State University of Rio de Janeiro, Rio de Janeiro, RJ, 20550-030, Brazil.

**** Corresponding author:***

Professor Egberto Gaspar de Moura - PhD
Departamento de Ciências Fisiológicas – 5°andar
Instituto de Biologia - Universidade do Estado do Rio de Janeiro
Av. 28 de Setembro, 87- Rio de Janeiro, RJ, 20550-030 - Brazil
Phone #: (+55.21) 25876434, FAX #: (+55.21) 25876129
E-mail: egberto@pq.cnpq.br

SHORT TITLE: Early weaning and leptin action: resveratrol effect
KEY WORDS: early weaning, programming, resveratrol, leptin resistance

ABSTRACT

Obesity and its clustering of cardiovascular risk factors are increasing worldwide. Resveratrol has been shown to improve cardiovascular disease and much speculation about its use in the treatment of obesity has emerged in the last years. In this study, we evaluated the effects of resveratrol (res) over visceral obesity, hyperleptinemia and insulin and leptin

resistance in a developmental plasticity model of obesity in adult early weaned rats. To induce early weaning, lactating dams were separated into two groups: early weaning (EW) – dams were wrapped with a bandage to interrupt lactation in the last 3 d of lactation and control (C) – dams whose pups had free access to milk during the entire lactation period (21 d). At 150 days-old, EW animals were subdivided into: EW+res (30mg/kg/BW) during 30 days and EW treated with diluent solution both by gavage. Resveratrol prevented the higher body weight, hyperphagia, visceral obesity, hyperleptinemia, hyperglycemia, insulin resistance and hypoadiponectinemia at adulthood in animals that were early weaned. Leptin resistance associated with lower JAK2 and pSTAT3 and higher NPY in hypothalamus of EW rats was normalized by resveratrol, and this seems to be independent of SOCS3 normalization. The present results suggest that resveratrol is useful as therapeutic tool in treating obesity as this compound could modulate fat accumulation and appetite control.

INTRODUCTION

Adverse conditions during the fetal or early post-natal life can alter an organism's physiology and metabolism contributing to the development of metabolic diseases in later life (Moura et al., 2008, Fernandez-Twinn & Ozanne, 2010). This phenomenon is termed metabolic programming and was proposed by Barker that suggested an association between low size at birth and an increased risk of adult-onset type 2 diabetes, hypertension and cardiovascular disease (Barker, 2007). The current term “developmental plasticity” has been proposed instead of metabolic programming, which suggests a more probabilistic than deterministic phenomenon (Gluckman & Hanson, 2007). A large number of studies have supported this theory and suggested that an adequate nutrition not only during gestation but also in lactation is critical to establish the future endocrine and metabolic status. Maternal malnutrition during lactation or early weaning have been associated with obesity, insulin resistance, hyperleptinaemia and leptin resistance at adulthood in rats (Passos et al., 2000; de Moura et al., 2009; Lisboa et al., 2006; Bonomo et al., 2007; Lisboa et al., 2010). We previously showed in a model of early weaning without maternal separation that pups had malnutrition at weaning and developed visceral obesity and some metabolic syndrome components, as well, hyperleptinaemia and central leptin resistance at adulthood (Lima et al, 2011). Studies have suggested that breastfeeding is potentially useful for population-based strategies aimed at obesity prevention. In humans, exclusive breastfeeding and up to 6 month of age were associated with reduced risk of obesity at child and adulthood (Arenz et al., 2004; Owen et al., 2006, de Armas et al., 2009).

Obesity is characterized by increased storage of fatty acids in an expanded adipose tissue mass and a deregulated expression of adipocytokines (Galic et al., 2010). Leptin, produced mainly by adipocytes is an important adipocytokine with a significant role in the regulation of food intake and energy expenditure (Rahmouni, 2010; Friedman, 2011). The hypothalamus is the most important tissue responsive to the anorectic leptin actions (Ahima & Antwi, 2008), where leptin binds to its long form receptor (OBRb) and activates the janus tyrosine kinase 2 (JAK2) signal transducer and activator of transcription 3 (STAT3) intracellular pathway. The activation of the JAK2/STAT3 pathway stimulates the suppressor of cytokine signaling 3 (SOCS3), an inhibitor of the leptin signaling pathway (Sahu, 2003; Ahima & Osei, 2004). Besides obese humans and animals have high leptin levels, its action in central nervous system is generally impaired since they frequently develop a central leptin resistance (Morton & Schwartz, 2011; Rahmouni, 2010; El-Haschimi et al., 2000). Central leptin resistance is associated with a reduction of expression and/or activation of the OBRb, JAK2, and STAT3 as well as an increase in SOCS3 expression (Sahu et al., 2003).

Resveratrol (3,4',5-trihydroxystilbene) is a natural phytoalexin detected in several plant species, including grapes, peanuts, berries and pines that has been investigated since the 90's by its cardiovascular protective properties implicated in the "French Paradox" (Lekli et al., 2010). Studies have shown that resveratrol improved insulin sensitivity and lowers body weight in obese rodents (Baur et al., 2006; Lekli et al, 2008; Sharma et al., 2010; Gómez-Zorita et al., 2012) which has led to much speculation about its use as therapeutic supplement to treat obesity in humans. However to the best of our knowledge there is no *in vivo* study about the mechanism by which resveratrol reduces food intake. Our main hypothesis is that through the serum leptin reduction, resveratrol improves leptin sensitivity at the hypothalamic level in obesity. Thus, considering the anti-obesity actions of resveratrol we evaluated its possible beneficial effects in preventing hyperleptinaemia and leptin resistance, which have previously identified (Lima et al, 2011) in an experimental model of obesity programmed by the interruption of breastfeeding in the last 3 days of lactation.

METHODS

Experimental model of early weaning and oral treatment with resveratrol

Wistar rats were kept in an environmentally controlled room (25±1ºC and 12:12-h light-dark cycle; lights on from 7:00 A.M. to 7:00 P.M.). Three-month-old virgin rats were caged with male rats in the proportion of 3:1. After mating, each pregnant rat was placed in an individual cage with free access to food and water until delivery. This experimental model

was approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro (CEA/017/2009), according to the Brazilian Law issued on November 8, 2008, which regulates the use of animals in teaching and research activities in Brazil (Marques et al. 2009).

After birth, 20 lactating rats were randomly divided into two groups: Early weaning (EW, n = 10) – dams were lightly anaesthetized with thiopental (0.06 mg/ml per 100 g) and wrapped with a bandage to interrupt lactation in the last 3 days of lactation; Control (C, n = 10) – dams whose pups had a standard lactation period, i.e. weaning at 21 days of lactation.

In postnatal day (PN) 150, two EW offspring of each litter were randomly subdivided into two groups: EW + resveratrol (EW+Res, n = 10) - rats received resveratrol (30 mg/kg/d); EW+ vehicle (EW, n = 10) rats received 0.5% (w/v) aqueous methylcellulose (vehicle). The Control group (n = 10) received 0.5% (w/v) aqueous methylcellulose (vehicle). Animals received resveratrol or vehicle once a day by gavage during 30 days. Resveratrol was suspended into carboxymethylcellulose solution because of its low solubility in water (Das et al. 2008; Franco et al., 2010) and this suspension was prepared freshly and daily before the gavage. From PN 21 until PN 180, body weight (BW) and food intake (g) were monitored every 4 day.

In PN 180, animals were euthanized with a non-lethal dose of thiopental (0.06 mg/ml per 100g of BW) to collect blood, carcass, visceral fat mass and hypothalamus samples. The blood was collected by cardiac puncture, and visceral fat (epididymal, mesenteric, and retroperitoneal regions) and hypothalamus were removed. Plasma and tissue samples were frozen at -80°C until analysis.

Western Blotting Analysis

JAK2, pSTAT3, SOCS3, NPY hypothalamic contents were analyzed by Western Blotting using actin as an internal control. Hypothalamus samples were homogenized in ice-cold lysis buffer (50mM-HEPES, 1mM-MgCl2, 10mM-EDTA, Triton X-100 1%, pH 6,4) containing Complete Protease Inhibitor Cocktail Tablets (Roche®, Branford, CT, USA). After centrifugation (7500 g for 5 min), supernatants were stored at -80°C. Protein concentrations were determined by the BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, USA). Samples (30 mg total protein) were electrophoresed in 10% or 12% tris-glycine SDS polyacrylamide gels and transferred onto nitrocellulose membranes (Hybond ECL; Amersham Pharmacia Biotech, Bucks, UK). Membranes were blocked with 2% albumin in T–TBS (0.02 M Tris/0,15 M NaCl, pH 7.5, containing 0,1% Tween 20) for 1 h. The following specific primary antibodies were used: anti-JAK2, anti-pSTAT3, anti-SOCS3, anti-NPY and

anti-actin. Membranes were incubated with primary antibodies at a 1:500 to 1:1000 dilution in T–TBS for 1 h. After washing 3 times with T–TBS, the blots were incubated with the corresponding secondary antibody (1:5000; peroxidase-conjugated IgG; Santa Cruz Biotechnology, Inc.) for 1 h and then with streptavidin (1:5000; Zymed, San Francisco, CA, USA) for 1 h. Targeted proteins were developed with enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and then exposed to X-ray film. Images were scanned and bands were quantified by densitometry using Image J 1.34 software (Wayne Rasband National Institute of Health, Bethesda, MA, USA).

Serum hormones measurement

Blood samples were centrifuged (1500 g for 20 min) to obtain serum, which was kept at -80C until assay. All determinations were performed in one assay. Leptin was measured with a specific radioimmunoassay (RIA) kit (Linco Research, St Charles, MO, USA) with a range of detection from 0.5 to 50 ng/ml; the intra-assay variation was 2.9%. Insulin was determined using a RIA kit (ICN Pharmaceuticals, Inc., Orangeburg, NY, USA) with an assay sensitivity of 0.1 ng/ml and an intra-assay variation of 4.1%. Serum adiponectin was measured using an ELISA kit (Millipore, Billerica, MA, USA) with an assay sensitivity of 0.155 ng/ml.

Adipocytes morphometric analysis

Visceral (epididymal) white adipose tissue was fixed (freshly prepared 1.27 mol/L formaldehyde, 0.1 M phosphate-buffered saline, pH 7.2), embedded in paraffin, sectioned (5 μm of thickness) and stained with hematoxylin-eosin. The cross-sectional area of the adipocytes was measured on digital images acquired at random (TIFF format, 36-bit color, 1360x1024 pixels) with an Olympus DP71 camera and an Olympus BX40 epifluorescence microscope (Olympus, Tokyo, Japan), and analyzed with the software Image-Pro Plus version 5.0 (Media Cybernetics, Silver Spring, MD, USA). Some of the original color images were converted to gray scale images, for documentation purposes, by using the Adobe Photoshop software. At least 50 adipocytes per animal (n = 5) were randomly measured, totaling 250 adipocytes per group.

Glucose homeostasis evaluation

Fasting blood glucose was determined from the tail vein of fasting rats using a glucometer (ACCU-CHEK Advantage; Roche Diagnostics, Mannhein, Germany).

Insulin sensitivity was assessed by:

HOMA-IR = insulin μU/ml * serum glucose mmol/22.5

where HOMA-IR is homeostasis model assessment of insulin resistance.

Statistical analysis

Results are reported as mean ± SEM. Differences among the groups were analyzed by One-way ANOVA followed by Newman Keuls post-test. Differences were considered significant at $p<0.05$.

RESULTS

As demonstrated in Figure 1, EW programmed animals to higher body weight, hyperphagia and visceral obesity at adulthood (+9% fig. 1A, +18% fig. 1C, +44% fig. 1D, respectively; $p<0.05$). Resveratrol treatment prevented these alterations in EW+Res group which had body weight, body weight gain, visceral fat mass and food intake similar to C group.

As expected, EW offspring had higher glycemia and HOMA-IR (+38% and 62%, figs.2A and 2B, $p<0.05$) associated with lower serum adiponectin (-22%, fig. 2D). These parameters were reversed by resveratrol in EW+Res group. No change in serum insulin was found among the groups.

EW offspring showed hyperleptinaemia (+64%, fig. 3A) and morphometric analysis showed that these animals had higher epididymal adipocyte area (+34%, fig. 3B). EW+Res rats showed serum leptin and area of epididymal adipocyte similar to those found in C group.

Protein content of the leptin signaling pathway in the hypothalamus was shown in Figure 4. Adult EW offspring displayed lower hypothalamic JAK2 (-27%, fig. 4A; $p<0.05$), pSTAT3 (-40%, fig. 4B; $p<0.05$) and higher SOCS3 (+53%, fig. 4C; $p<0.05$) contents, and resveratrol treatment normalized JAK2 and pSTAT3 in EW+Res but not SOCS3 content. In addition, EW rats showed higher hypothalamic content of NPY (+39%, fig. 4D; $p<0.05$) and resveratrol normalized it.

DISCUSSION

Obesity and overweight pose a major risk for chronic diseases, including type 2 diabetes, cardiovascular disease and hypertension (WHO, 2011). This clustering of cardiovascular risk factors is known as metabolic syndrome (Sarzani et al., 2008). Animal models and *in vitro* experiments have shown beneficial properties of resveratrol indicating that this compound may be helpful in preventing and treating some metabolic diseases (Xia &

Weng, 2010; Prasad , 2010; Chachay et al., 2011). In this study, rats programmed by early weaning were used as an experimental model of obesity associated with some characteristic of metabolic syndrome (Lima et al., 2011). Resveratrol administration at a dose of 30 mg/kg/BW during 30 days in EW rats prevented the higher body weight, visceral obesity, hyperleptinemia, central leptin resistance and hyperglycemia as well as hypoadiponectinemia that feature this experimental model of programming.

EW adult rats had a small increase in BW associated with an important increase in visceral fat and total body fat (Lima et al., 2011). We confirmed these findings here and showed that EW resveratrol treated rats did not have increased BW or visceral fat mass probably due to a food intake reduction found in these animals. Studies have shown that resveratrol acts in different steps of adipocyte life cell cycle, inhibiting cell proliferation during early preadipocyte development, differentiation and lipid accumulation in later preadipocyte as well as promotes lipolysis and apoptosis in mature adipocytes (Rayalam et al., 2008; Szkudelska et al., 2009a; Baile et al., 2011). In Obese Zucker rats, resveratrol at both doses of 15 or 45 mg/kg/BW per day for 6 weeks reduced final body weight and visceral fat pads weight (Gómez-Zorita et al., 2012). Lagouge et al. (2006) showed that resveratrol supplementation for 15 weeks significantly decreases body weight gain and visceral fat-pad weights in high-fat diet fed mice. However, also in high-fat fed mice, resveratrol (about 20 mg/kg) did not reduce body weight (Baur et al. 2006) and had no effects on food intake or body fat (Tauriainen et al., 2011). Besides the lower VFM, EW rats treated with resveratrol had lower epididymal adipocyte area compared with EW rats suggesting that resveratrol with this change may alter the flow of free fatty acids and adipocytokines from these animals. Resveratrol has been shown to up regulate expression of genes that modulate mitochondrial function promoting fatty acid oxidation (Zhang, 2006; Gerhart-Hines et al., 2007). Indeed, resveratrol down regulates the expression of transcription factors involved in adipocyte metabolism such as PPARγ, fatty acid synthase (FAS), hormone-sensitive lipase (HSL), and lipoprotein lipase (LPL) (Baile et al., 2011). In visceral adipose tissue of obese humans, resveratrol decreased PPARγ and increased adiponectin expression (Costa Cdos et al., 2011). In obese Zucker rats, resveratrol increased adiponectin levels (Rivera et al., 2009) as well in a mice model of obesity induced by high fat diet (Rogers et al., 2008). Also in our study, adiponectin levels were restored by resveratrol. Hyperglycemia, higher HOMA-IR and hypoadiponectinemia despite normal insulin levels suggested insulin resistance in EW programmed rats, as we previously reported (Lima et al., 2011; Lopes Nobre et al., 2011). Resveratrol prevented the higher glucose levels and HOMA by normalizing serum

adiponectin in EW treated rats. The possible mechanism by which resveratrol improves glycemia in our study, could be the reported increasing of GLUT4 translocation and enhancing Akt phosphorylation with stimulation of glucose uptake in skeletal muscle cells (Minakawa et al., 2011).

There is a positive correlation between circulating leptin levels and total body fat mass, which could explain the hyperleptinemia in obesity (Lonnqvist et al., 1997). However, despite the high levels of leptin, which should reduce food intake and fat stores, a leptin resistance generally occurs in obesity (Galic et al., 2010; Morton & Schwartz, 2011). Resveratrol prevented the hyperleptinemia in EW+Res rats, probably due to a reduction in both body weight and visceral fat. Studies have shown that resveratrol seems to prevent obesity and reduce plasma leptin in rodents on a high-caloric diet (Baur et al., 2006). In addition, resveratrol inhibited leptin secretion from adipocyte *in vitro* (Szkudelska et al., 2009b) and suppressed leptin expression in adipose tissues in high-fat diet fed mice (Kim et al., 2011). Resveratrol also normalized hypothalamic contents of JAK2 and pSTAT3 and this could be explained by the normalization of serum leptin. Despite resveratrol changed JAK2 and STAT3 activity, SOCS3 remained increased in EW+Res. We previously showed in adult EW rats that calcium supplementation prevented hyperleptinemia and central leptin resistance also normalizing JAK2 and pSTAT3 without changing the higher SOCS3 (Lopes Nobre et al., 2011). Other molecules, such as phosphotyrosine phosphatase (PTP)-1B, can probably inhibit leptin signaling pathway (Zabolotny et al., 2002). Studies demonstrated that resveratrol, through activation of sirtuin (SIRT)-1, could suppress the expression of PTP-1B (Ghanim et al., 2010). Thus, we cannot discard a direct resveratrol effect on the leptin signaling pathway independent of its effect lowering leptin production.

Neuropeptide Y (NPY) is an orexigenic neuromodulator secreted by neurons in the central and peripheral nervous systems that normally is inhibited by leptin in the arcuate hypothalamic nucleus (Valassi et al., 2008). In this study, we showed that EW animals had high hypothalamic levels of NPY that could be related to the leptin resistance. Thus, the normalization of NPY by resveratrol could be explained by the improvement of leptin sensitivity, normalizing food intake in these animals. The present findings could be reinforced by the study of Kim et al. (2010) that demonstrated that one-time intraperitoneal injection of resveratrol (100 mg/kg) suppressed food intake during 24 and 48 h in C57BL/6J mice and that resveratrol downregulated NPY and AgRP expression in hypothalamic N29-4 cell culture. To our knowledge this is the first study that investigates the effects of resveratrol *in vivo* upon hypothalamic content of leptin signaling pathway proteins and NPY.

In conclusion, resveratrol prevented the visceral fat accumulation in adult rats programmed by early weaning and consequently regulated leptin and adiponectin levels that contributed to correction of insulin resistance, central leptin resistance and food intake in these animals. These present results suggest the importance of the future use of resveratrol as therapeutic tool in treating obesity. However, further research is required to verify other mechanisms besides those reported here as well as safety use in human studies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research was supported by the 'National Council for Scientific and Technological Development' (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq), the 'Carlos Chagas Filho Research Foundation of the State of Rio de Janeiro' (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro – FAPERJ) and Coordination for the Enhancement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nı´vel Superior – CAPES).

Author contribution statement

Conception and design: J.G.F., P.C.L., E.G.M.

Collection, analysis and interpretation of data: J.G.F., A.C.R., N.L.S., T.A.S.A., N.P.S.

Drafting and/or revising the article critically for important intellectual content: J.G.F., E.O., P.C.L., M.C. F. P., E.G.M.

Acknowledgements

All authors are grateful to Ms Mônica Gaspar de Moura and Mr Ulisses Risso Siqueira for their technical assistance.

REFERENCES

Ahima RS & Antwi DA 2008 Brain regulation of appetite and satiety. *Endocrinology and Metabolism Clinics of North America* **37** 811-823.

Ahima RS & Osei SY 2004 Leptin signaling. *Physiology & Behavior* **81** 223-241.

Arenz S, Rückerl R, Koletzko B & von Kries R 2004 Breast-feeding and childhood obesity-a systematic review. *International Journal of Obesity and Related Metabolic Disorders* **28** 1247-1256.

Baile CA, Yang JY, Rayalam S, Hartzell DL, Lai CY, Andersen C & Della-Fera MA 2011 *Effect of resveratrol on fat mobilization. Annals of New York Academy of Sciences* **1215** 40-47.

Barker DJ 2007 The origins of the developmental origins theory. *Journal of Internal Medicine* 261, 412-417.

Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K et al 2006 Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **444** 337-342.

Bonomo IT, Lisboa PC, Pereira AR, Passos MC & de Moura EG 2007 Prolactin inhibition in dams during lactation programs for overweight and leptin resistance in adult offspring. *Journal of Endocrinolology* **192** 339-344.

Chachay VS, Kirkpatrick CM, Hickman IJ, Ferguson M, Prins JB & Martin JH 2011 Resveratrol-pills to replace a healthy diet? *British Journal of Clinical Pharmacology* **72** 27-38.

Costa Cdos S, Rohden F, Hammes TO, Margis R, Bortolotto JW, Padoin AV, Mottin CC & Guaragna RM 2011 Resveratrol upregulated SIRT1, FOXO1, and adiponectin and downregulated PPARγ1-3 mRNA expression in human visceral adipocytes. *Obesity Surgery* **21** 356-361.

Das S, Lin HS, Ho PC & Ng KY 2008 The impact of aqueous solubility and dose on the pharmacokinetic profiles of resveratrol. *Pharmacology Research* **25** 2593-2600.

de Armas MG, Megías SM, Modino SC, Bolaños PI, Guardiola PD & Alvarez TM 2009 Importance of breastfeeding in the prevalence of metabolic syndrome and degree of childhood obesity. *Endocrinología y Nutrición* **56** 400-403.

de Moura EG, Bonomo IT, Nogueira-Neto JF, de Oliveira E, Trevenzoli IH, Reis AM, Passos MC & Lisboa PC 2009 Maternal prolactin inhibition during lactation programs for met abolic syndrome in adult progeny. *Journal of Physiology* **587** 4919-4129.

de Moura EG, Lisboa PC & Passos MC 2008 Neonatal programming of neuroimmunomodulation-role of adipocytokines and neuropeptides. *Neuroimmunomodulation 2008* **15** 176-188.

El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C &Flier JS 2000 Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *Journal of Clinical Investigation* **105** 1827-1832.

Fernandez-Twinn DS & Ozanne SE 2010 Early life nutrition and metabolic programming. *Annals of The New York Academy of Sciences* **1212** 78-96.

Franco JG, de Moura EG, Koury JC, Trotta PA, Cordeiro A, Souza LL, Almeida NA, Lima Nda S, Pazos-Moura CC, Lisboa PC et al 2010 Resveratrol reduces lipid peroxidation and increases sirtuin 1 expression in adult animals programmed by neonatal protein restriction. *Journal of Endocrinology* **207** 319-328.

Friedman JM 2011 Leptin and the regulation of body weight. *The Keio Journal of Medicine* **60** 1-9.

Galic S, Oakhill JS & Steinberg GR 2010 Adipose tissue as an endocrine organ. *Molecular and Cellular Endocrinology* **316** 129-139.

Gerhart-Hines Z, Rodgers JT, Bare O, Lerin C, Kim SH, Mostoslavsky R, Alt FW, Wu Z & Puigserver P 2007 Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. *Embo Journal* **26** 1913-1923.

Ghanim H, Sia CL, Abuaysheh S, Korzeniewski K, Patnaik P, Marumganti A, Chaudhuri A & Dandona P 2010 An antiinflammatory and reactive oxygen species suppressive effects of an extract of Polygonum cuspidatum containing resveratrol. *Journal of Clinical Endocrinology and Metabolism* **95** 1-8

Gluckman PD & Hanson MA 2007 Developmental plasticity and human disease: research directions. *Journal of International Medicine* **261** 461-471.

Gómez-Zorita S, Fernández-Quintela A, Macarulla MT, Aguirre L, Hijona E, Bujanda L, Milagro F, Martínez JA & Portillo MP 2009 Resveratrol attenuates steatosis in obese Zucker rats by decreasing fatty acid availability and reducing oxidative stress. *The British Journal of Nutrition* **107** 202-210.

Kim S, Jin Y, Choi Y & Park T 2011 Resveratrol exerts anti-obesity effects via mechanisms involving down-regulation of adipogenic and inflammatory processes in mice. *Biochemical Pharmacology* **81** 1343-1351.

Kim SJ, Lee YH, Han MD, Mar W, Kim WK & Nam KW 2010 Resveratrol, purified from the stem of Vitis coignetiae Pulliat, inhibits food intake in C57BL/6J Mice. *Archives of Pharmacal Research* **33** 775-780.

Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P et al 2006 Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* **127** 1109-1122.

Lekli I, Ray D & Das DK 2010 Longevity nutrients resveratrol, wines and grapes. *Genes & Nutrition* **5** 55-60.

Lekli I, Szabo G, Juhasz B, Das S, Das M, Varga E, Szendrei L, Gesztelyi R, Varadi J, Bak I et al 2008 A Protective mechanisms of resveratrol against ischemia-reperfusion-induced damage in hearts obtained from Zucker obese rats: the role of GLUT-4 and endothelin. *American Journal Physiology. Heart and Circulatory Physiology* **294** 859-66.

Lima Nda S, de Moura EG, Passos MC, Nogueira Neto FJ, Reis AM, de Oliveira E & Lisboa PC 2011 Early weaning causes undernutrition for a short period and programmes some metabolic syndrome components and leptin resistance in adult rat offspring. *British Journal of Nutritional* **105** 1405-1413.

Lisboa PC, Passos MC, Dutra SC, Bonomo IT, Denolato AT, Reis AM & Moura EG 2006 Leptin and prolactin, but not corticosterone, modulate body weight and thyroid function in protein-malnourished lactating rats. *Hormone and Metabolic Research* **38** 295-299.

Lisboa PC, Pires L, de Oliveira E, Lima NS, Bonomo IT, Reis AM, Passos MC & Moura EG 2010 Prolactin inhibition at mid-lactation influences adiposity and thyroid function in adult rats. *Hormone and Metabolic Research* **42** 562-569.

Lönnqvist F, Wennlund A & Arner P 1997 Relationship between circulating leptin and peripheral fat distribution in obese subjects. *International Journal of Obesity and Related Metabolic Disorders* **21** 255-260.

Lopes Nobre J, Lisboa PC, da Silva Lima N, Franco JG, Firmino Nogueira Neto J, de Moura EG & de Oliveira E 2011 Calcium supplementation prevents obesity, hyperleptinaemia and hyperglycaemia in adult rats programmed by early weaning. *British Journal of Nutrition* **9**:1-10.

Marques RG, Morales MM & Petroianu A 2009 Brazilian law for scientific use of animals. *Acta Cirúrgica Brasileira* **24** 69-74.

Minakawa M, Kawano A, Miura Y & Yagasaki K 2011 Hypoglycemic effect of resveratrol in type 2 diabetic model db/db mice and its actions in cultured L6 myotubes and RIN-5F pancreatic β-cells. Journal of Clinical Biochemistry and Nutrition **48** 237-244.

Morton GJ & Schwartz MW 2011 Leptin and the central nervous system control of glucose metabolism. *Physiological Reviews* **91** 389-411.

Owen CG, Martin RM, Whincup PH, Smith GD & Cook DG 2006 Does breastfeeding influence risk of type 2 diabetes in later life? A quantitative analysis of published evidence. *American Journal of Clinical Nutrition* **84** 1043-1054

Passos MCF, Ramos CF & Moura EG 2000 Short and long term effects of malnutrition in rats during lactation on the body weight of offspring. *Nutrition Reserach* **20** 1603-1612.

Prasad K 2010 Natural products in regression and slowing of progression of atherosclerosis. *Current Pharmaceutical Biotechnology* **11** 794-800.

Rahmouni K 2010 Leptin-Induced Sympathetic Nerve Activation: Signaling Mechanisms and Cardiovascular Consequences in Obesity. *Current Hypertensio Reviews* **6** 104-209.

Rayalam S, Yang JY, Ambati S, Della-Fera MA & Baile CA 2008 Resveratrol induces apoptosis and inhibits adipogenesis in 3T3-L1 adipocytes. *Phytotherapy Research* **22** 1367-1371.

Rivera L, Morón R, Zarzuelo A & Galisteo M 2009 Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats. *Biochemical Pharmacology* **77** 1053-1063

Rogers CQ, Ajmo JM & You M 2008 Adiponectin and alcoholic fatty liver disease. *IUBMB Life* **60** 790-797.

Sahu A 2003 Leptin signaling in the hypothalamus: emphasis on energy homeostasis and leptin resistance. *Frontiers in Neuroendocrinology* **24** 225-253.

Sarzani R, Salvi F, Dessi-Fulgheri P & Rappelli A 2008 Reninangiotensin system, natriuretic peptides, obesity, metabolic syndrome, and hypertension: an integrated view in humans. *Journal of Hypertension* **26** 831–843.

Sharma S, Misra CS, Arumugam S, Roy S, Shah V, Davis JA, Shirumalla RK & Ray A 2010 Antidiabetic activity of resveratrol, a known SIRT1 activator in a genetic model for type-2 diabetes. *Phytotherapy Research* **25** 67-73.

Szkudelska K, Nogowski L & Szkudelski T 2009a Resveratrol, a naturally occurring diphenolic compound, affects lipogenesis, lipolysis and the antilipolytic action of insulin in isolated rat adipocytes. *Journal of Steroid and Biochemistry Molecular Biology* **113** 17-24.

Szkudelska K, Nogowski L & Szkudelski T 2009b The inhibitory effect of resveratrol on leptin secretion from rat adipocytes. *European Journal of Clinical Investigation* **39** 899-905.

Tauriainen E, Luostarinen M, Martonen E, Finckenberg P, Kovalainen M, Huotari A, Herzig KH, Lecklin A & Mervaala E 2011 Distinct effects of calorie restriction and resveratrol on diet-induced obesity and Fatty liver formation. *Journal of Nutrition and Metabolism* **2011** 525094.

Valassi E, Scacchi M & Cavagnini F 2008 Neuroendocrine control of food intake. *Nutrition, Metabolism and Cardiovascular Disease* **18** 158-168.

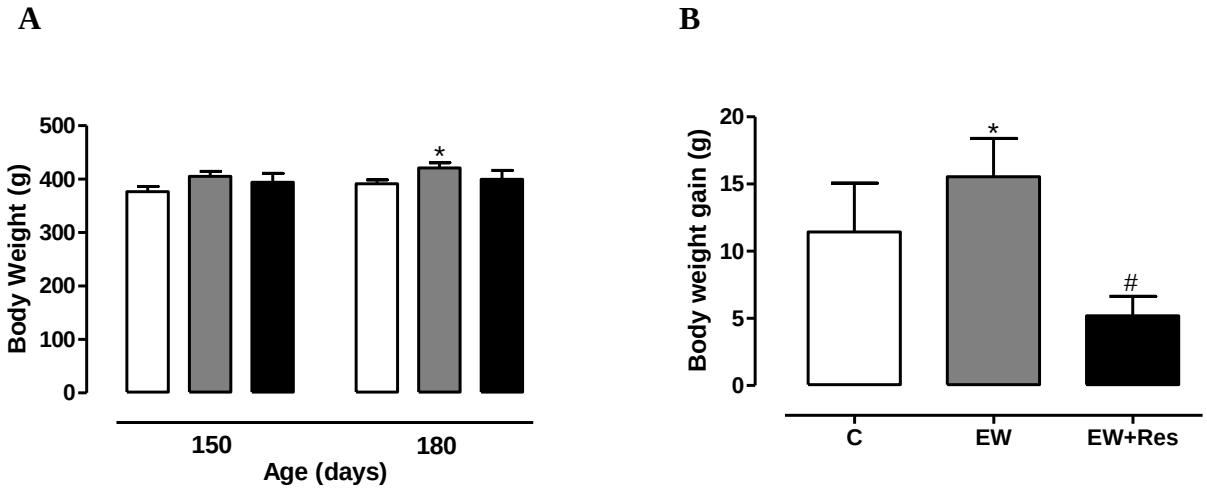
World Health Organization (WHO) 2011 Obesity and Overweight. Fact sheet nº 311. <http://www.who.int/mediacentre/factsheets/fs311/en/index.html>

Xia X & Weng J 2010 Targeting metabolic syndrome: candidate natural agents. *Journal of Diabetes* **2** 243-249.

Zabolotny JM, Bence-Hanulec KK, Stricker-Krongrad A, Haj F, Wang Y, Minokoshi Y, KimYB, Elmquist JK, Tartaglia LA, Kahn BB et al 2002 PTP1B regulates leptin signal transduction in vivo. *Developmental Cell* **2** 489-495.

Zhang J 2006 Resveratrol inhibits insulin responses in a SirT1-independent pathway. *Biochemical Journal* **397** 519-527.

Figure 1



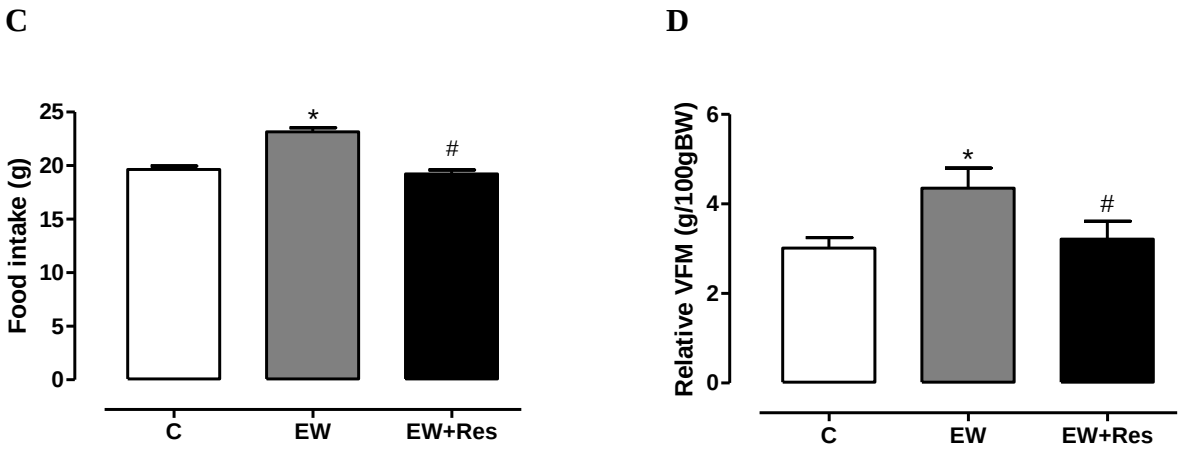
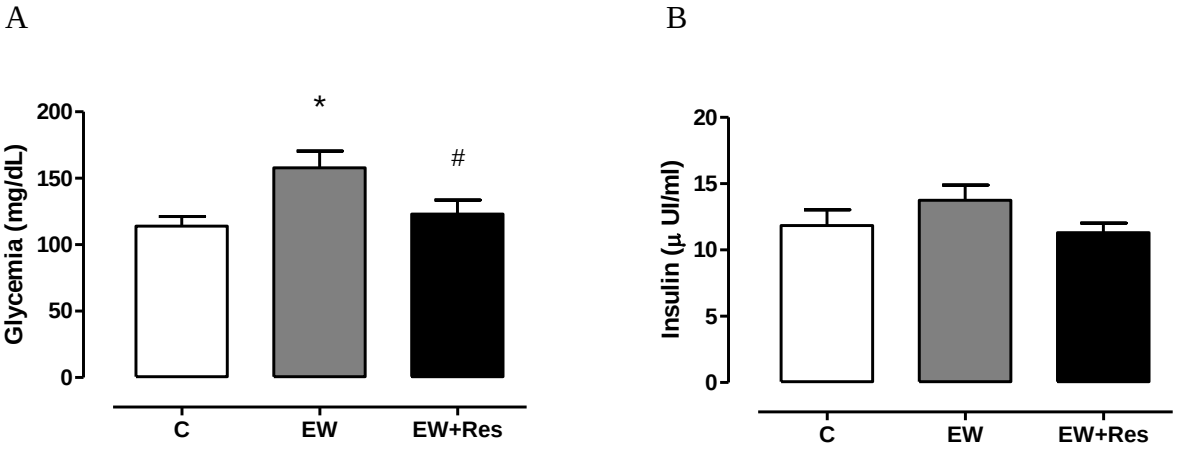


Figure 1. A) Body weight at 150 d (first day of Res treatment) and at 180 d; B) body weight gain in 30 days; C) food intake in 30 days and D) relative visceral fat mass of adult control (C) and early weaning offspring treated (EW+Res) or no with resveratrol (EW) for 30 days. Values are means, with their standard errors represented by vertical bars, n=10. Statistical significance was determined by one-way ANOVA and Newman-Keuls post-test; $p<0,05$; * vs C; # vs EW

Figure 2



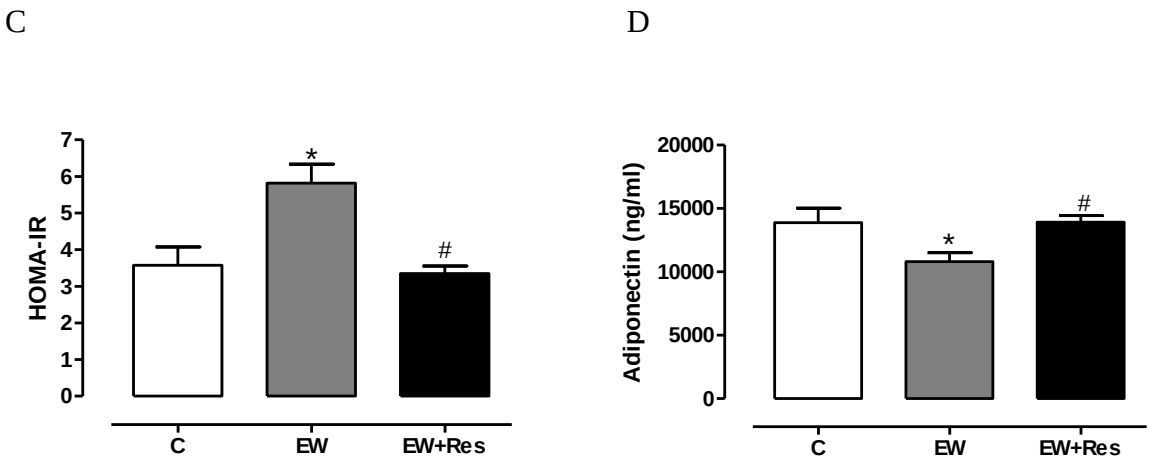
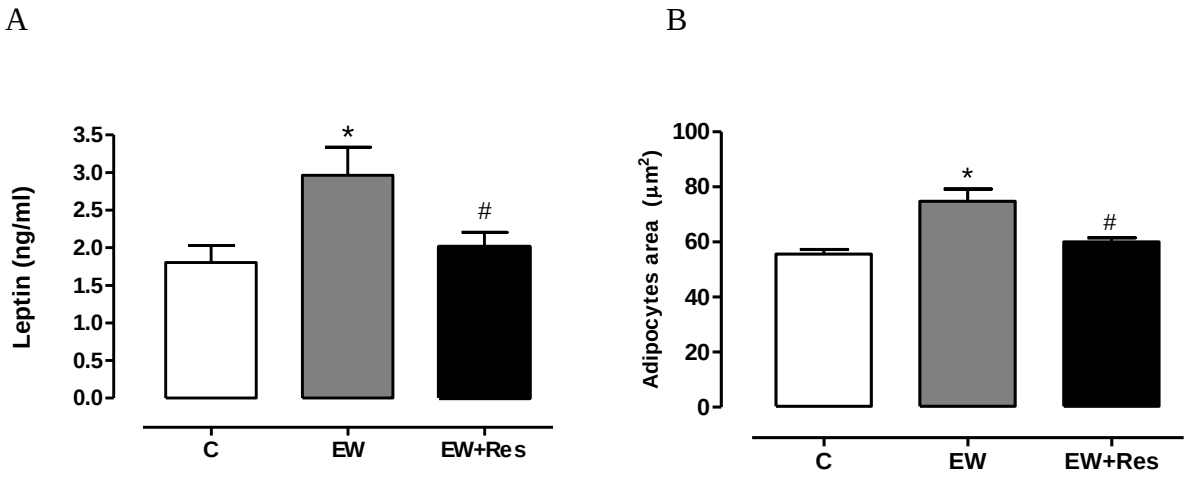


Figure 2. A) Glycaemia; B) insulinemia; C) homeostasis model assessment of insulin resistance (HOMA-IR) and D) adiponectinemia of adult control (C) and early weaning offspring treated (EW+Res) or no with resveratrol (EW) for 30 days. Values are means, with their standard errors represented by vertical bars, n=10. Statistical significance was determined by one-way ANOVA and Newman-Keuls post-test; p<0,05; * vs C; # vs EW

Figure 3



C

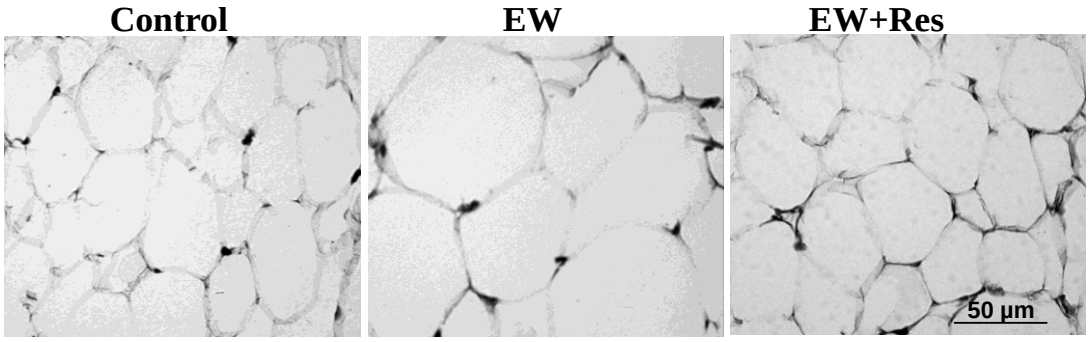


Figure 3. A) Serum leptin (n = 10) B) and mean cross-sectional area of adipocytes (n = 5) B). C) Photomicrographs of the white adipose tissue (epididymal fat) with same magnification (40x) and stained with hematoxylin-eosin (H&E). Adipocytes with normal size both in Control (C) and early weaning that received resveratrol (EW+Res) and hypertrophic adipocytes in early weaning rats (EW). Values are means, with their standard errors represented by vertical bars. Statistical significance was determined by one-way ANOVA and Newman-Keuls post-test; $p<0,05$; * vs C; # vs EW.

Figure 4

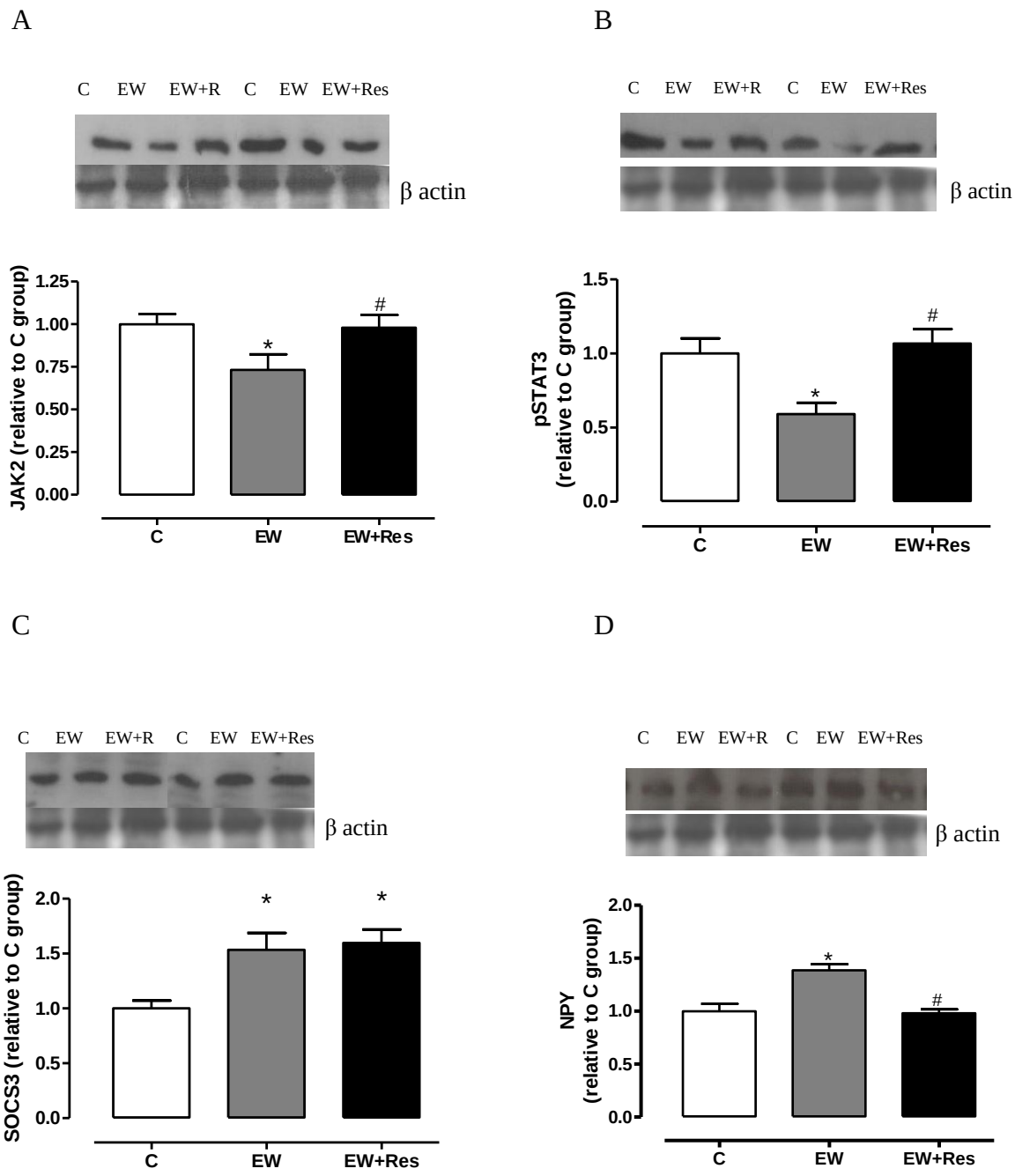


Figure 4. A) Hypothalamic content of janus tyrosine kinase 2 (JAK2); B) phosphorylated STAT (pSTAT); C) suppressor of cytokine signalling 3 (SOCS3) and D) NPY. Actin content was used as the control loading. Values are means with standard errors, represented by vertical bars, n=7. Statistical significance was determined by one-way ANOVA and Newman-Keuls post-test; $p<0,05$; * vs C; # vs EW.

4 DISCUSSÃO

No presente estudo demonstramos que tanto a desnutrição, provocada pelo desmame precoce, quanto a supernutrição, causada pela redução do número de filhotes, causou nas proles adultas aumento de massa corporal, obesidade do tipo visceral e hiperfagia, corroborando com nossos achados anteriores (Rodrigues et al., 2009; Conceição et al., 2011; Lima et al., 2011; Lopes Nobre et al., 2011; Rodrigues et al, 2011) além de importante alterações no estresse oxidativo que são dados originais para estes dois modelos.

O estresse oxidativo tem sido proposto como um importante mecanismo causador de resistência à insulina, hipertensão e doença cardiovascular (Otani, 2011; Roberts & Sindhu, 2009). Acredita-se que o aumento do estresse oxidativo contribua para a instalação de inflamação sistêmica com consequente desregulação da produção de adipocinas (Otani, 2011). Como em ambos os modelos de plasticidade ontogenética (desmame precoce e supernutrição) os animais apresentaram componentes da SM na idade adulta, um dos objetivos deste trabalho foi avaliar o estresse oxidativo nesses animais.

Com o aumento alarmante da prevalência de obesidade em diferentes regiões no mundo, diversas estratégias de tratamento têm sido propostas nos últimos anos. Contudo, combater este grave problema de saúde pública permanece um grande desafio. Como a intervenção dietética é considerada como a primeira opção no tratamento da obesidade, polifenóis derivados de plantas, como o resveratrol, têm sido sugeridos como estratégias terapêuticas coadjuvantes e, por essa razão, têm despertado bastante interesse nos últimos anos. Dessa maneira, também foi objetivo deste trabalho estudar os efeitos antioxidantes do resveratrol e as possíveis consequências sobre componentes da SM em ratos adultos programados pelo desmame precoce.

Recentemente, mostramos que ratos SN apresentam adipócitos hipertróficos e sabe-se que este tipo de adipócito se relaciona com aumento do risco cardiovascular. No entanto, foi visto que a secreção de leptina pelos adipócitos está diminuída na idade adulta, sugerindo uma disfunção deste tecido nesses animais (Conceição et al., 2011). Anteriormente foi demonstrado em seres humanos que a leptina se correlaciona negativamente com a severidade da SM (Paz-Filho et al., 2009). Assim, é provável que ocorra uma exaustão da produção da leptina pelo tecido adiposo dos ratos SN (Conceição et al., 2011), assim como pode haver exaustão da célula beta pancreática na resistência à insulina. A leptina, além de atuar na regulação de ingestão de alimentos e na manutenção do peso corporal, pode atuar no tecido

adiposo promovendo lipólise e adipogênese. Conceição et al. (2011) sugeriram que a secreção diminuída de leptina, pode estar relacionada a hipertrofia dos adipócitos nos animais SN.

Em nosso primeiro trabalho apresentado (Artigo 1), mostramos que os animais programados pela supernutrição na lactação tiveram aumento do estresse oxidativo no plasma e no fígado, associado com resistência à insulina, acúmulo de gordura e infiltrado inflamatório hepáticos. Os animais SN apresentaram obesidade e hiperfagia persistentes até a idade adulta, corroborando com achados anteriores em nosso laboratório (Rodrigues et al., 2009; Rodrigues et al., 2011; Conceição et al., 2011). A hiperfagia nestes animais pode ser explicada pela resistência à leptina, identificada na idade adulta pela diminuição de pSTAT e aumento de SOCS3 hipotalâmicos, apesar da normoleptinemia (Rodrigues et al., 2011). O excesso de nutrientes eleva a utilização da cadeia de transporte de elétrons mitocondrial, aumentando da geração de ERO (Rudich et al, 2007; de Oliveira et al., 2010). Os animais SN apresentaram maior dano oxidativo e diminuição da atividade da SOD, CAT e GPx no fígado. Uma vez que estes animais apresentaram grande quantidade de tecido adiposo visceral e área aumentada dos adipócitos, é provável que ocorra a liberação desregulada de citocinas inflamatórias que contribuam ainda mais para o estresse oxidativo. TNFα e IL-6 são capazes de reduzir a sinalização à insulina através de indução de SOCS-3 e da fosforilação de IRS1 em resíduos de serina (Mooney et al., 2001; Popa et al., 2007). O estresse oxidativo provavelmente contribuiu para resistência à insulina no fígado destes animais evidenciada pelo menor conteúdo de IRβ, IRS1, pIRS1, PI3-K e Akt1. A relação entre estresse oxidativo e diabetes vem sendo proposta por diversos autores (Roberts & Sindhu, 2009; Otani, 2011). As ERO são capazes de inibir a sinalização da insulina através da ativação de JNK (kinase c-jun N-terminal kinase) com consequente fosforilação em resíduos de serina do substrato do receptor de insulina 1 (IRS1) (Hirosumi et al., 2002), diminuição de IRS1 e IRS2 e diminuição da fosforilação de Akt (Archuleta et al., 2009). Outros autores mostraram que apesar da normoinsulinemia, animais superalimentados na lactação apresentaram resistência à insulina no adipócito (Rodrigues et al., 2007) e no coração (Martins et al. 2008). Recentemente, a hipermetilação do promotor do receptor de insulina foi relatada neste modelo de plasticidade ontogenética (Plagemann et al., 2010). Portanto, é possível também que o tecido adiposo dos animais programados apresente resistência à insulina, prejudicando o armazenamento de lipídeos nos adipócitos, que então se acumulam na circulação ou mesmo se depositam em outros tecidos.

Sabe-se que o estresse oxidativo pode promover injúria em diferentes tecidos, incluindo o fígado (Rolo et al., 2012). Evidenciamos nos animais SN, quando observamos a morfologia hepática, a presença de microesteatose e de infiltrado inflamatório aos 180 dias. A

esteatose não alcoólica (NAFLD - nonalcoholic fat liver disease) caracteriza-se pela deposição de gordura no fígado, sendo sua causa não diretamente relacionada ao consumo de etanol e está fortemente associada à obesidade, principalmente à obesidade visceral (Gabriely & Barzilai, 2003). O adipócito visceral é lipoliticamente mais ativo que o subcutâneo e a liberação de ácidos graxos livres, assim como de adipocinas diretamente na veia porta expõe o fígado a estas moléculas, favorecendo o desenvolvimento de esteatose (Saito et al., 2007). Portanto, a supernutrição neonatal induzida pela redução do tamanho da ninhada causou obesidade com acúmulo de gordura visceral, hiperfagia, estresse oxidativo e esteatose hepática nas proles na vida adulta.

No segundo trabalho desta tese (Artigo 2), evidenciamos aumento do estresse oxidativo também em ratos desmamados precocemente. Neste modelo, utilizamos o resveratrol, um polifenol estilbeno com atividade antioxidante, para testar a seguinte hipótese: se modulação do estresse oxidativo pode prevenir ou melhorar componentes da SM previamente identificados neste modelo de plasticidade ontogenética (Lima et al., 2011; Lopes Nobre et al., 2011).

Além das características já relatadas, como obesidade visceral, hiperfagia e hipertrigliceridemia (Lima et al., 2011; Lopes Nobre et al., 2011), neste estudo evidenciamos que ratos adultos programados pelo desmame precoce tiveram hipertensão arterial, diminuição de HDL-c e aumento de LDL-c, acúmulo de trigilicerídeos hepáticos e esteatose na vida adulta. Associado a essas alterações metabólicas, estes animais tiveram aumento de estresse oxidativo. O resveratrol preveniu o aumento do estresse oxidativo, assim como o desenvolvimento de hipertensão arterial, dislipidemia e esteatose nos animais DP. Ainda, mostramos no 3º artigo desta tese (Artigo 3) que o resveratrol normalizou a leptinemia, a adiponectinemia, a glicemia e a resistência à insulina nestes animais. Além disso, corrigiu a resistência central à leptina e a hiperfagia.

O estresse oxidativo está envolvido na gênese de doenças cardiovasculares e um potente mecanismo proposto é a disfunção endotelial. Na hipertensão arterial sistêmica (HAS), ocorre aumento da produção intravascular de ERO e ERN com participação importante das enzimas NADPH oxidase, XO, Cox e eNOS. Além disso, a HAS cursa com o declínio na atividade do óxido nítrico que pode ser devido a diminuição na expressão ou atividade de eNOS, ausência de substrato ou cofator para eNOS ou ainda degradação acelerada desta enzima (Cai & Harrison, 2008). A HAS nos animais DP pode ser associada também ao aumento da concentração sérica de leptina. A leptina atua diretamente no coração e nos vasos sanguíneos via receptores específicos estimulando a frequência cardíaca e a

pressão arterial e através da estimulação central do sistema nervoso simpático (Rahmouni et al., 2010). Além disso, a hipoadiponectinemia pode estar envolvida no aumento da PA, uma vez que estudos clínicos e experimentais mostram que a hipoadiponectinemia é um fator de risco independente para o desenvolvimento de hipertensão associada à obesidade (Nishida et al., 2007). O excesso de gordura visceral e a consequente liberação de AGL e citocinas, além do estresse oxidativo contribuíram para o desenvolvimento de esteatose nestes animais, assim como de dislipidemia. A esteatose é causada pelo maior influxo de AGL no fígado. Os AGL em excesso favorecem produção hepática de VLDL e TG, bem como a hidrólise das partículas de LDL repletas de TG pela lipase hepática, com consequente formação de moléculas de LDL pequenas e densas e de HDL do tipo 3 (carregadas de TG) que podem rapidamente serem degradadas e excretadas pelos rins, comprometendo a função cardioprotetora desta lipoproteína. Este mecanismo pode explicar o aumento de TG e LDL-c e a diminuição de HDL-c encontrados nos animais DP. Ainda, o influxo de AGL no fígado prejudica a capacidade oxidativa mitocondrial, aumentando o estado reduzido da cadeia de transporte de elétrons (ETC) e estimulando vias peroxisomais e microssomais da oxidação de AGL, o que eleva a produção de ERO (Rolo et al., 2012).

O resveratrol normalizou a concentração de TBARS tanto no plasma quanto no fígado e de SOD no plasma, e aumentou a atividade plasmática da CAT. A diminuição da produção de radicais livres pelo resveratrol é atribuída, principalmente, à modulação do sistema antioxidante enzimático, através da inibição da enzima pró-antioxidante NADPH oxidase (Orallo et al., 2002; Soylemez et al., 2008; Soylemez et al., 2009) e ativação de enzimas antioxidantes catalase, SOD e GPx (Ungvari et al., 2007; Spanier et al., 2009). Considerando a participação de SOD e CAT na interceptação de ERO, podemos sugerir que o resveratrol normalizou a geração de ERO e, contribuindo para a normalização da PA e dos TG e LDL-c plasmáticos. O aumento da atividade da SOD pelo resveratrol evitou o desacoplamento da enzima eNOS e aumentou a biodisponibilidade de óxido nítrico (NO) em ratos hipertensos (Bhatt et al., 2011). Sabe-se que a eNOS desacoplada não perde a capacidade de receber elétrons, doando-os um a um ao seu substrato O_2 e consequentemente, gera superóxido ao invés de NO. Portanto, podemos sugerir que através da melhora da função endotelial, o resveratrol normalizou a PA. Além disso a normalização da leptina, mostrada no Artigo 3, também pode ter contribuido para a normalização da PA. Alguns estudos têm mostrado efeitos do resveratrol sobre a mobilização de lipídeos, diminuindo o acúmulo de gordura tanto em adipócitos isolados (Lagouge et al., 2006), quanto *in vivo* no tecido adiposo e no fígado (Baur et al., 2006; Lagouge et al., 2006; Shang et al., 2008, Bujanda et al., 2008; Rivera et al.,

2009) e aumentando a oxidação de ácidos graxos no músculo esquelético (Baur et al., 2006; Lagouge et al., 2006). Os efeitos do resveratrol sobre os lipídeos plasmáticos podem envolver ainda a redução da expressão de hidroxi metil gutamil CoA (HMG CoA) redutase (Cho et al., 2008) e o aumento da excreção de sais biliares nas fezes (Miura et al., 2003). Rivéra et al. (2009) mostraram que o resveratrol reduziu os TG plasmáticos em ratos obesos. Gómez-Zorita et al. (2012) também encontraram o efeito do resveratrol em ratos Zucker obesos na diminuição dos TG hepáticos e na melhora da esteatose. A prevenção da obesidade visceral nos animais DP também sugere uma menor liberação de AGL e citocinas contribuindo para a normalização dos lipídeos plasmáticos, assim como a redução do acúmulo de TG hepáticos e prevenção de esteatose nestes animais.

O resveratrol também preveniu a resistência à insulina nos animais DP. Apesar de não provocar alteração na insulina plasmática, o resveratrol normalizou a adiponectina e a glicemia. Diversos estudos mostraram que o resveratrol aumentou adiponectina em adipócitos isolados (Costa et al., 2011) e *in vivo* (Gómez-Zorita et al., 2012) e a captação de glicose no músculo esquelético através do aumento da translocação do GLUT4 e da fosforilação da AKT (Minakawa et al., 2011). Portanto, mesmo não alterando sua concentração, o resveratrol aumentou a sensibilidade à insulina tanto através da normalização da adiponectinemia quanto pelo aumento da captação de glicose.

Os animais programados pelo DP apresentaram diminuição de JAK2 e pSTAT3 e aumento de SOCS3 e NPY no hipotálamo indicando resistência à leptina (Lima et al., 2011 e Younes-Rapozo et al., 2012) . O resveratrol corrigiu essa resistência, assim como a ingestão de ração durante o período de tratamento. Ao que se saiba, este foi o primeiro estudo a avaliar o efeito do resveratrol sobre a sinalização da leptina e o NPY no hipotálamo no qual mostramos a normalização das proteínas JAK2, pSTAT3 e do NPY (Artigo 3). O conteúdo de SOCS3, entretanto permaneceu aumentado. Em um estudo anterior, os animais DP que receberam suplementação de cálcio tiveram correção da hiperfagia e da resistência central à leptina também sem normalização do conteúdo de SOCS3, que permaneceu aumentado (Lopes Nobre et al., 2011). A via da leptina pode ser regulada negativamente também pela fosfatase PTP-1B. Embora não tenhamos avaliado essa fosfatase neste estudo, é possível ela esteja reduzida, pois sabe-se que a sirtuína 1 (SIRT1) suprime sua expressão e que o resveratrol ativa a SIRT1. Anteriormente, demonstramos que o resveratrol aumentou a expressão de SIRT1 em ratos Wistar (Franco et al., 2010). A redução da ingestão foi demonstrada em camundongos injetados via I.P. com resveratrol (100 mg/kg) por Kim et al. (2009) junto com o aumento da expressão de NPY e AgRP *in vitro* em células hipotalâmicas.

Portanto, parece haver um efeito direto central do resveratrol sobre o controle alimentar, que neste estudo foi mostrado pela primeira vez *in vivo*. Não podemos deixar de atribuir a normalização da leptina sérica à correção da hiperfagia e da resistência central a este hormônio.

Comparando os dois modelos estudados (SN *vs* DP) quanto ao aumento do estresse oxidativo, podemos verificar que na supernutrição houve uma redução mais importante na atividade das enzimas antioxidantes no fígado destes animais, que se relacionou com a resistência à ação da insulina neste órgão. Nos animais DP, somente a atividade da GPx esteve diminuída no fígado, no entanto houve uma redução significativa da atividade da SOD e da GPx, esta porém não significativa, no plasma. Em ambos os modelos, não encontramos alteração significativa no conteúdo hepático das enzimas antioxidantes. A esteatose se relacionou com o aumento da lipoperoxidação no fígado tanto no SN quanto no DP.

A programação do dano oxidativo e da defesa antioxidante também foi vista em ratos cujas mães foram alimentadas com dieta rica em gordura (Zhang et al., 2011) ou com dieta pobre em proteína durante a gestação (Theys et al., 2009), sugerindo que a capacidade antioxidante e o dano oxidativo podem ser programados *in utero* (Strakovsky & Pan, 2011). No presente estudo mostramos que o estresse oxidativo pode também ser programado durante a lactação, seja pelo excesso ou pela falta de nutrientes (Artigos 1 e 2), sugerindo que genes envolvidos na regulação deste podem ser epigeneticamente regulados no período neonatal. Nossos achados sugerem ainda que o resveratrol pode ser útil no tratamento da obesidade e que estudos direcionados a avaliar os mecanismos modulados pelo resveratrol em animais obesos, bem como o uso deste composto em humanos, são necessários.

5 **CONCLUSÃO**

A supernutrição na lactação e o desmame precoce programaram o aumento do estresse oxidativo, que se associou com a presença de obesidade visceral e complicações metabólicas, tais como a esteatose hepática na vida adulta.

O resveratrol administrado via oral por 30 dias nos ratos adultos programados pelo DP preveniu o estresse oxidativo e distúrbios metabólicos como a resistência à insulina, a dislipidemia e a esteatose. Os efeitos do resveratrol nos animais DP provavelmente foram mediados pela prevenção da obesidade visceral e pela normalização da secreção de adipocinas, como a leptina. A normalização da leptinemia, assim como da sua ação no hipotálamo, controlou ainda a hiperfagia característica dos animais programados pelo DP.

REFERÊNCIAS

Ahima RS, Lazar MA. Adipokines and the peripheral and neural control of energy balance. *Mol Endocrinol*. 2008; 22: 1023-1031.

Ahima RS. Central actions of adipocyte hormones. *Trends Endocrinol Metab.* 2005;16: 307-313.

Archuleta TL, Lemieux AM, Saengsirisuwan V, Teachey MK, Lindborg KA, Kim JS, Henriksen EJ. Oxidant stress-induced loss of IRS-1 and IRS-2 proteins in rat skeletal muscle: role of p38 MAPK. *Free Radic Biol Med*. 2009; 47: 1486-1493.

Arenz S, Rückerl R, Koletzko B, von Kries R. Breast-feeding and childhood obesity - a systematic review. *Int J Obes Relat Metab Disord.* 2004; 28(10): 1247-1256.

Baile CA, Yang JY, Rayalam S, Hartzell DL, Lai CY, Andersen C, Della-Fera MA. Effect of resveratrol on fat mobilization. *Ann N Y Acad Sci.* 2011; 1215:40-47.

Banks WA. Leptin transport across the blood-brain barrier: implications for the cause and treatment of obesity. *Curr Pharm Des*. 2001; 7: 125-133.

Barker D. Commentary: birthweight and coronary heart disease in a historical cohort. *Int J Epidemiol*. 2006; 35: 886-887.

Barker DJ. The fetal and infant origins of disease. *Eur J Clin Invest*. 1995; 25(7): 457-463.

Barker DJ. The origins of the developmental origins theory. *J Intern Med*. 2007; 261: 412-417.

Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the in vivo evidence. *Nat Rev Drug Discov*. 2006; 5(6): 493-506.

Bertelli AA, Giovannini L, Stradi R, Bertelli A, Tillement JP. Plasma, urine and tissue levels of trans- and cis-resveratrol (3,4',5-trihydroxystilbene) after short-term or prolonged administration of red wine to rats. *Int J Tissue React*. 1996, 18: 67-71.

Bhatt SR, Lokhandwala MF, Banday AA. Resveratrol prevents endothelial nitric oxide synthase uncoupling and attenuates development of hypertension in spontaneously hypertensive rats. *Eur J Pharmacol*. 2011; 667(1-3): 258-264.

Bjorbaek C, El-Haschimi K, Frantz JD, Flier JS. The role of SOCS-3 in leptin signaling and leptin resistance. *J Biol Chem*. 1999; 274: 30059-30065.

Bjorbaek C, Kahn BB. Leptin signaling in the central nervous system and the periphery. *Recent Prog Horm Res.* 2004; 59: 305-331.

Bonomo IT, Lisboa PC, Passos MC, Alves SB, Reis AM, de Moura EG. Prolactin inhibition at the end of lactation programs for a central hypothyroidism in adult rat. *J Endocrinol.* 2008; 198(2): 331-337.

Bonomo IT, Lisboa PC, Passos MC, Pazos-Moura CC, Reis AM, Moura EG. Prolactin inhibition in lactating rats changes leptin transfer through the milk. *Horm Metab Res*. 2005; 37(4): 220-225.

Bonomo IT, Lisboa PC, Pereira AR, Passos MC, de Moura EG. Prolactin inhibition in dams during lactation programs for overweight and leptin resistance in adult offspring. *J Endocrinol*. 2007; 192(2): 339-344.

Boullu-Ciocca S, Dutour A, Guillaume V, Achard V, Oliver C, Grino M. Postnatal diet-induced obesity in rats upregulates systemic and adipose tissue glucocorticoid metabolism during development and in adulthood: its relationship with the metabolic syndrome. *Diabetes*. 2005; 54: 197-203.

Bradamante S, Barenghi L, Villa A. Cardiovascular protective effects of resveratrol. *Cardiovasc Drug Rev.* 2004; 22: 169–188.

Brasil. Ministério da Saúde. II Pesquisa nacional de aleitamento materno nas capitais brasileiras e Distrito Federal. Brasília, Ministério da Saúde, 2009. Disponível em: http://bvsms.saude.gov.br/bvs/publicacoes/pesquisa_prevalencia_aleitamento_materno.pdf Acesso em: 10.01.2012.

Bujanda L, Hijona E, Larzabal M, Beraza M, Aldazabal P, García-Urkia N, Sarasqueta C, Cosme A, Irastorza B, González A, Arenas JI Jr. Resveratrol inhibits nonalcoholic fatty liver disease in rats. *BMC Gastroenterol*. 2008; 8: 40.

Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases. The role of oxidant stress. *Circ Res*. 2002; 87: 840-844.

Candeia NM. Health education in the prevention of the risk of premature weaning. *Rev Saude Publica.* 1983; 17(2): 71-82.

Carrascoza KC, Costa Júnior AL, Moraes ABA. Fatores que influenciam o desmame precoce e a extensão do aleitamento materno. *Estudos de Psicologia*. 2005; 22(4): 433-440.

Carvalheira JB, Torsoni MA, Ueno M, Amaral ME, Araujo EP, Velloso LA, Gontijo JA, Saad MJ. Cross-talk between the insulin and leptin signaling systems in rat hypothalamus. *Obes Res*. 2005; 13: 48-57.

Chachay VS, Kirkpatrick CM, Hickman IJ, Ferguson M, Prins JB, Martin JH. Resveratrol--pills to replace a healthy diet? *Br J Clin Pharmacol*. 2011; 72(1): 27-38.

Chen B, Wei J, Wang W, Cui G, Zhao Y, Zhu X, Zhu M, Guo W, and Yu J. Identification of signaling pathways involved in aberrant production of adipokines in adipocytes undergoing oxidative stress. *Arch Med Res.* 2009; 40: 241-248.

Cho IJ, Ahn JY, Kim S, Choi MS, Ha TY. Resveratrol attenuates the expression of HMG-CoA reductase mRNA in hamsters. *Biochem Biophys Res Commun*. 2008; 367: 190-194.

Conceição EP, Trevenzoli IH, Oliveira E, Franco JG, Carlos AS, Nascimento-Saba CC, Moura EG, Lisboa PC. Higher white adipocyte area and lower leptin production in adult rats overfed during lactation. *Horm Metab Res*. 2011; 43(7): 513-516.

Conde WL, Borges C. The risk of incidence and persistence of obesity among Brazilian adults according to their nutritional status at the end of adolescence. *Rev Bras Epidemiol.* 2011; 14(1): 71-79.

Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL & et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med*. 1996; 334: 292-295.

Costa Cdos S, Rohden F, Hammes TO, Margis R, Bortolotto JW, Padoin AV, Mottin CC, Guaragna RM. Resveratrol upregulated SIRT1, FOXO1, and adiponectin and downregulated PPARγ1-3 mRNA expression in human visceral adipocytes. *Obes Surg.* 2011; 21(3): 356-361.

Davidowa H, Plagemann A. Insulin resistance of hypothalamic arcuate neurons in neonatally overfed rats. *Neuroreport*. 2007; 18: 521-524.

de Armas MG, Megías SM, Modino SC, Bolaños PI, Guardiola PD, Alvarez TM. Importance of breastfeeding in the prevalence of metabolic syndrome and degree of childhood obesity. *Endocrinol Nutr*. 2009; 56(8): 400-403.

de Moura EG, Bonomo IT, Nogueira-Neto JF, de Oliveira E, Trevenzoli IH, Reis AM, Passos MC, Lisboa PC. Maternal prolactin inhibition during lactation programs for metabolic syndrome in adult progeny. *J Physiol*. 2009; 587(20): 4919-4129.

de Moura EG, Lisboa PC, Passos MC. Neonatal programming of neuroimmunomodulation: role of adipocytokines and neuropeptides. *Neuroimmunomodulation.* 2008; 15(3): 176-188.

de Oliveira PR, da Costa CA, de Bem GF, de Cavalho LC, de Souza MA, de Lemos Neto M, da Cunha Sousa PJ, de Moura RS, Resende AC. Effects of an extract obtained from fruits of Euterpe oleracea Mart. in the components of metabolic syndrome induced in C57BL/6J mice fed a high-fat diet. *J Cardiovasc Pharmacol*. 2010; 56: 619-626.

de Onis M, Blössner M, Borghi E. Global prevalence and trends of overweight and obesity among preschool children. *Am J Clin Nutr*. 2010; 92: 1257-1264.

Devaraj S, Jialal I. Antioxidants and vitamins to reduce cardiovascular disease. *Curr Atheroscler Rep*. 2000; 2: 342-51.

Dong Z. Molecular mechanism of the chemopreventive effect of resveratrol. *Mutat Res*. 2003, 523-524:145-150.

dos Santos Oliveira L, de Lima DP, da Silva AA, da Silva MC, de Souza SL, Manhães-de-Castro R. Early weaning programs rats to have a dietary preference for fat and palatable foods in adulthood. *Behav Processes*. 2011; 86(1): 75-80.

Dröge W. Free radicals in the physiological control of cell function. *Physiol Rev*. 2002; 82: 47-95.

Dutra SCP, Passos MCF, Lisboa PC, Santos RS, Cabanelas AP, Pazos-Moura CC, Moura EG. Liver deiodinase activity is increased in adult rats whose mothers were submitted to malnutrition during lactation. *Horm Metab Res.* 2003; 35: 268-270.

Ector BJ, Magee JB, Hegwood CP, et al. Resveratrol concentration in muscadine berries, juice, pomace, purees, seeds, and wines. *Am J Enol Vitic*. 1996; 7: 57–62.

Efstathiou SP, Skeva II, Zorbala E, Georgiou E, Mountokalakis TD. Metabolic Syndrome in Adolescence: Can it be Predicted from Natal and Parental Profile? The Prediction of Metabolic Syndrome in Adolescence (PREMA) Study. *Circulation.* 2012.

Escobar, AMU, Ogawa AR, Hiratsuka , Kawashita MY, Teruya PY, Grisi S, Tomikawa SO. Aleitamento materno e condições socioeconômico-culturais: fatores que levam ao desmame precoce. Rev. Bras. Saude Mater. Infant. Recife, v. 2, n. 3, 2002 . Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1519-38292002000300006&lng=pt&nrm=iso. Acesso em 10.01.2012.

Fagundes AT, Moura EG, Passos MC, Oliveira E, Toste FP, Bonomo IT, Trevenzoli IH, Garcia RM, Lisboa PC. Maternal low-protein diet during lactation programmes body composition and glucose homeostasis in the adult rat offspring. *Br J Nutr.* 2007; 98(5): 922-928.

Fagundes AT, Moura EG, Passos MC, Santos-Silva AP, de Oliveira E, Trevenzoli IH, Casimiro-Lopes G, Nogueira-Neto JF, Lisboa PC. Temporal evaluation of body composition, glucose homeostasis and lipid profile of male rats programmed by maternal protein restriction during lactation. *Horm Metab Res.* 2009; 41: 866-873.

Faleiros, FTV, Trezza, EMC, Carandina, L. Aleitamento materno: fatores de influência na sua decisão e duração. Rev. Nutr. Campinas, v.19, n. 5, 2006. Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1415-52732006000500010&lng=en&nrm=iso. Acesso em 10.01.2012.

Fan Z, Zhang ZX, Li Y, Wang Z, Xu T, Gong X, Zhou X, Wen H, Zeng Y. Relationship between birth size and coronary heart disease in China. *Ann Med*. 2010; 42(8): 596-602.

Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, Esquivel-Soto J, Morales-González A, Esquivel-Chirino C, Durante-Montiel I, Sánchez-Rivera G, Valadez-Vega C, Morales-González JA. Inflammation, oxidative stress, and obesity. *Int J Mol Sci.* 2011; 12(5): 3117-3132.

Fernandez-Twinn DS & Ozanne SE. Early life nutrition and metabolic programming. *Ann N Y Acad Sci.* 2010; 1212: 78-96.

Fewtrell MS. Breast-feeding and later risk of CVD and obesity: evidence from randomised trials. *Proc Nutr Soc.* 2011; 70(4): 472-477.

Finaud J, Scislowski V, Lac G, Durand D, Vidalin H, Robert A, Filaire E. Antioxidant status and oxidative stress in professional rugby players: evolution throughout a season. *Int J Sports Med*. 2006; 27: 87-93.

Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of Obesity and Trends in the Distribution of Body Mass Index Among US Adults, 1999-2010. *JAMA*, 2012.

Fraga MC, Moura EG, Silva JO, Bonomo IT, Filgueiras CC, Abreu-Villaça Y, Passos MC, Lisboa PC, Manhães AC. Maternal prolactin inhibition at the end of lactation affects learning/memory and anxiety-like behaviors but not novelty-seeking in adult rat progeny. *Pharmacol Biochem Behav.* 2011; 100(1): 165-173.

Franco JG, de Moura EG, Koury JC, Trotta PA, Cordeiro A, Souza LL, Almeida NA, Lima Nda S, Pazos-Moura CC, Lisboa PC, Passos MC. Resveratrol reduces lipid peroxidation and increases sirtuin 1 expression in adult animals programmed by neonatal protein restriction. *J Endocrinol.* 2010; 207(3): 319-328.

Fridovich I. Superoxide radical and superoxide dismutases. *Annu Rev Biochem.* 1995; 64: 97–112.

Fujita K, Nishizawa H, Funahashi T, Shimomura I, Shimabukuro M. Systemic oxidative stress is associated with visceral fat accumulation and the metabolic syndrome. *Circulation Journal*. 2006; 70(11): 1437-1442.

Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, and Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest*. 2004; 114: 1752-1761.

Gabriely I, Barzilai N. Surgical removal of visceral adipose tissue: effects on insulin action. *Curr Diab Rep*. 2003; 3(3): 201-206.

Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. *Mol Cell Endocrinol*. 2010; 316(2): 129-319.

Gluckman PD & Hanson MA. The developmental origins of the metabolic syndrome. *Trends Endocrinol Metab*. 2004; 15: 183-187.

Gluckman PD, Hanson MA. Developmental plasticity and human disease: research directions. *J Intern Med.* 2007; 261(5): 461-471.

Gnoni GV, Paglialonga G. Resveratrol inhibits fatty acids and triacylglycerol synthesis in rat hepatocytes. *Eur J Clin Invest.* 2009; 39: 211–218.

Gómez-Zorita S, Fernández-Quintela A, Macarulla MT, Aguirre L, Hijona E, Bujanda L, Milagro F, Martínez JA, Portillo MP. Resveratrol attenuates steatosis in obese Zucker rats by decreasing fatty acid availability and reducing oxidative stress. *Br J Nutr*. 2012; 107(2): 202-210.

Grove KL, Smith MS. Ontogeny of the hypothalamic neuropeptide Y system. *Physiol Behav*. 2003; 79: 47–63.

Gu X, Creasy L, Kester A, Zeece M. Capillary electrophoretic determination of resveratrol in wines. *J Agric Food Chem*. 1999; 47(8): 3223-3227.

Gupta N, Goel K, Shah P, Misra A. Childhood Obesity in Developing Countries: Epidemiology, Determinants, and Prevention. *Endocr Rev.* 2012.

Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia*. 1992; 35(7): 595-601.

Halliwell B. Free radicals and antioxidants: a personal view. *Nutr Rev*. 1994, 52: 253-65.

Halliwell, B. The antioxidant paradox. *The Lancet*. 2000; 355: 1179-1180.

Harder T, Bergmann R, Kallischnigg G, Plagemann A. Duration of breastfeeding and risk of overweight: a meta-analysis. *Am J Epidemiol*. 2005; 162: 397-403.

Hattori Y, Akimoto K, Gross SS, Hattori S, and Kasai K. Angiotensin-II-induced oxidative stress elicits hypoadiponectinaemia in rats. *Diabetologia*. 2005; 48: 1066-1074.

Hileman SM, Pierroz DD, Masuzaki H, Bjorbaek C, El-Haschimi K, Banks WA, Flier JS. Characterizaton of short isoforms of the leptin receptor in rat cerebral microvessels and of brain uptake of leptin in mouse models of obesity. *Endocrinology*. 2002; 143: 775-783.

Hirosumi J, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. *Nature*. 2002; 420: 333-336.

Hoffman DJ, Sawaya AL, Verreschi I, Tucker KL, Roberts SB. Why are nutritionally stunted children at increased risk of obesity? Studies of metabolic rate and fat oxidation in shantytown children from São Paulo, Brazil. *Am J Clin Nutr*. 2000a; 72: 702-707.

Hoffman DJ, Roberts SB, Verreschi I, Martins PA, de Nascimento C, Tucker KL, Sawaya AL. Regulation of energy intake may be impaired in nutritionally stunted children from the shantytowns of São Paulo, Brazil. *J Nutr*. 2000b; 130: 2265-2270.

Houpt KA, Epstein AN. Ontogeny of controls of food intake in the rat: GI fill and glucoprivation. *Am J Physiol*. 1973; 225: 58-66.

Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang LL, Scherer B, Sinclair DA. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. *Nature.* 2003; 425: 191–196.

Hukshorn CJ, Lindeman JH, Toet KH, Saris WH, Eilers PH, Westerterp-Plantenga MS, Kooistra T. Leptin and the proinflammatory state associated with human obesity. *J. Clin. Endocrinol. Metab.* 2004; 89: 1773-1778.

Instituto Brasileiro de Geografia e Estatística (IBGE). POF 2008 2009 - Antropometria e estado nutricional de crianças, adolescentes e adultos no Brasil, 2010. Disponível em: http://www.ibge.gov.br/home/estatistica/populacao/condicaodevida/pof/2008_2009_encaa/pof_20082009_encaa.pdf Acesso em 11.01.2012.

Kastin AJ, Pan W, Maness LM, Koletsky RJ, Ernsberger P. Decreased transport of leptin across the blood-brain barrier in rats lacking the short form of the leptin receptor. *Peptides* 1999; 20: 1449-1453.

Kathirvel E, Morgan K, French SW, Morgan TR. Overexpression of liver-specific cytochrome P4502E1 impairs hepatic insulin signaling in a transgenic mouse model of nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol. 2009; 21: 973-983.

Kikusui T, Mori Y. Behavioural and neurochemical consequences of early weaning in rodents. *J Neuroendocrinol.* 2009; 21(4): 427-431.

Kikusui T, Nakamura K, Kakuma Y, Yuji M. Early weaning augments neuroendocrine stress responses in mice. *Behav Brain Res.* 2006; 175: 96-103.

Kim SJ, Lee YH, Han MD, Mar W, Kim WK, Nam KW. Resveratrol, purified from the stem of Vitis coignetiae Pulliat, inhibits food intake in C57BL/6J Mice. *Arch Pharm Res.* 2010; 33: 775-780.

Koubova J, Guarente L. How does calorie restriction work? *Genes Dev*. 2003; 17: 313-321.

Kramer MS, Guo T, Platt RW, Sevkovskaya Z, Dzikovich I, Collet JP, Shapiro S, Chalmers B, Hodnett E, Vanilovich I, Mezen I, Ducruet T, Shishko G, Bogdanovich N. Infant growth and health outcomes associated with 3 compared with 6 mo of exclusive breastfeeding. *Am J Clin Nutr.* 2003; 78: 291-295.

Kramer MS, Matush L, Vanilovich I, Platt RW, Bogdanovich N, Sevkovskaya Z, Dzikovich I, Shishko G, Collet JP, Martin RM, Smith GD, Gillman MW, Chalmers B, Hodnett E, Shapiro S. Effects of prolonged and exclusive breastfeeding on child height, weight, adiposity, and blood pressure at age 6.5 y: evidence from a large randomized trial. *Am J Clin Nutr*. 2007; 86: 1717-1721.

Kramer MS, Matush L, Vanilovich I, Platt RW, Bogdanovich N, Sevkovskaya Z, Dzikovich I, Shishko G, Collet JP, Martin RM, Smith GD, Gillman MW, Chalmers B, Hodnett E, Shapiro S. A randomized breast-feeding promotion intervention did not reduce child obesity in Belarus. *J Nutr.* 2009; 139(2): 417-421.

Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* 2006; 127: 1109–1122.

Lappas M, Yee K, Permezel M, Rice GE. Release and regulation of leptin, resistin and adiponectin from human placenta, fetal membranes, and maternal adipose tissue and skeletal muscle from normal and gestational diabetes mellitus-complicated pregnancies. *J Endocrinol*. 2005; 186: 457-465.

Lei número 11770 de 8 de setembro de 2008. Disponível em: http://www.planalto.gov.br/ccivil_03/_Ato2007-2010/2008/Lei/L11770.htm

Lima Nda S, de Moura EG, Passos MC, Nogueira Neto FJ, Reis AM, de Oliveira E, Lisboa PC. Early weaning causes undernutrition for a short period and programmes some metabolic syndrome components and leptin resistance in adult rat offspring. *Br J Nutr.* 2011; 105(9): 1405-1413.

Lins MC, de Moura EG, Lisboa PC, Bonomo IT, Passos MC. Effects of maternal leptin treatment during lactation on the body weight and leptin resistance of adult offspring. *Regul Pept*. 2005; 127(1-3): 197-202.

Lisboa PC, Passos MC, Dutra SC, Bonomo IT, Denolato AT, Reis AM, Moura EG. Leptin and prolactin, but not corticosterone, modulate body weight and thyroid function in protein-malnourished lactating rats. *Horm Metab Res.* 2006; 38(5): 295-299.

Lisboa PC, Pires L, de Oliveira E, Lima NS, Bonomo IT, Reis AM, Passos MC, Moura EG. Prolactin inhibition at mid-lactation influences adiposity and thyroid function in adult rats. *Horm Metab Res.* 2010; 42(8): 562-569.

Liu D, Caldji C, Sharma S, Plotsky PM, Meaney MJ. Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinepherine release in the hypothalamic paraventricular nucleus. *J Neuroendocrinol*. 2000; 12: 5-12.

Lopes Nobre J, Lisboa PC, da Silva Lima N, Franco JG, Firmino Nogueira Neto J, de Moura EG, de Oliveira E. Calcium supplementation prevents obesity, hyperleptinaemia and hyperglycaemia in adult rats programmed by early weaning. *Br J Nutr.* 2011.

Lopez M, Tovar S, Vazquez MJ, Nogueiras R, Seoane LM, García M, Señarís RM, Diéguez C. Perinatal overfeeding in rats results in increased levels of plasma leptin but unchanged cerebrospinal leptin in adulthood. *Int J Obes*. 2007; 31: 371-377.

Louis XL, Thandapilly SJ, Mohankumar SK, Yu L, Taylor CG, Zahradka P, Netticadan T. Treatment with low-dose resveratrol reverses cardiac impairment in obese prone but not in obese resistant rats. J Nutr Biochem. 2011.

Lustig RH. The neuroendocrinology of obesity. *Endocrinol Metab Clin North Am.* 2001; *3*0: 765-785.

Markus MA, Morris BJ. Resveratrol in prevention and treatment of common clinical conditions of aging. *Clin Interv Aging*. 2008; 3: 331-339.

Martins MR, Vieira AKG, de Souza EPG, Moura AS. Early overnutrition impaired insulin signaling in the heart of adult Swiss mice. *J Endocrinol*. 2008; 198: 591-598.

Mascarenhas ML, Albernaz EP, Silva MB, Silveira RB. Prevalence of exclusive and its determiners in the first 3 months of life in the South of Brazil. *Pediatr (Rio J).* 2006; 82(4): 289-294.

Mates MM, Perez-Gomez C, Nunez de Castro I. Antioxidant enzymes and human diseases. *Clinical Biochemistry*. 1999, 8: 595–603.

Maury E, Brichard SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Mol Cell Endocrinol*. 2010; 314: 1–16.

McMillen IC, Adam CL, Mühlhäusler BS. Early origins of obesity: programming the appetite regulatory system. *J Physiol*. 2005; 565(1): 9-17.

Melo MCN, Taddei JAAC, Diniz-Santos DR, Camilo Vieira C, Carneiro NB, Melo, RF, Silva LR. Incidence of Diarrhea in Children Living in Urban Slums in Salvador, Brazil. *The Brazilian Journal of Infectious Diseases*. 2008; 12(1): 89-93.

Michels KB, Willett WC, Graubard BI, Vaidya RL, Cantwell MM, Sansbury LB, Forman MR. A longitudinal study of infant feeding and obesity throughout life course. *Int J Obes*. 2007; 31: 1078-1085.

Minakawa M, Kawano A, Miura Y, Yagasaki K. Hypoglycemic effect of resveratrol in type 2 diabetic model db/db mice and its actions in cultured L6 myotubes and RIN-5F pancreatic β-cells. *J Clin Biochem Nutr*. 2011; 48(3): 237-244.

Miura D, Miura Y, Yagasaki K. Hypolipidemic action of dietary resveratrol, a phytoalexin in grapes and red wine, in hepatomabearing rats. *Life Sci*. 2003; 73:1393-1400.

Mooney RA, Senn J, Cameron S, Inamdar N, Boivin LM, Shang Y, Furlanetto RW. Suppressors of cytokine signalling-1 and 6 associate with and inhibit the insulin receptor. A potential mechanism for cytokine-mediated insulin resistance. *J Biol Chem*. 2001; 276: 25889-25893.

Morton GJ, Schwartz MW. Leptin and the central nervous system control of glucose metabolism. *Physiol Rev*. 2011; 91(2): 389-411.

Moura EG, Lisboa PC, Custódio CM, Nunes MT, Souza KP, Passos MCF. Malnutrition during lactation changes growth hormone mRNA expression in offspring at weaning and adulthood. *J Nutr Biochem*. 2007; 18(2): 134-139.

Must A. Does overweight in childhood have an impact on adult health? *Nutr Rev*. 2003; 61:139–142.

National Center for Health Statistics. Health, United States, 2010: With Special Features on Death and Dying. Hyattsville, MD, U.S. Department of Health and Human Services, 2011.

Nishida M, Funahashi T, Shimomura I. Pathophysiological significance of adiponectin. *Med Mol Morphol*. 2007; 40(2): 55-67.

Niswender KD, Baskin DG , Schwartz MW. Insulin and its evolving partnership with leptin in the hypothalamic control of energy homeostasis. *Trends Endocrinol Metab*. 2004; 15: 362-369.

Nordberg J, Arnér E. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic Biol Med*. 2001; 31(11): 1287-1312.

Ogden CL, Carroll MD, Curtin LR, Lamb MM, Flegal KM. Prevalence of high body mass index in US children and adolescents, 2007–2008. *JAMA.* 2010; 303(3): 242-249.

Ono M, Kikusui T, Sasaki N, Ichikawa M, Mori Y, Murakami-Murofushi K. Early weaning induces anxiety and precocious myelination in the anterior part of the basolateral amygdala of male Balb/c mice. *Neuroscience.* 2008; 156(4): 1103-1110.

Orallo F, Alvarez E, Camiña M, Leiro JM, Gómez E, Fernández P. The possible implication of trans-Resveratrol in the cardioprotective effects of long-term moderate wine consumption. *Mol Pharmacol.* 2002; 61(2): 294-302.

Osaki LH, Figueiredo PM, Alvares EP, Gama P. EGFR is involved in control of gastric cell proliferation through activation of MAPK and Src signalling pathways in early-weaned rats. *Cell Prolif.* 2011; 44(2): 174-182.

Otani H. Oxidative stress as pathogenesis of cardiovascular risk associated with metabolic syndrome. *Antioxid Redox Signal.* 2011; 15(7): 1911-1926.

Owen CG, Whincup PH, Orfei L, Chou QA, Rudnicka AR, Wathern AK, Kaye SJ, Eriksson JG, Osmond C, Cook DG. Is body mass index before middle age related to coronary heart disease risk in later life? Evidence from observational studies. *Int J Obes(Lond).* 2009; 33(8): 866-877.

Palou M, Sánchez J, Rodríguez AM, Priego T, Picó C, Palou A. Induction of NPY/AgRP orexigenic peptide expression in rat hypothalamus is an early event in fasting: relationship with circulating leptin, insulin and glucose. *Cell Physiol Biochem.* 2009; 23(1-3): 115-24.

Passos MA, Passos MC, Oliveira E, Trotta PA, Nogueira-Neto JF, Bonomo IT, Lisboa PC, de Moura EG. Maternal prolactin inhibition during lactation is associated to renal dysfunction in their adult rat offspring. *Horm Metab Res.* 2011; 43(9): 636-664.

Passos MC, Toste FP, Dutra SC, Trotta PA, Toste FP, Lisboa PC, de Moura EG. Role of neonatal hyperleptinaemia on serum adiponectin and suppressor of cytokine signalling-3 expression in young rats. *Br J Nutr.* 2009; 101(2): 250-256.

Passos MCF, Ramos CF, Moura EG. Short and long term effects of malnutrition in rats during lactation on the body weight of offspring. *Nutrition Res.* 2000; 20: 1603-1612.

Passos MCF, Vicente LL, Lisboa PC, Moura EG. Absence of anorectic effect to acute peripheral leptin treatment in adult animals whose mothers were malnourished during lactation. *Horm Metab Res*. 2004; 36: 625-629.

Paz-Filho GJ , Volaco A , Suplicy HL , Radominski RB , Boguszewski CL. Decrease in leptin production by the adipose tissue in obesity associated with severe metabolic syndrome. *Arq Bras Endocrinol Metabol.* 2009; 53: 1088 -1095.

Pereira-Toste F, Toste FP, Oliveira E, Trotta PA, Lisboa PC, de Moura EG, Passos MC. Early maternal hyperleptinemia programs adipogenic phenotype in rats. *Horm Metab Res*. 2009; 41(12): 874-879.

Pervaiz S, Holme AL. Resveratrol: its biologic targets and functional activity. *Antioxid Redox Signal*. 2009; 11: 2851-2897.

Pervaiz S. Chemotherapeutic potential of the chemopreventive phytoalexin resveratrol. *Drug Resist Updat*. 2004; 7: 333-344.

Pinney SE, Simmons RA. Metabolic programming, epigenetics, and gestational diabetes mellitus. *Curr Diab Rep.* 2012; 12(1): 67-74.

Plagemann A, Harder T, Brunn M, Harder A, Roepke K, Wittrock-Staar M, Ziska T, Schellong K, Rodekamp E, Melchior K, Dudenhausen JW. Hypothalamic proopiomelanocortin promoter methylation becomes altered by early overfeeding: an epigenetic model of obesity and the metabolic syndrome. *J Physiol.* 2009; 587(20): 4963-4976.

Plagemann A, Heidrich I, Gotz F, Rohde W, Dorner G. Obesity and enhanced diabetes and cardiovascular risk in adult rats due to early postnatal overfeeding. *Exp Clin Endocrinol.* 1992; 99: 154-158.

Plagemann A, Roepke K, Harder T, Brunn M, Harder A, Wittrock-Staar M, Ziska T, Schellong K, Rodekamp E, Melchior K, Dudenhausen JW. Epigenetic malprogramming of the insulin receptor promoter due to developmental overfeeding. *J Perinat Med*. 2010; 38(4): 393-400.

Plaut SM, Davis JM. Effects of mother-litter separation on survival, growth, and brain amino acid levels. *Physiol Behav.* 1972; 8(1): 43-51.

Popa C, Riel PLCM, Meer WM, Stalenhoef AFH. The role of TNF-α in chronic inflammatory conditions, intermediary metabolism, and cardiovascular risk. *J Lipid Res*. 2007; 48: 751-762.

Pozzo Miller LD, Aoki A. Postnatal development of the hypothalamic ventromedial nucleus: neurons and synapses. *Cell Mol Neurobiol*. 1992; 12: 121-129.

Rahmouni, K. Leptin-Induced Sympathetic Nerve Activation: Signaling Mechanisms and Cardiovascular Consequences in Obesity. *Curr Hypertens Rev*. 2010; 6(2): 104–209.

Ravelli GP, Stein ZA & Susser MW. Obesity in young men after famine exposure in utero and early infancy. *N Engl J Med*. 1976; 295: 349-353.

Reilly JJ, Methven E, McDowell ZC, Hacking B, Alexander D, Stewart L, Kelnar CJ. Health consequences of obesity. *Arch Dis Child.* 2003; 88: 748–752.

Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*. 1992; 339(8808):1523-1526.

Rivera L, Morón R, Zarzuelo A, Galisteo M. Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats. *Biochem Pharmacol*. 2009; 77(6): 1053-1063.

Robb EL, Winkelmolen L, Visanji N, Brotchie J, Stuart JA. Dietary resveratrol administration increases MnSOD expression and activity in mouse brain. *Biochem Biophys Res Commun*. 2008; 372(1): 254-259.

Roberts CK, Sindhu KK. Oxidative stress and metabolic syndrome. *Life Sci.* 2009; 84(21-22): 705-712.

Robertson, K.D. DNA methylation, methyltransferases, and cancer. *Oncogene.* 20:3139-3155, 2001.

Rodrigues AL, de Moura EG, Passos MC, Dutra SC, Lisboa PC. Postnatal early overnutrition changes the leptin signalling pathway in the hypothalamic-pituitary-thyroid axis of young and adult rats. *J Physiol.* 2009; 587(11): 2647-2661.

Rodrigues AL, de Moura EG, Passos MC, Trevenzoli IH, da Conceição EP, Bonono IT, Neto JF, Lisboa PC. Postnatal early overfeeding induces hypothalamic higher SOCS3 expression and lower STAT3 activity in adult rats. *J Nutr Biochem*. 2011; 22(2): 109-117.

Rodrigues AL, De Souza EP, Da Silva SV, Rodrigues DS, Nascimento AB, Barja-Fidalgo C, De Freitas MS. Low expression of insulin signaling molecules impairs glucose uptake in adipocytes after early overnutrition. *J Endocrinol*. 2007; 195: 485-494.

Rolo AP, Teodoro JS, Palmeira CM. Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. *Free Radic Biol Med*. 2012; 52(1):59-69.

Rudich A, Kanety H, Bashan N. Adipose stress-sensing kinases: linking obesity to malfunction. *Trends Endocrinol Metab.* 2007; 18: 291-299.

Ryan AS. Breastfeeding and the risk of childhood obesity. *Coll Antropol*. 2007; 31(1): 19-28.

Sahu A. Leptin signaling in the hypothalamus: emphasis on energy homeostasis and leptin resistance. *Front Neuroendocrinol*. 2003; 24: 225-253.

Saito T, Misawa K, Kawata S. *Fatty liver and non-alcoholic steatohepatitis. Intern Med*. 2007; 46(2): 101-103.

Sakurai T, Izawa T, Kizaki T, Ogasawara JE, Shirato K, Imaizumi K, Takahashi K, Ishida H, and Ohno H. Exercise training decreases expression of inflammation-related adipokines through reduction of oxidative stress in rat white adipose tissue. *Biochem Biophys Res Commun.* 2009; 379: 605-609.

Savoia C & Schiffrin EL. Significance of recently identified peptides in hypertension: endothelin, natriuretic peptides, adrenomedullin, leptin. *Med Clin North Am*. 2004; 88: 39-62.

Sawaya AL, Martins P, Hoffman D, Roberts SB. The link between childhood undernutrition and risk of chronic diseases in adulthood: a case study of Brazil. 2003; *Nutr. Rev.* 61: 168-175.

Scanferla de Siqueira R, Monteiro CA. Breastfeeding and obesity in school-age children from families of high socioeconomic status. *Rev Saude Publica*. 2007; 41(1): 5-12.

Schmatz R, Perreira LB, Stefanello N, Mazzanti C, Spanevello R, Gutierres J, Bagatini M, Martins CC, Abdalla FH, Daci da Silva Serres J, Zanini D, Vieira JM, Cardoso AM, Schetinger MR, Morsch VM. Effects of resveratrol on biomarkers of oxidative stress and on the activity of delta aminolevulinic acid dehydratase in liver and kidney of streptozotocin-induced diabetic rats. *Biochimie*. 2012; 94(2): 374-383.

Shang J, Chen LL, Xiao FX, Sun H, Ding HC, Xiao H. Resveratrol improves non-alcoholic fatty liver disease by activating AMP-activated protein kinase. *Acta Pharmacol Sin*. 2008; 29(6): 698-706.

Sichieri R, Siqueira KS, Moura AS. Obesity and abdominal fatness associated with undernutrition early in life in a survey in Rio de Janeiro. *Int. J. Obes. Relat. Metab. Disord*. 2000a; 24: 614-618.

Sichieri R, Siqueira KS, Pereira RA, Ascherio A. Short stature and hypertension in the city of Rio de Janeiro, Brazil. *Public. Health. Nutr*. 2000b; 3: 77-82.

Sichieri R, Souza RA. Strategies for obesity prevention in children and adolescents. *Cad Saude Publica*. 2008; 24(2): 209-223.

Sies H. Strategies of antioxidant defense. *Eur J Biochem.* 1993; 215: 213-219.

Simmons R. Developmental origins of adult metabolic disease: concepts and controversies. *Trends Endocrinol Metab.* 2005; 16: 390-394.

Soares AF, Guichardant M, Cozzone D, Bernoud-Hubac N, Bouzaidi-Tiali N, Lagarde M, and Geloen A. Effects of oxidative stress on adiponectin secretion and lactate production in 3T3-L1 adipocytes. *Free Radic Biol Med*. 38: 882-889, 2005.
Soylemez S, Gurdal H, Sepici A, Akar F. The effect of long-term resveratrol treatment on relaxation to estrogen in aortae from male and female rats: role of nitric oxide and superoxide. *Vascul Pharmacol.* 2008; 49(2-3): 97-105.

Soylemez S, Sepici A, Akar F.Resveratrol supplementation gender independently improves endothelial reactivity and suppresses superoxide production in healthy rats. *Cardiovasc Drugs Ther*. 2009; 23(6): 449-458.

Spanier G, Xu H, Xia N, Tobias S, Deng S, Wojnowski L, Forstermann U, Li H. Resveratrol reduces endothelial oxidative stress by modulating the gene expression of superoxide dismutase 1 (SOD1), glutathione peroxidase 1 (GPx1) and NADPH oxidase subunit (Nox4). *J Physiol Pharmacol*. 2009; 60(4):111-116.

Steffes M, Gross M, Lee D, Schreiner P, Jacobs D. Adiponectin, visceral fat, oxidative stress and early macrovascular disease: The coronary artery risk development in young adults study. *Obesity*. 2006; 14: 319-326.

Strakovsky RS, Pan YX. In Utero Oxidative Stress Epigenetically Programs Antioxidant Defense Capacity and Adulthood Diseases. *Antioxid Redox Signal.* 2012.

Su HC, Hung LM, Chen JK. Resveratrol, a red wine antioxidant, possesses an insulin-like effect in streptozotocin-induced diabetic rats. *Am J Physiol Endocrinol Metab.* 2006; 290: 1339–1346.

Szkudelska K, Nogowski L, Szkudelski T. The inhibitory effect of resveratrol on leptin secretion from rat adipocytes. *Eur J Clin Invest*. 2009; 39: 899-905.

Szkudelski T. Resveratrol inhibits insulin secretion from rat pancreatic islets. *Eur J Pharmacol.* 2006; 55: 176–181.

Tate, P.H., Bird, A.P. Effects of DNA methylation on DNA-binding proteins and gene expression. *Curr. Opin. Genet. Dev*. 1993; 3: 226-231.

Tatlidede E, Sehirli O, Velioğlu-Oğünc A, Cetinel S, Yeğen BC, Yarat A, Süleymanoğlu S, Sener G. Resveratrol treatment protects against doxorubicin-induced cardiotoxicity by alleviating oxidative damage. *Free Radic Res*. 2009; 43(3): 195-205.

Tauriainen E, Luostarinen M, Martonen E, Finckenberg P, Kovalainen M, Huotari A, Herzig KH, Lecklin A, Mervaala E. Distinct effects of calorie restriction and resveratrol on diet-induced obesity and Fatty liver formation. J Nutr *Metab*. 2011; 2011:525094.

Teixeira C, Passos M, Ramos C, Dutra S, Moura E. Leptin serum concentration, food intake and body weight in rats whose mothers were exposed to malnutrition during lactation. *J Nutr Biochem*. 2002; 13(8): 493

Theys N, Clippe A, Bouckenooghe T, Reusens B, Remacle C. Early low protein diet aggravates unbalance between antioxidant enzymes leading to islet dysfunction. *PLoS One*. 4(7): e6110, 2009.

Toste FP, de Moura EG, Lisboa PC, Fagundes AT, de Oliveira E, Passos MC. Neonatal leptin treatment programmes leptin hypothalamic resistance and intermediary metabolic parameters in adult rats. *Br J Nutr*. 2006; 95(4): 830-837.

Trevenzoli IH, Rodrigues AL, Oliveira E, Thole AA, Carvalho L, Figueiredo MS, Toste FP, Neto JF, Passos MC, Lisboa PC, Moura EG. Leptin treatment during lactation programs leptin synthesis, intermediate metabolism, and liver microsteatosis in adult rats. *Horm Metab Res.* 2010; 42(7): 483-490.

Trevenzoli IH, Valle MM, Machado FB, Garcia RM, Passos MC, Lisboa PC, Moura EG. Neonatal hyperleptinaemia programmes adrenal medullary function in adult rats: effects on cardiovascular parameters. *J Physiol*. 2007; 580: 629-637.

Ungvari Z, Orosz Z, Rivera A, Labinskyy N, Xiangmin Z, Olson S, Podlutsky A, Csiszar A. Resveratrol increases vascular oxidative stress resistance. *Am J Physiol Heart Circ Physiol*. 2007; 292: 2417-2424.

Uriarte N, Breigeiron MK, Benetti F, Rosa XF, Lucion AB. Effects of maternal care on the development, emotionality, and reproductive functions in male and female rats. *Dev Psychobiol.* 2007; 49(5): 451-462.

Valassi E, Scacchi, M, Cavagnini, F. Neuroendocrine control of food intake. *Nutr Metab Cardiovasc Dis.* 2008; 18(2): 158-168.

Vicente LL, Moura EG, Lisboa PC, Costa AMA, Amadeu T, Mandarim-de-Lacerda CA, Passos MCF. Malnutrition during lactation is associated with higher expression of leptin receptor in pituitary of the adult offspring. *Nutrition.* 2004; 20: 924-928.

Vitrac X, Bornet A, Vanderlinde R, Valls J, Richard T, Delaunay JC, Mérillon JM, Teissédre PL. Determination of stilbenes (delta-viniferin, trans-astringin, trans-piceid, cis- and trans-resveratrol, epsilon-viniferin) in Brazilian wines. *J Agric Food Chem.* 2005; 53: 5664-5669.

Volpini, CCA, Moura, EC. Determinantes do desmame precoce no distrito noroeste de Campinas. Rev. Nutr. Campinas, v. 18, n. 3, 2005. Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1415-52732005000300003&lng=en&nrm=iso. Acesso em: 10.01.2012.

Weinberg F, Chandel NS. Reactive oxygen species-dependent signaling regulates cancer. *Cell Mol Life Sci*. 2009; 66(23): 3663-3673.

World Health Organization (WHO). Collaborative Study Team on the Role of Breastfeeding on the Prevention of Infant Mortality. Effect of breastfeeding on infant and child mortality due to infectious diseases in less developed countries: a pooled analysis. *Lance.* 2000; 355(9202): 451-455.

World Health Organization (WHO). Breastfeeding: a vital emergency response. Are you ready? 2010. Disponível em: http://www.who.int/mediacentre/news/statements/2009/world_breastfeeding_week_20090731/en/index.html Acesso em: 10.01.2012.

World Health Organization (WHO). Obesity and Overweight. Fact sheet nº 311. 2011. Disponível em: http://www.who.int/mediacentre/factsheets/fs311/en/index.html Acesso em: 09.01.12.

Yamamoto, H. K. Schoonjans & J. Auwerx. Sirtuin functions in health and disease. *Mol. Endocrinol*. 2007; 21: 1745–1755.

Yan LJ, Forster MJ. Chemical probes for analysis of carbonylated proteins: a review. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2010; 879(17-18):1308-1315.

Yar AS, Menevse S, Alp E. The effects of resveratrol on cyclooxygenase-1 and -2, nuclear factor kappa beta, matrix metalloproteinase-9, and sirtuin 1 mRNA expression in hearts of streptozotocin-induced diabetic rats. *Genet Mol Res*. 2011; 10(4): 2962-2975.

You S, Gotz F, Rohde W, Dorner G. Early postnatal overfeeding and diabetes susceptibility. *Exp Clin Endocrinol*. 1990; 96: 301-306.

Younes-Rapozo V, Moura EG, Lima NS, Barradas PC, Manhães AC, Oliveira E, Lisboa PC. Early weaning is associated with higher neuropeptide Y (NPY) and lower cocaine and amphetamine-regulated transcript (CART) expressions in paraventricular nucleus (PVN) at adulthood. *Br J Nutr.* 2012.

Yu BP. Cellular defenses against damage from reactive oxygen species. *Physiol. Rev.* 1994; 74: 139-162.

Yu J, Auwerx J. Protein deacetylation by SIRT1: An emerging key post-translational modification in metabolic regulation. *Pharmacol Res*. 2009; 62(1): 35-41.

Zabolotny JM, Bence-Hanulec KK, Stricker-Krongrad A, Haj F, Wang Y, Minokoshi Y, Kim YB, Elmquist JK, Tartaglia LA, Kahn BB, Neel BG. PTP1B regulates leptin signal transduction in vivo. *Dev Cell.* 2002; 2: 489-495.

Zhang X, Strakovsky R, Zhou D, Zhang Y, Pan YX. A maternal high-fat diet represses the expression of antioxidant defense genes and induces the cellular senescence pathway in the liver of male offspring rats. *J Nutr.* 2011; 141(7): 1254-1259.

Zheng J, Chen LL, Zhang HH, Hu X, Kong W, Hu D. Resveratrol improves insulin resistance of catch-up growth by increasing mitochondrial complexes and antioxidant function in skeletal muscle. *Metabolism*. 2011.