Universidade Federal de Pernambuco – UFPE
Centro Acadêmico de Vitória – CAV
Programa de Pós-Graduação em Nutrição, Atividade Física e Plasticidade Fenotípica – PPGNAFPF

Aiany Cibelle Simões Alves

TRATAMENTO COM FLUOXETINA EM RATOS NEONATOS: EFEITOS NA BIOENERGETICA MITOCONDRIAL E ESTRESSE OXIDATIVO NO FíGADO DE RATOS ADULTOS

Vitória de Santo Antão

2017

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Orientadora: Mariana Pinheiro Fernandes

Dissertação apresentada à Universidade Federal de Pernambuco, como parte das exigências do Programa de Pós-Graduação em Nutrição, Atividade Física e Plasticidade Fenotípica, área de concentração em Bases Experimentais da Plasticidade Fenotípica, para a obtenção do título de Mestre.

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AIANY CIBELLE SIMÕES ALVES

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Dissertação apresentada ao Programa de Pós-Graduação em Nutrição, Atividade Física e Plasticidade Fenotípica da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de Mestre.

Área de concentração: Bases Experimentais e Clínicas da Plasticidade Fenotípica.

Aprovada em: 23/02/2017.

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Dedico este trabalho...

Aos curiosos...

Aos observadores...

Aos investigadores...

Aos amantes da Ciência!

Afinal, observar é fundamental para se fazer ciência.

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RESUMO

Inibidores seletivos de recaptação de serotonina (ISRS) são uma classe de antidepressivos como a fluoxetina, citalopram, sertralina entre outros, que são prescritos para mulheres grávidas e lactantes para o tratamento de depressão, expondo fetos e crianças a droga durante períodos críticos de desenvolvimento. Estudos recentes demonstraram que a exposição ao tratamento com fluoxetina induz a produção excessiva de espécies reativas de oxigênio (EROs) e altera as defesas antioxidantes em vários tecidos, principalmente no fígado. Quando a fluoxetina é administrada por via intraperitoneal, o fármaco atinge rapidamente concentrações elevadas no fígado, que pode ter múltiplos efeitos potencialmente tóxicos no metabolismo energético mitocondrial hepático. O objetivo deste estudo foi avaliar os efeitos do tratamento farmacológico com fluoxetina durante a lactação na bioenergética mitocondrial e estresse oxidativo no fígado de ratos adultos. Para realizar este estudo, filhotes de ratos do 1º ao 21º dia pós-natal foram tratados com fluoxetina (grupo Fx) ou veículo (grupo controle, Ct). Foi avaliado o consumo de oxigênio mitocondrial, o controle respiratório, a produção mitocondrial de espécies reativas, integridade de membrana mitocondrial, biomarcadores de estresse oxidativo (malondealdeido-MDA, carbonilas e níveis de grupamentos sulfidrilas-SH), atividade de enzimas antioxidantes (atividade da superóxido dismutase-SOD, catalase-CAT e glutationa S-transferase-GST) e níveis de glutationa reduzida (GSH) no fígado de ratos machos aos 60 dias de idade. Nossos resultados mostraram, que o tratamento com Fx durante o período crítico do desenvolvimento resultou em peso corporal reduzido, melhora da capacidade respiratória mitocondrial, menor inchamento mitocondrial, diminuição de biomarcadores de estresse oxidativo (305% MDA, *p<0,05), aumento nos níveis de grupamentos sulfidrilas-SH (14% nos níveis de SH, *p<0,05) e aumento de defesas antioxidantes enzimáticas (115% SOD, *p<0,05; 94% CAT, **p<0,01 e 13% GST, *p<0,05), no fígado de ratos adultos. Nossos resultados sugerem que o tratamento farmacológico com fluoxetina durante um período crítico do desenvolvimento pode melhorar a capacidade respiratória mitocondrial e o metabolismo oxidativo do fígado de ratos na vida adulta.

Palavras-chaves: Fluoxetina. Serotonina. Mitocôndria. Estresse oxidativo. Fígado. Período crítico do desenvolvimento.

ABSTRACT

Selective serotonin reuptake inhibitors (SSRIs) are a class of antidepressants such as fluoxetine, citalopram, sertraline among others, which are often prescribed for treated and lactating women for the treatment of depression. Recent studies have shown that treatment with fluoxetine induces excessive production of reactive oxygen species (ROS) and changes as antioxidant defenses in various tissues, especially in the liver. When a fluoxetine is administered intraperitoneally, the drug rapidly elevates concentrations in the liver, which may have multiple potentially toxic effects not hepatic mitochondrial energy metabolism. The aim this study was to evaluate the effects of the pharmacological treatment with fluoxetine during lactation on the mitochondrial bioenergetics and oxidative stress in liver of adult rats. To perform this study, pups of rats from the 1st day to the 21st postnatal day treated with fluoxetine (Fx group) or vehicle (control group, Ct). We evaluated the mitochondrial oxygen consumption, a respiratory control ratio, a mitochondrial production of reactive species, mitochondrial membrane integrity, oxidative stress biomarkers (Malondialdehyde-MDA, carbonyls and SH-sulfhydryl group levels) and activity of antioxidant enzymes (superoxide dismutase-SOD, catalase-CAT and glutathione S-transferase-GST activity) and reduced glutathione levels (GSH) no liver of rats at 60 days of age. Our results showed that Fx treatment during the critical period of development resulted in reduced body weight, improved mitochondrial respiratory capacity, integrated and resistant mitochondrial membrane, decreased biomarkers of oxidative stress (305% MDA, *p<0,05), increased levels of sulfhydryl-SH groups (14% in SH levels, *p<0,05) and increased enzymatic antioxidant (115% SOD, *p<0,05; 94% CAT, **p<0,01 e 13% GST, ;*p<0,05) in the liver of adult rats. Our results suggest that pharmacological treatment with fluoxetine during critical periods of development may improve mitochondrial respiratory capacity and result in persistent changes in liver energy metabolism during later life.

Keywords: Fluoxetine. Serotonin. Mitochondria. Oxidative stress. Liver. Critical period of development.

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APRESENTAÇÃO

RESULTADOS

LISTA DE ABREVIATURAS

5-HIAA – 5-Hidroxiindolacético

5-HT – Serotonina

AIF – Fator de indução de apoptose

ALT –Enzima Alanina Aminotransferase

ANT – Translocador de nucleotídeo de adenina

AST – Enzima aspartato aminotransferase

AT(D)P – Adenosina tri (di) – fosfato

BCL-2 – Proteína Anti-apoptótica

BMCP1 – Proteína Transportadora Mitocondrial Cerebral 1

BMP - Proteína morfogenética do osso

BSA – Albumina de Soro Bovino

Ca^{2+}– Cálcio

CAT – Catalase

CCAC –Conselho Canadense de Cuidados com Animais

CCCP – Carbonilcianeto m-clorofenil-hidrazona

CDNB – 1-cloro-2,4 dinitrobenzeno

CsA – Ciclosporina A

Ct – Controle

Cu/Zn-SOD – Superóxido dismutase dependente de cobre e zinco

CYP450 – CytocromoP450

CyP-D –Ciclofilina D

DCF – Diclorofluoresceína

DNAmt – DNA mitocondrial

DNPH – 2,4-dinitrofenil-hidrazina

DNP-SG –DinitroFenil S Glutationa

DTNB – 5,5'-Ditiobis(2-nitrobenzóico)

EDTA – Ácido Etilenodiamino Tetra-Acético

EGTA – Ácido Etileno Glicol Tetra Acético

EPM – Erro Padrão da Média

ERNs – Espécies reativas de nitrogênio

EROs – Espécies reativas de oxigênio

ETC – Cadeia transportadora de elétrons

ETF – Flavoproteínas de transporte de elétrons

FGF - Fator de crescimento de fibroblastos

Fx – Fluoxetina

GD(D)P – Guanosina tri (di) – fosfato

GPx – Glutationa peroxidase

GR – Glutationa redutase

GSH – Glutationa reduzida

GSSG – Glutationa oxidada

GST – Glutationa S Transferase

H_2DCFDA – 5-(-6)-clorometil-2',7'-diclorodiidrofluoresceina diacetato

H_2O – Água

H_2O_2 – Peróxido de hidrogênio

HEPES – (N-(2-hidroxietil) piperazina-N'-(2-ácido etanosulfônico)

IR – Isquemia/ Reperfusão

ISRS/ SSRI – Inibidores Seletivos de Recaptação de Serotonina

K^+ – Potássio

K_2HPO_4 – Fosfato Monopotássico

KCl – Cloreto de potássio

MAO –Monoamina Oxidase

MDA – Malondialdeído

mg – Miligrama

MgCl – Cloreto de Magnesio

MnSOD – Superóxido dismutase dependente de manganês

MR – Metabólitos Reativos

Na^+ – Sódio

NaCl – Cloreto de Sódio

NAD^+ – Nicotinamida adenina dinuclotídeo (estado oxidado)

NADH – Nicotinamida adenina dinuclotídeo (estado reduzido)

$NADP^+$ – Nicotinamida adenina dinuclotídeo fosfato (estado oxidado)

NADPH – Nicotinamida adenina dinuclotídeo fosfato (estado reduzido)

NO^- – Óxido nítrico

O_2^- – Ânion superóxido

O_2 – Oxigênio

OH^- – Radical hidroxil

$ONOO^-$ – Peroxinitrito

OPT – O-Ftaldialdeído

Pi – Fosfato inorgânico

PMSF –Fenilmetilsulfonilfluoride

POMC – Pró-ópio-melanocortina

PTPM/ MPTP – Poro de transição de permeabilidade mitocondrial

ROS – Espécies Reativas de Oxigênio

sc – Subcutânea

SelCys – Selenocisteínas

SERT – Proteína Transportadora de Serotonina

SH - Sulfidrilas

Smac – Segundo ativador mitocondrial de caspases

SNC – Sistema Nervoso Cental

TBA –Ácido Tiobarbitúrico

TCA –Triclocoacético

TPH –Triptofato Hidroxilase

TPM/ MPT – Transição de permeabilidade mitocondrial

UCPs - Proteína desacopladoraMitocodrial

UQ – Ubiquinona (coenzima Q oxidada)

$UQH^{\bullet}$ – Radical semiquinona

UQH_2 – Ubiquinona (coenzima Q reduzida)

VDAC – Canal de ânion voltagem dependente

VMAT2 – Transportador de Monoamina

ΔpH – Gradiente químico de prótons

ΔΨ – Potencial elétrico

$\Delta\Psi_m$– Potencial elétrico de membrana mitocondrial

SUMÁRIO

1 INTRODUÇÃO

Os inibidores seletivos de recaptação da serotonina (ISRS) são uma classe de antidepressivos como a fluoxetina, o citalopram, a sertralina entre outros, que são frequentemente prescritos a mulheres grávidas e lactantes diagnosticadas com depressão, expondo assim fetos e lactentes a fármacos durante períodos críticos de desenvolvimento (FLESCHLER; PESKIN, 2008). Alguns autores demonstraram que a fluoxetina (Fx) pode atravessar a placenta humana e sua presença no leite materno pode induzir efeitos nocivos sobre o desenvolvimento de fetos e recém-nascidos (DAVANZO et al., 2011; FRANCIS-OLIVEIRA et al., 2013). Apesar da baixa concentração produzida e atuação no cérebro, a serotonina está associada a uma variedade de funções no sistema nervoso central, uma vez que atua no controle da energia para a modulação de vários comportamentos (HALLIDAY; BAKER; HARPER, 1995).

O órgão responsável pela ativação da fluoxetina é o fígado, onde sofre uma extensa conversão metabólica, levando à formação do metabólito ativo norfluoxetina entre vários outros metabólitos pelo citocromo P450 (ALTAMURA; MORO; PERCUDANI, 1994; A et al., 2003). Devido à inibição do seu próprio metabolismo, a eliminação da fluoxetina e da norfluoxetina do corpo é extremamente lenta (CREWE et al., 1992). Quando a fluoxetina é administrada intraperitonealmente, o fármaco atinge rapidamente concentrações elevadas no fígado. Fluoxetina e norfluoxetina mostraram efeitos potencialmente tóxicos no metabolismo energético em mitocôndrias de fígado de rato (SOUZA et al., 1994). Isto parece ser uma consequência da solubilização do fármaco e / ou dos seus metabólitos na membrana mitocondrial interna. No entanto, a base molecular da hepatotoxicidade induzida pela fluoxetina (FRIEDENBERG; ROTHSTEIN, 1996; JOHNSTON; WHEELER, 1997; CAI et al., 1999) ainda não é bem compreendida.

A mitocôndria é a principal organela do metabolismo energético celular, responsável pela grande maioria da síntese de adenosina-5-trifosfato (ATP) por meio da fosforilação oxidativa. Nos últimos anos, além da sua função conhecida de gerar energia para a célula, as mitocôndrias emergiram como organelas equipadas

com sofisticada maquinaria para mediar o fluxo de cálcio através da membrana mitocondrial interna, além disso estão envolvidas nas vias de sinalização, lesão e morte celular por apoptose (FIGUEIRA et al., 2013; PERNAS; SCORRANO, 2016a). O metabolismo energético mitocondrial é reconhecido como a principal fonte de espécies reativas de oxigênio (EROS) celular, como o ânion superóxido (O_2-) (HALLIWELL; GUTTERIDGE, 1990). O ânion superóxido, dá origem a outras espécies reativas de oxigênio e nitrogênio, por diferentes reações. A dismutação de O_2- pela enzima antioxidante superoxido desmutase (Mn-SOD na matriz e Cu / Zn-SOD no espaço intermembranar), presente nas mitocôndrias e no citosol, produz o peróxido de hidrogênio (H2O2) (NOHL; GILLE; STANIEK, 2005). O H_2O_2 é permeável às membranas e pode ser convertido em oxigênio e água pelas enzimas antioxidantes, catalase (CAT) e glutationa peroxidase (HALLIWELL; GUTTERIDGE, 1990). Em condições fisiológicas, a produção de EROS desempenha uma função primordial de segundo mensageiro, regulando a expressão de genes sensíveis a sinais redox e alterações na homeostase celular através da síntese de moléculas fisiologicamente ativas. No entanto, em altas concentrações, EROs podem ser importantes mediadores de danos às estruturas celulares de ácidos nucleicos, lipídios e proteínas (CADENAS; DAVIES, 2000).

Os efeitos da exposição a fármacos antidepressivos no estado redox celular permanecem controversos. Numerosos estudos demonstraram que esses fármacos induzem estresse oxidativo em vários tecidos e tipos celulares (MORETTI et al., 2012; DE LONG et al., 2014; SONEI et al., 2016). O tratamento crônico com fluoxetina, sertralina ou tioacetamida, inibidores seletivos da recaptação da serotonina (ISRS), pode aumentar significativamente biomarcadores de estresse oxidativo no cérebro e no fígado (INKIELEWICZ-STEPNIAK, 2011; ABDEL SALAM et al., 2013; ZLATKOVIC et al., 2014). Sob estresse crônico, a fluoxetina altera o sistema antioxidante e promove a sinalização apoptótica em ratos *Wistar* (DJORDJEVIC et al., 2011). Em contraste, Aksu et al. (2014), estudando o papel potencial da fluoxetina como antioxidante no modelo de rim isquemia-reperfusão (IR), relatou que o pré-tratamento com fluoxetina restaurou significativamente o equilíbrio redox e diminuiu as medidas de inflamação no rim (AKSU et al., 2014); além disso, a administração crônica de fluoxetina a animais estressados por restrição durante 21 dias impediu o dano oxidativo induzido pelo estresse com uma

eficácia semelhante à curcuma, utilizado como padrão, uma vez que integra propriedades antioxidantes e antidepressivas, como evidenciado pelo aumento significativo de componentes antioxidantes no cérebro e no fígado (ZAFIR; BANU, 2007).

Assim, o presente estudo teve como objetivo avaliar os efeitos do tratamento farmacológico de ratos machos com fluoxetina durante o período crítico do desenvolvimento na bioenergetica mitocondrial e estresse oxidativo no fígado de ratos adultos.

2 REVISÃO DA LITERATURA

2.1 Período Crítico do desenvolvimento, Serotonina e Inibidores Seletivos de Recaptação de Serotonina

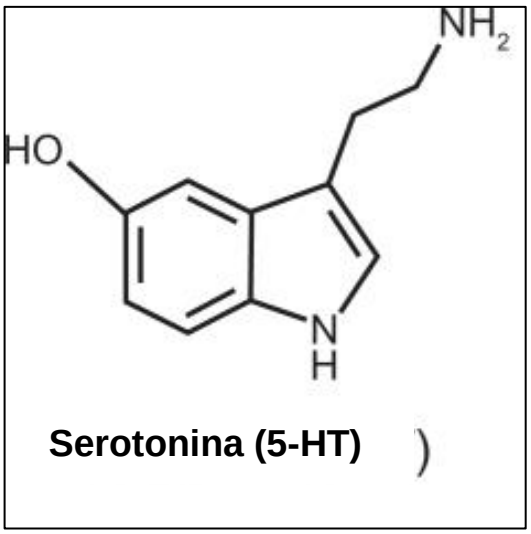
O período de crescimento e desenvolvimento do organismo compreende eventos biológicos de grande proliferação e diferenciação celular (MORGANE; MOKLER; GALLER, 2002). Durante esta janela temporal os eventos de desenvolvimento ocorrem numa grande velocidade e são extremamente sensíveis a estímulos provindos do ambiente, esse período é conhecido como período crítico do desenvolvimento (DOBBING, 1970). Em humanos, o período crítico de desenvolvimento compreende a fase pré-natal, em particular do último trimestre da gestação até os cinco primeiros anos de vida (MORGANE et al., 1978; MORGANE; MOKLER; GALLER, 2002), em roedores, esta fase perdura até as três primeiras semanas de vida pós-natal (MORGANE et al., 1978).

O período crítico de desenvolvimento vem sendo estudado ao longo dos anos em modelos experimentais que utilizam a manipulação farmacológica neonatal do sistema serotoninérgico e seus efeitos em fases tardias da vida (MANHÃES DE CASTRO et al., 2001; MENDES-DA-SILVA et al., 2002; DEIRÓ et al., 2004). Estes estudos reservam atenção especial à exposição neonatal de inibidores seletivos de recaptação (ISRS) e suas conseqüências. A administração crônica de citalopram, um antidepressivo ISRS, promoveu prejuízo no crescimento somático e maturação de reflexos e de características físicas (DEIRÓ et al., 2004) levando a diminuição do comportamento agressivo de ratos adultos (MANHÃES DE CASTRO et al., 2001). O uso de fluoxetina alterou a sensibilidade de receptores e reduziu o número de neurônios serotoninérgicos desses animais (HJORTH et al., 2000; MENDES-DA-SILVA et al., 2002).

A serotonina ou 5-hidroxitriptamina (5-HT) foi descoberta desde o ano de 1930 quando Ersparmer começou a estudar a distribuição de um tipo celular chamada células enterocromafins, que se coravam com um reagente para indóis. As maiores concentrações foram observadas na mucosa gastrointestinal e em seguida nas plaquetas e no Sistema Nervoso Central (SNC) (ERSPAMER, 1986). A esta substância, eles chamaram de *enteraminas*. Pager e colaboradores foram os

primeiros a isolar e caracterizar quimicamente essa substância que atuava de forma vasoconstrictora e era liberada pelas plaquetas no sangue no processo de coagulação. Eles a denominaram então de serotonina ou simplesmente 5-HT (RAPPORT; GREEN; PAGE, 1948). Contudo só em 1976, Pager demonstrou ser a mesma substância encontrada por Ersparmer em 1930.

Figura 1- Estrutura química da serotonina.



Adaptado de (BELLO; LIANG, 2011)

Os neurônios serotoninérgicos são encontrados numa ampla variedade de organismos. Nos mamíferos, estão entre os primeiros neurônios que são diferenciados durante o desenvolvimento, e compreendem uma complexa rede neuronal distribuídos no cérebro (MAZER et al., 1997; LESCH; WAIDER, 2012). Dados experimentais indicam que a 5-HT pode atuar como uma via de sinalização encefálica do feto durante períodos críticos de desenvolvimento. Reconhece-se que a 5-HT é sintetizada no início do período embrionário e os seus receptores são expressos precocemente. O encéfalo do feto recebe além da 5-HT endógena àquela proveniente da placenta da mãe, enfatizando ainda mais a importância da 5- HT no desenvolvimento embrionário precoce do cérebro. A contribuição dessas interações materno-placentário-fetal parece ser crítica para a formação de circuitos cerebrais e para as suas funções a longo prazo (SULLIVAN; MENDOZA; CAPITANIO, 2011). Estudos utilizando modelos genéticos em ratos revelam que os níveis excessivos de 5-HT no encéfalo alteram o correto desenvolvimento do córtex somatosensorial (CASES et al., 1996; PERSICO et al., 2001). Por outro lado, a depleção de 5-HT no

cérebro leva a defeitos comportamentais e funcionais no SNC (HENDRICKS et al., 2003; SAVELIEVA et al., 2008; ALENINA et al., 2009). Apenas 2% da 5-HT é produzida no SNC nos núcleos da rafe localizados no tronco encefálico (NASYROVA et al., 2009). Nos seres humanos, assim como na maioria das outras espécies de mamíferos, a serotonina pode ser produzida, na primeira etapa de sua síntese,por duas enzimas distintas, a triptofanohidroxilase (TPH) 1 e 2 (COTE et al., 2007). TPH1, está localizado na glândula pineal e células enterocromafins do intestino, sendo responsável por sintetizar a maior parte da serotonina encontrada no organismo. TPH2, que é restrita aos neurônios dos núcleos da rafe e do sistema nervoso entérico, é responsável pela síntese do restante da serotonina (ERSPAMER, 1954; HOYER; HANNON; MARTIN, 2002). A síntese da 5-HT se dá a partir do aminoácido essencial triptofano. Na primeira etapa, o aminoácido essencial é hidroxilado pela enzima TPH tendo como produto o 5-hidroxitriptofano (5-HTT) (CLARK; WEISSBACH; UDENFRIEND, 1954). Na sequência, o 5-hidroxitriptofano é descarboxilado pela triptofano descarboxilase, formando a 5-HT (CLARK; WEISSBACH; UDENFRIEND, 1954).

A síntese de serotonina está sujeita a variações diárias e muda dependendo da espécie estudada (diurna ou noturna), bem como do órgão de estudo. Em animais diurnos foi observado que no período de luz, os níveis são mais elevados do que na noite no hipotálamo e hipocampo (GARAU et al., 2006). No entanto, Sun et al. mostrou que na pineal de ratos (animais noturnos), existem três fases na produção de 5-HT: uma primeira fase com níveis elevados e constantes durante o dia, uma segunda fase com um novo aumento acentuado na síntese e libertação de 5- HT no início do período noturno e uma terceira fase com uma diminuição dos níveis de serotonina durante o resto da noite. A diminuição nos níveis de 5-HT durante o período escuro é porque neste período o neurotransmissor é convertido em melatonina, um hormônio sintetizado principalmente durante a escuridão na glândula pineal. Estes processos são controlados por diferentes receptores (SUN et al., 2002).

Devido à extensa distribuição do sistema serotoninérgico no sistema nervoso central, a 5-HT influencia uma ampla variedade de funções fisiológicas tais como a regulação do sistema cardiovascular (NEBIGIL et al., 2003), a respiração (MIYATA et al., 2000), e sistema gastrointestinal (KATO; FUJIWARA; YOSHIDA, 1999), além

de desempenhar um papel importante no comportamento, depressão, regulação do humor, função cognitiva, ansiedade, sono, apetite, função sexual, fluxo sanguíneo para o cérebro e muitas outras funções (SANCHEZ et al., 2008).

Os neurônios serotoninérgicos exercem seus efeitos através da ação de 7 classes de receptores (5-HT1A, 1B, 1D, 1E, 1F, 2A, 2B, 2C, 3, 4, 5A, 5B, 6 e 7) e um transportador de serotonina (SERT). Exceto o receptor 5-HT3, que passa por um canal iônico permeável a cátions, todos os receptores da 5-HT são acoplados à proteína G e diversos são os fatores que determinam a intensidade e duração de sua sinalização, sendo a quantidade de 5-HT liberada na fenda sináptica o principal deles (MILLAN et al., 2008).

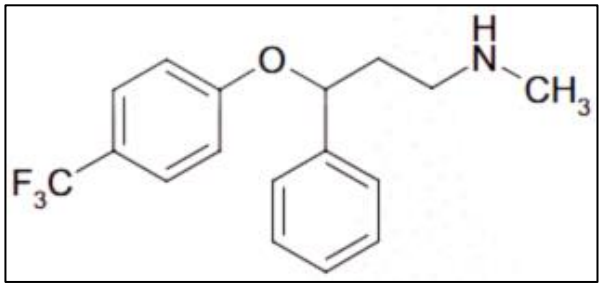
Para finalizar a sua ação, a 5-HT é recaptada pela molécula SERT, um complexo molecular com 13 laços transmembranares localizados na região pré-sináptica e nas membranas somatodendríticas da maioria dos neurônios serotoninérgicos. Uma vez no espaço intermembranar, a 5-HT é subsequentemente absorvida pelo transportador de monoamina (VMAT2) e armazenada no sistema sináptico em vesículas para reutilização. Outra via importante de finalização da atividade serotoninérgica é o processo pelo qual a 5-HT é absorvida pelas células gliais circundantes e degradada pela enzima monoamino-oxidase-A (MAO-A)(YOUDIM; EDMONDSON; TIPTON, 2006). Existem dois tipos de MAO, a MAO-A e B. A primeira é responsável pela metabolização da 5-HT encefálica. A MAO-B age primordialmente sobre a 5-HT periférica (plaquetas, células enterocromafins). A 5-HT sofre ação da MAO formando o aldeído 5-hidroxindolacetaldeído que por sua vez pode ser convertido em ácido 5-hidroxiindolacético (5-HIAA) pela enzima aldeído desidrogenase ou por uma via alternativa que consiste na redução pela ação da enzima aldeído redutase do acetaldeído a álccol, o 5-hidroxitriptofol. No entanto esta via é normalmente insignificante. O 5-HIAA do cérebro e dos locais periféricos de armazenamento e metabolismo da 5-HT é excretado na urina juntamente com pequenas quantidades de sulfato de 5-hidroxitriptofol ou conjugados de glicuronídeos (SANDLER; REVELEY; GLOVER, 1981). Nesse processo de metabolização da 5-HT, as MAOs presentes na membrana externa das mitocôndrias produzem espécies reativas de oxigênio (MANNI et al., 2016).

Inibidores seletivos da recaptação da serotonina (ISRS) inibem este ciclo contínuo de recaptação e são frequentemente usados para o tratamento de

disturbios neurológicos, transtornos alimentares (bulimia nervosa, anorexia nervosa, distúrbio alimentar compulsivo) e distúrbio disfórico pré-menstrual. ISRSs, incluindo fluoxetina (Prozac), sertralina (Zoloft), paroxetina (Paxil), fluvoxamina (Luvox) e citalopram (Celexa), são comumente utilizados pelo efeitos colaterais mínimos e boa tolerabilidade (MASAND; GUPTA, 1999; FLESCHLER; PESKIN, 2008).

A fluoxetina é um fármaco largamente prescrito para tratamento de distúrbios neurológicos, como depressão e ansiedade (BEASLEY et al., 2000). Quimicamente, é (±)-N-metil-3-fenil-3- [4- (trifluorometil) fenoxi] propan-1-amina (figura 2). A fluoxetina liga-se ao SERT, bloqueando a recaptação do neurotransmissor para a fenda pré-sináptica, o que resulta em aumentos agudos nos níveis de serotonina extracelular. No entanto, o início dos efeitos terapêuticos é retardado por 3-4 semanas. A fluoxetina é metabolizada pelas enzimas do citocromo P450 (CYP450) localizado na membrana mitocondrial interna, com as enzimas CYP2CP e CYP2D6 desempenhando um papel importante. O principal metabolito é a norfluoxetina, que é biologicamente ativa com afinidade igual para o SERT (VASWANI; LINDA; RAMESH, 2003). Nos seres humanos, a fluoxetina tem uma meia-vida de 1-4 dias enquanto a norfluoxetina tem uma meia-vida mais longa de 7-15 dias. Além disso, a fluoxetina e a norfluoxetina inibem seu próprio metabolismo através de interações com as enzimas hepáticas do CYP450, particularmente o CYP2D6 (PRESKORN, 1997; HIEMKE; HARTTER, 2000). Assim, a dosagem cumulativa, como é frequentemente utilizada clinicamente, resulta em diferentes concentrações sanguíneas e farmacocinéticas do que a dosagem aguda (SAWYER; HOWELL, 2011).

Figura 2 - Estrutura química da fluoxetina.



Adaptado de (MAERTENS et al., 1999).

A fluoxetina pode ser considerada uma droga bem-sucedida para tratamento com base na sua relação segurança, eficácia favorável e tempo de meia vida longo. No entanto, sangramento, lesão pulmonar e cardiotoxicidade tem sido notificados durante a terapêutica com fluoxetina (BEASLEY et al., 2000). Vários autores relataram que a fluoxetina induz hepatotoxicidade e afeta a atividade de enzimas no fígado (FRIEDENBERG; ROTHSTEIN, 1996), além de dano oxidativo hepático (INKIELEWICZ-STEPNIAK, 2011).

2.2 Fígado e alterações hepáticas pelo tratamento com fluoxetina

O fígado é um grande órgão, localizado no hipocôndrio direito, pesa entre 1,3 kg e 1,5 kg, nos homens, e 1,2 kg nas mulheres. É um órgão extremamente vascularizado, recebendo sangue proveniente da veia porta e pela artéria hepática. É composto praticamente de hepatócitos, o restante é constituído de *células de Kuppfer* (um tipo de macrófago) e *células estreladas*, são essas células estreladas que podem se transformar em fibroblastos e produzirem colágeno, um caso que parece estar relacionado com o desenvolvimento de fibrose e cirrose hepática (WANG et al., 2017).

O início do desenvolvimento de fígado começa por volta do 8° dia embrionário a partir da endoderme do intestino anterior. O desenvolvimento do fígado a partir das células da endoderme é ditada por duas citocinas cruciais, o fator de crescimento de fibroblastos (FGF) a partir do coração em desenvolvimento e proteína morfogenética do osso (BMP) a partir do septo mesênquima transverso. As células da endoderme do intestino anterior são células progenitoras que dá origem aos hepatócitos e são chamados "hepatoblastos" durante o desenvolvimento do fígado (MIYAJIMA; TANAKA; ITOH, 2014). Esses hepatoblastos se comportam como células estaminais, capazes de auto-duplicação, dando origem a hepatócitos e células ductais (HUCH; DOLLE, 2016). Durante o período de gestação e lactação, enzimas metabólicas como tirosina aminotranferase, glicoquinase e aspartato transcarbomoilase passam por uma grande oscilação em termos de atividades (GEBHARDT, 1992); é nessa fase que insultos sofridos podem levar a prejuízos morfológicos e bioquímicos. Durante a idade adulta, a renovação celular é bastante

lenta, menos de 1 em 10.000 hepatócitos são mitóticos, sugerindo um tempo de renovação de pelo menos um ano. No entanto, o fígado é um órgão altamente capaz de restabelecer completamente sua função após uma lesão (KOPP; GROMPE; SANDER, 2016).

O fígado funciona como o centro de distribuição do organismo, exporta nutrientes nas proporções corretas para outros órgãos, diminuindo as flutuações do metabolismo causadas pela ingestão de alimentos e processando o excesso de grupos amino em uréia e outros produtos para serem excretados pelos rins. O fígado exerce também a função de armazenamento de nutrientes, entre eles o ferro e a vitamina A; além de ser um órgão extremamente adaptável quanto ao metabolismo total do organismo, com notável flexibilidade metabólica, providenciando substratos energéticos de acordo com a demanda do corpo, a partir da modulação neural e reguladores endócrinos. Diferentes sistemas de estoques energéticos são utilizados em momentos de oscilação da ingesta de nutrientes e demanda energética. Graças a uma orquestrada gama de enzimas hepáticas de síntese e degradação 5 a 10 vezes maior que de outros tecidos (MIYAJIMA; TANAKA; ITOH, 2014).

Este órgão tem uma alta capacidade de estocar glicogênio e liberar a glicose através da glicogenólise. O estoque de glicogênio é capaz de manter a glicemia adequada por 24 a 48 horas, representando cerca de 250 a 500 mg de glicose. Além de desempenhar um papel importante no metabolismo lipídico que consiste na regulação da conversão do excesso de carboidratos em ácidos graxos, esterificação dos ácidos graxos para formar triglicerídeos de transporte e armazenamento, e sintetizar proteínas de transporte (lipoproteínas). O fígado também tem participação importante no metabolismo das proteínas. Esse órgão é capaz de produzir uma variedade de proteínas que inclui, proteínas de coagulação e proteínas ligantes envolvidas no transporte no plasma (albumina, transferrina, lipoproteínas e haptoglobina, uma proteína de fase aguda que se liga irreversivelmente às hemoglobinas após hemólise) (RUI, 2014).

O fígado é o principal local de metabolismo, desintoxicação e bioativação de produtos químicos e drogras pelas enzimas do CYP450, extremamente abundantes neste órgão (FENG; HE, 2013). Os CYP das famílias 1 a 4 estão bem associados ao metabolismo farmacêutico e são altamente expressos em hepatócitos de mamíferos,

mais especificamente, CYP3A4, CYP2D6, CYP2C9 e CYP1A2 são responsáveis por 50%, 25%, 15% e 5% do metabolismo de fármacos humanos, respectivamente (HEMERYCK; BELPAIRE, 2002; SMITH et al., 2012). Essas enzimas do CYP450 induzem a ativação metabólica de inúmeras lesões por compostos xenobióticos, formando metabólitos reativos (MR) que se ligam covalentemente a macromoléculas celulares (TANG; LU, 2010). Os MR tem vários destinos possíveis durante o metabolismo hepático, em primeiro lugar, estes compostos podem reagir com nucleófilos proximais de resíduos de aminoácidos no local da enzima, causando mecanismo de inibição da enzima (MCCONN; ZHAO, 2004). Segundo, podem reagir com componentes celulares tais como proteínas, DNA e membranas (por exemplo, membrana mitocondrial), resultando em estresse ixidativo celular (KALGUTKAR; DIDIUK, 2009). Em terceiro lugar, MRs podem ser inibidores de glutationa reduzida (GSH), importante tiol não protéico no sistema de defesas antioxidantes através de processos químicos ou mediados por enzimas (NAKAYAMA et al., 2011).

A fluoxetina, um ISRS é metabolizada no fígado, onde sofre extensa conversão metabólica, levando à formação do metabolito ativo norfluoxetina entre vários outros metabólitos (ALTAMURA; MORO; PERCUDANI, 1994). Devido à inibição do seu próprio metabolismo, a eliminação da fluoxetina e norfluoxetina do corpo é extremamente lenta (CREWE et al., 1992). Quando a fluoxetina é administrada intraperitonealmente, o fármaco atinge altas concentrações no fígado, exercendo efeitos potencialmente tóxicos sobre o metabolismo mitocondrial hepático (SOUZA et al., 1994). O que parece está associado a sua solubilização ou dos seus metabólitos na membrana mitocondrial interna (DJORDJEVIC et al., 2011).

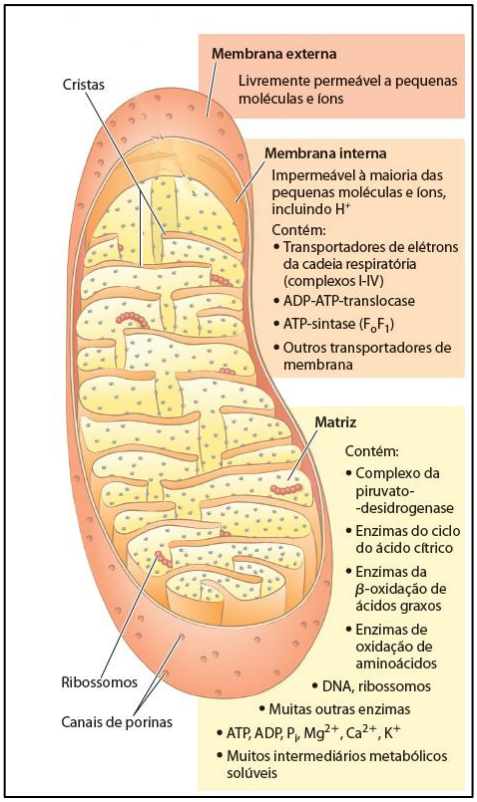
Muitos estudos focaram nos efeitos deletérios da exposição à fluoxetina no fígado pelos níveis elevados de aminotransferases (BEASLEY et al., 2000) e estresse oxidativo (INKIELEWICZ-STEPNIAK, 2011). As aminotransferases (ALT e AST) são enzimas que frequentemente indicam lesão hepática pela sensibilidade a danos nas membranas citoplasmáticas e/ou mitocondriais. Aumento na atividade de ALT é um importante indicador de doenças hepáticas e são mais específicas para as lesões hepáticas que a AST, devido à localização celular desta enzima. As células hepáticas contêm mais AST do que ALT, mas ALT é confinada ao citoplasma onde sua concentração é maior do que a AST (BEASLEY et al., 2000). Além disso, altas doses de fluoxetina interferem no metabolismo energético em mitocôndrias de fígado

de rato (SOUZA et al., 1994), levando ao aumento de biomarcadores de estresse oxidativo (ZLATKOVIC et al., 2014).

2.3 Mitocôndrias, estresse oxidativo e Fluoxetina

As mitocôndrias, tem duas membranas, a membrana externa é prontamente permeável a moléculas pequenas, enquanto a membrana interna é extremamente impermeável a maioria das moléculas pequenas. É na membrana mitocondrial interna que estão alojados os complexos da cadeia transportadora de elétrons. A matriz mitocondrial, delimitada pela membrana interna, contém todas as vias de oxidação de combustível celular (adenosina-5-trifosfato -ATP), exceto a glicólise (KALUDERCIC; GIORGIO, 2016).

Figura 3 - Estrutura anatômica da mitocôndria. Setas indicam membrana externa, membrana interna, matriz mitocondrial e seus constituintes; ribossomos, canais de porina e cristas.



Adaptado de (NELSON, 2011).

Devido a sua origem bacteriana, as mitocôndrias têm o seu próprio genoma, e são capazes de se auto-replicar (BRUNI; LIGHTOWLERS; CHRZANOWSKA-

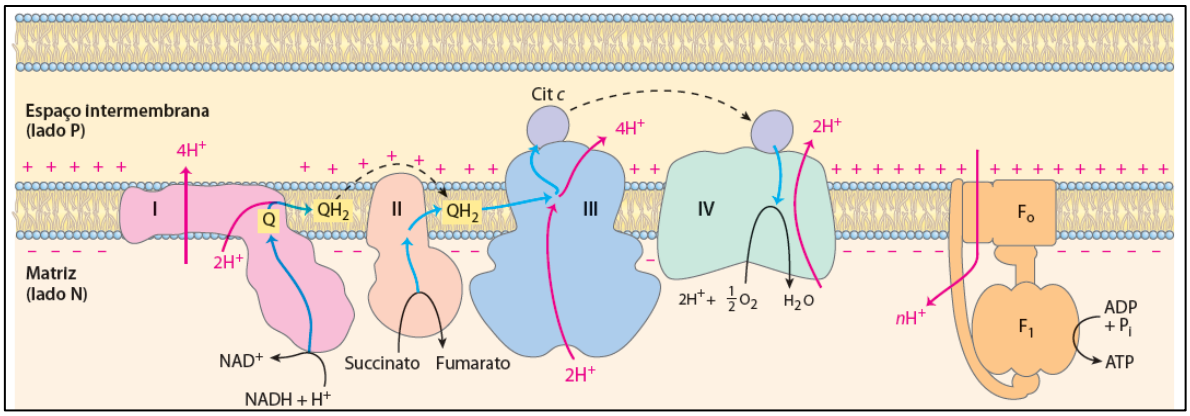
LIGHTOWLERS, 2016). A biogênese mitocondrial pode ser definida como o crescimento e a divisão de mitocôndrias pré-existentes. Nesse processo, proteínas mitocondriais são codificadas no núcleo pelo genoma nuclear. O DNA mitocondrial (DNAmt) é uma molécula circular de cadeia dupla de que contêm os genes que codificam as subunidades dos complexos da cadeia de transporte de elétrons I, III, IV e V. A biogênese mitocondrial requer a síntese coordenada e importação de proteínas codificadas pelo genoma nuclear e sintetizadas pelos ribossomos citosólicos. O crescimento de novas mitocôndrias pode ser influenciado por estresse ambiental, tais como o exercício, restrição calórica, baixa temperatura, estresse oxidativo e é acompanhada não só por variação em números, mas também em tamanho e massa (JORNAYVAZ; SHULMAN, 2010).

A mitocôndria é a principal executora do metabolismo energético celular, responsável pela grande maioria da síntese de ATP. É nela que estão dispostos os quatro complexos transportadores de elétrons, que impulsionados pelo catabolismo de nutrientes energéticos de carboidratos, lipídios e proteínas, resulta em um grande número de reações de oxido-redução para maximizar a conservação de energia. O resultado final dessas reações é a redução do oxigênio à água. Esses transportadores de elétrons são acoplados a síntese de ATP por um gradiente de potencial eletroquímico de prótons nas mitocôndrias (TAHARA; NAVARETE; KOWALTOWSKI, 2009; FIGUEIRA et al., 2013).

Os transportadores de elétrons da cadeia respiratória mitocondrial são organizados em complexos supramoleculares dentro da membrana interna. O complexo I, também chamado de NADH-desidrogenase é um complexo multienzimático composto por uma grande cadeia polipeptídica, incluindo flavoproteínas e centros de ferro-enxofre; ele catalisa dois processos simultâneos e obrigatoriamente acoplados, a transferência exergônica para a ubiquinona de um íon hidreto do NADH e um próton para matriz mitocondrial e a transferência endergônica de quatro prótons para o espaço intermembranas (VAN DER LAAN; HORVATH; PFANNER, 2016).O complexo II, também chamado de succinato-desidrogenase, é complexo multienzimático do ciclo do ácido cítrico, nele contém uma flavoproteína transferidora de elétrons através dos centros ferro-enxofre até a ubiquinona e um sítio de ligação para o succinato. O complexo III, chamado de complexo citocromo *bc,* acopla a transferência de elétrons do ubiquinol para o citocromo *c* com o

transporte vetorial de prótons da matriz para o espaço intermembranas. O complexo IV, conhecido como citocromo c-oxidase, carrega elétrons do citocromo *c* para o oxigênio, reduzindo-o completamente a água e bombeando dois prótons para o espaço intermembranas, como ilustrado na figura 2 (JEZEK; HLAVATA, 2005).

Figura 4 - Complexos I, II, III e IV e ATPsintase.

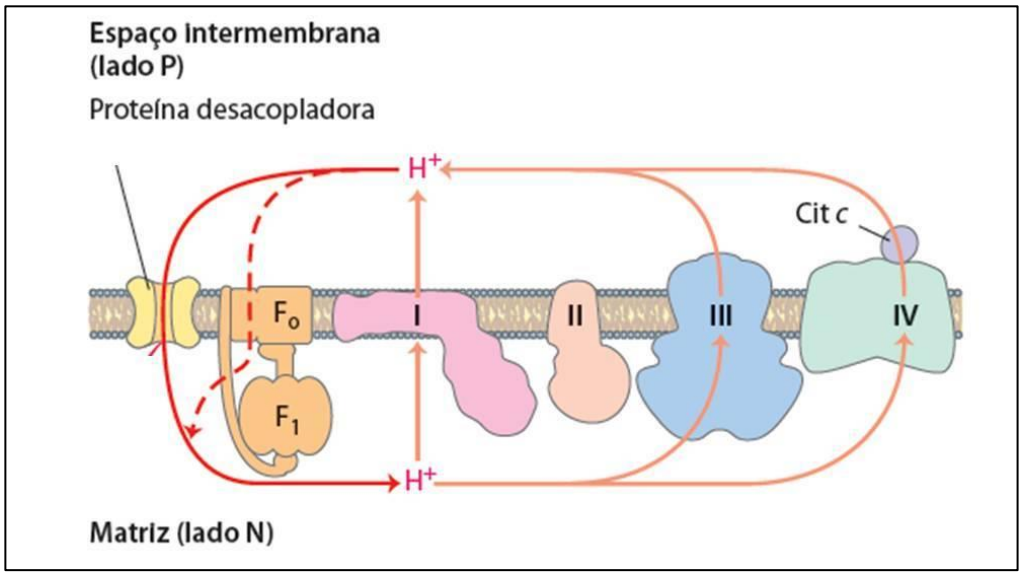


Adaptado de (NELSON, 2011).

O bombeamento de prótons pelos quatro complexos gera um gradiente de prótons que fornece energia para a síntese de ATP a partir do ADP e de P_i pela ATP-sintase na membrana interna. A força próton-motriz proporciona catálise rotacional da ATP-sintase promovendo a liberação de ATP a partir de ADP e Pi (VAZQUEZ-ACEVEDO et al., 2016).

Existe ainda, na membrana mitocondrial interna, transportadores de cátions capazes de desacoplar o metabolismo oxidativo mitocondrial, diminuindo a eficiência da fosforilação oxidativa (GARLID et al., 1996). Há cerca de 40 transportadores de cátions mitocondriais, incluindo as UCPs, que são comumente encontrados entre os eucariotos. A ativação de UCPs resulta na reentrada de prótons H^+ do espaço intermembrana de volta para a matriz mitocondrial (SLUSE; JARMUSZKIEWICZ, 2002). Ânion de ácidos graxos livres são considerados ativadores de UCPs, enquanto nucleotídeos de purina são considerados inibidores. Os mecanismos exatos pelos quais esses transportadores medeiam à saída de H^+ através da membrana interna mitocondrial ainda são controversos (SLUSE et al., 2006), entretanto, a literatura tem demonstrado que as proteínas desacopladoras podem desempenhar um papel de antioxidante, diminuindo a liberação de ânions superóxidos (BARTOSZ, 2009).

Figura 5 - Proteínas desacopladoras (UCPs) atuando no retorno de prótons para matriz mitocondrial.



Adaptado de (NELSON, 2011).

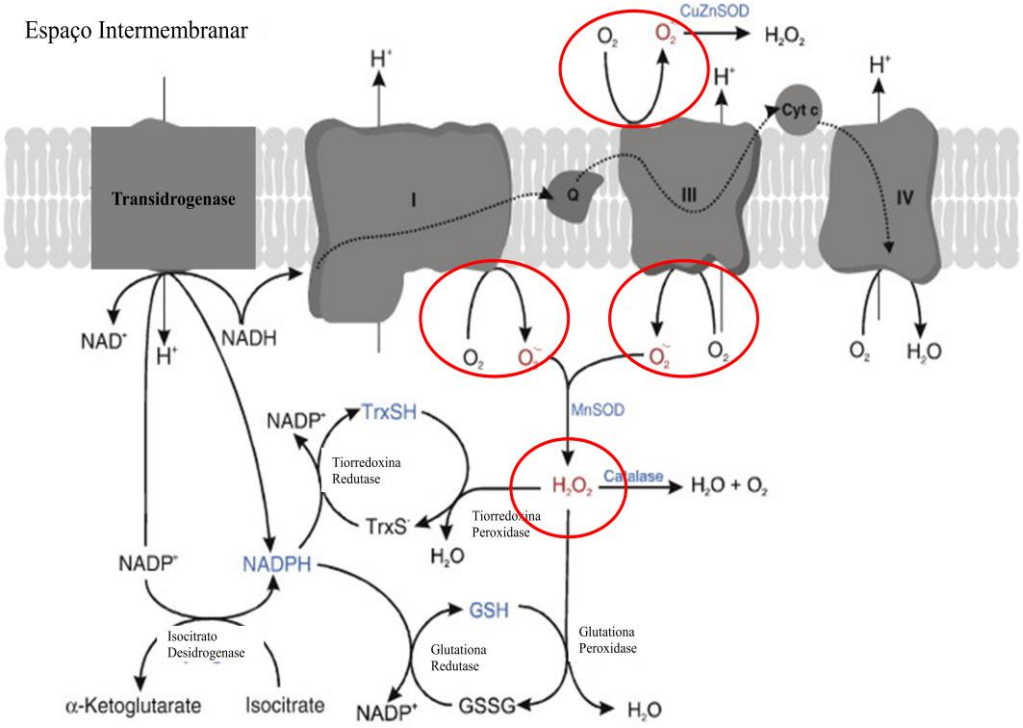
As mitocôndrias são capazes de integrar de forma autônoma e responder a estímulos e demandas celulares, remodelando sua morfologia. Estes processos definem a dinâmica mitocondrial transmutando sinais citosólicos em funções compatíveis com as necessidades celulares pela remodelação de suas cristas, fusão, fissão e autofagia (PERNAS; SCORRANO, 2016b).

As cristas podem variar em comprimento, espessura, alinhamento lateral, rigidez, e angularidade. Estas membranas podem ser submetidas a grandes alterações, dependendo do estado do ambiente; estas alterações morfológicas podem ser acompanhadas por níveis elevados de proteínas de cadeia respiratória e supercomplexos, que em conjunto aumentam a atividade da fosforilação oxidativa (COGLIATI et al., 2013). As mitocôndrias também alteram a sua morfologia pelo processo de fusão, o qual pode ocorrer em menos de 2 min e permite uma transferência de informações através do intercâmbio de DNAmt, proteínas, lipídios, e metabolitos; principalmente para manutenção de uma população saudável mitocondrial (NUNNARI et al., 1997). Outro método pelo qual uma mitocôndria pode responder a sinais citosólicos e ambientais e alterar a sua morfologia é pelo processo de fissão, um evento de divisão que produz uma ou mais mitocôndrias filhas (CHAN, 2012).

Nos últimos anos, além de sua conhecida função de gerar energia para a célula, as mitocôndrias têm emergido como organela responsável por processos de sinalização, injuria e morte celular (INADA et al., 2008). Vários desses processos de morte celular resultam na liberação de proteínas mitocondriais, como o citocromo *c,* fator de indução de apoptose (AIF), segundo ativador mitocondrial de caspases (Smac) promovendo eventos de sinalização citosólicos dependente ou não de caspases (CIRCU; AW, 2010). Na via intrínseca da apoptose, sinais de morte alcançam a mitocôndria, os quais induzem a abertura do poro de transição de permeabilidade mitocondrial (PTPM), levando ao colapso do potencial elétrico da membrana mitocondrial interna ($\Delta\psi$), bem como a transição de permeabilidade mitocondrial (TPM) e perda da homeostase bioquímica das células; como a síntese de ATP é comprometida, NADH, NADPH e glutationa (GSH) são oxidados, e um excesso de ROS é produzido, o que pode provocar a oxidação de lipídios, ácidos nucleicos e proteína (SINHA et al., 2013).

O metabolismo energético mitocondrial é reconhecido como a principal fonte de EROs celular na maioria das células de eucariotos, cerca de 0,1-2% de todo O_2 consumido fisiologicamente é convertido em ânion superóxido (O_2^-) (STANIEK; NOHL, 2000; QUIJANO et al., 2015). A geração de O_2^- acontece em pelo menos cinco sítios da cadeia transportadora de elétrons: nos complexos I e III, na ubiquinona (UQ), no grupo prostético Flavina no complexo I, na transferência de elétrons na flavoproteína (ETF), UQ oxido redutase, e na glicerol 3-fosfato desidrogenase. Desses sítios, três são bem caracterizados no que diz respeito ao mecanismo de geração de O_2^-, a UQ no complexo III, a UQ e a flavina no complexo I (CARDOSO et al., 2012), gerando cerca de 2-5 % de ânion superóxido via mitocondrial (ADAM-VIZI; CHINOPOULOS, 2006). Além da produção de O_2^- pela CTE há ainda produção de EROs pela oxoglutarato desidrogenase e pelas monoamina oxidase via mitocondrial (BAO et al., 2009; ADAM-VIZI; STARKOV, 2010).

Figura 6 - Geração de EROs pelos complexos I e III na membrana mitocondrial interna e produção de peróxido de hidrogênio como resultado da ação da enzima antioxidante superóxido dismutase dependente de manganês (MnSOD).



Adaptado de (KOWALTOWSKI et al., 2009).

O complexo I pode produzir ânion superóxido tanto no sítio de ligação da Flavina, quanto no sítio de ligação da UQ. Esses sítios de ligação do complexo I são considerados um importante doador de elétrons do O_2 para o $O_2^{\cdot-}$ (INDO et al., 2015a). Além disso, o centro ferro-enxofre do complexo I, também tem sido proposto como um doador de elétrons ao O_2, direta ou indiretamente através da semiquinona (LENAZ, 2012). O superóxido também pode ser formado durante o transporte reverso de elétrons, da UQ para o complexo I, esse mecanismo é inibido pala rotenona(MURPHY, 2009a).

A produção de $O_2^{\cdot-}$ pelo complexo III está relacionada com o seu mecanismo particular de transferência de elétrons, o ciclo-Q, ao passo que a transferência de elétrons para o citocromo *b* é retardado pelo gradiente elétrico através da membrana mitocondrial interna e o tempo de meia-vida da UQ é prolongado, permitindo a redução do O_2 para formar o $O_2^{\cdot-}$(JEZEK; HLAVATA, 2005). A produção de $O_2^{\cdot-}$ pelo complexo III é dependente da meia-vida de UQ, que pode ser inibido por

substâncias que aumentem a meia-vida da UQ, através da inibição de bloqueadores de UQH_2 diminuindo a entrega de elétrons ao complexo II e reduzindo a formação de $O_{2^{\cdot}}$ (CAPE; BOWMAN; KRAMER, 2007).

O ânion superóxido, principal EROs produzido na mitocôndria, dá origem a outras espécies reativas de oxigênio e nitrogênio, por reações distintas. A dismutação do $O_{2^{\cdot}}$ acontece principalmente pela ação da enzima antioxidante superóxido dismutase (Mn-SOD na matriz e Cu/Zn-SOD no espaço intermembranas), presente na mitocôndria e no citosol (NOHL; GILLE; STANIEK, 2005), produzindo peróxido de hidrogênio (H_2O_2), por sua vez, pode reagir com íons cobre ou ferro e produzir radicais hidroxil ($OH\cdot$), uma espécie altamente reativa, através da reação de Fenton (FENTON, 1894). Do mesmo modo, na presença de metais de transição, parte desse superóxido pode reagir com o H_2O_2 e também produzir radical hidroxil (HABER; WEISS, 1934). Além disso, o $O_{2^{\cdot}}$ pode reagir com o óxido nítrico NO^{-} para formar peroxinitrito ($ONOO^{-}$) (VALKO et al., 2007; FIGUEIRA et al., 2013).

Em condições fisiológicas, a produção de EROs desempenha funções primordiais de segundo mensageiro, com regulação da expressão de genes sensíveis aos sinais redox e alterações na homeostase celular, através da síntese de moléculas fisiologicamente ativas (CADENAS; DAVIES, 2000). Entretanto, em concentrações elevadas, EROs podem ser importantes mediadores de danos nas estruturas celulares, de ácidos nucleicos (OZAWA, 1999), lipídios (SPITELLER, 2002) e proteínas (CADENAS; DAVIES, 2000). O radical hidroxil pode reagir com praticamente todos os componentes da molécula de DNA, danificando tanto os nucleotídeos de purina e de pirimidina e também a desoxirribose. Modificações permanentes no material genético resultante de "danos oxidativos" representam o primeiro passo envolvido na mutagênese, carcinogênese e envelhecimento (BARTOSZ, 2009). Além disso, a literatura tem demonstrado que EROs mitocondriais estão amplamente envolvidos em várias doenças humanas e condições degenerativas (FUKUI; MORAES, 2008; DORIGHELLO et al., 2016), do mesmo modo, a produção de EROs está relacionada a indução de modificações pós-traducionais da atividade catalítica da ATP sintase em varias condições fisiopatológicas (KALUDERCIC; GIORGIO, 2016).

As mitocôndrias são equipadas com uma maquinaria sofisticada para mediar o fluxo de Ca^{2+} através da membrana mitocondrial interna, esse sistema é composto por canais, proteínas reguladoras e uma matriz de Ca^{2+} caracterizada como sistema tampão. O uniporte de Ca^{2+} mitocondrial é o principal mediador do transporte de Ca^{2+} para a matriz mitocondrial, um transporte passivo e unidirecional de Ca^{2+} através da membrana mitocondrial interna, um processo que é conduzido pelo gradiente eletroquímico ($\Delta\psi_m$) em mitocôndrias. A absorção do Ca^{2+} mitocondrial tem sido relacionada a uma variedade de funções celulares, incluindo exocitose, transcrição genética, regulação do ciclo celular, respiração e morte celular (KEVIN FOSKETT; MADESH, 2014).

Existem dois mecanismos fisiológicos para liberação do cálcio mitocondrial, um dependente de Na^+ mediado por um translocador de Na^+/Ca^{2+} e outro independente de Na^+ mediado por um translocador H^+/Ca^{2+}. Um terceiro mecanismo de abertura, chamado PTPM, é ativado sob condições fisiopatológicas específicas durante uma sobrecarga de Ca^{2+} por longos períodos de tempo (SANTO-DOMINGO; WIEDERKEHR; DE MARCHI, 2015). Esses movimentos do Ca^{2+} são acionados direta ou indiretamente por meio de hidrólise de ATP, tornando as suas funções de sinalização altamente dependente do estado de energia da célula (GLANCY; BALABAN, 2012a). Assim, defeitos em processos de fornecimento de ATP podem levar a desregulação da sinalização do Ca^{2+} e podem comprometer o funcionamento celular (BERRIDGE; BOOTMAN; RODERICK, 2003).

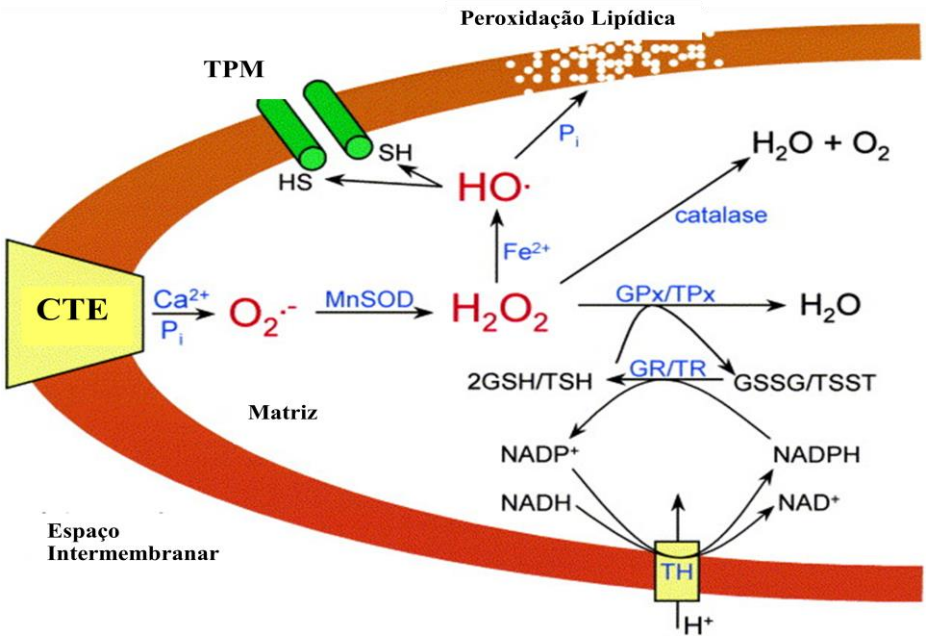
De modo geral, o cálcio aparece como um efetor positivo das funções mitocondriais e, perturbações no seu equilíbrio mitocondrial ou citosólico implicará em diversas vias metabólicas celulares (HIDALGO; DONOSO, 2008). Tanto na matriz mitocondrial quanto na membrana mitocondrial interna, existem enzimas que são ativadas alostericamente pelo cálcio, como a α-cetoglutarato desidrogenase, a isocitrato desidrogenase e a piruvato desidrogenase. Também atua como um estimulador da ATP-sintase, da α-glicerofosfato desidrogenase e do translocador de nucleotídeos de adenina (ANT) (BROOKES et al., 2004), estimulando a bioenergética e elevando os níveis de NADH (GLANCY; BALABAN, 2012b). A formação de complexos de cálcio com outros íons inorgânicos, como o fosfato, é de extrema importância para que a mitocôndria acumule cálcio sem alterar as suas funções e, desta forma, prevenir a dissipação do $\Delta\Psi_m$ (CARAFOLI, 2010).

Alterações na permeabilidade da membrana mitocondrial interna induzidas por Ca^{2+} podem ocorrer em consequência da ação de EROs geradas na mitocôndria(VERCESI; HOFFMANN; et al., 1993; VERCESI; MORENO; et al., 1993). A combinação entre sobrecarga de Ca^{2+} na matriz mitocondrial e estresse oxidativo está relacionada ao processo de TPM, caracterizado pela abertura de um poro na membrana mitocondrial interna (KOWALTOWSKI; CASTILHO; VERCESI, 2001). Entretanto, as mitocôndrias são mais suscetíveis ao processo de TPM quando seus sistemas antioxidantes, representados principalmente por NADPH, estão esgotados (RONCHI et al., 2015).

A abertura do PTPM promove o colapso do gradiente eletroquímico de prótons ($\Delta\mu H^{+}$) com despolarização mitocondrial, seguido de inibição respiratória e geração de EROs, hidrólise de ATP e inchamento mitocondrial, além do extravasamento de proteínas pró-apoptóticas para o citosol, como citocromo *c* e caspases, liberação de cálcio, induzindo assim a morte celular (KOWALTOWSKI; VERCESI, 1999; BAINES, 2009; LEMASTERS et al., 2009; ZOROV et al., 2009; CARAFOLI, 2010).

O processo de transição de permeabilidade mitocondrial (TPM) é considerado uma das principais causas de morte celular sob uma variedade de estados patológicos, incluindo isquemia e reperfusão (HALESTRAP; PASDOIS, 2009), doenças neurodegenerativas (NICHOLLS, 2009; RASHEED; TABASSUM; PARVEZ, 2016), lesão cerebral traumática (MBYE et al., 2009) e toxicidade a droga (RUSSMANN; KULLAK-UBLICK; GRATTAGLIANO, 2009), além de diversas doenças crônico-degenerativas, tais como câncer, Parkinson, diabetes tipo II (KOWALTOWSKI; CASTILHO; VERCESI, 2001; KOWALTOWSKI et al., 2009).

Figura 7 - O acúmulo de EROs mitocondrial pode provocar a abertura do poro de transição de permeabilidade mitocondrial. Além disso, na presença de cálcio e fosfato inorgânico, à produção de EROs aumenta devido à inativação de vias antioxidantes.



(KOWALTOWSKI; CASTILHO; VERCESI, 2001).

Na literatura existem relatos controversos da relação entre exposição ao tratamento com ISRS e alterações no metabolismo energético celular. Agostinho et al. avaliaram os efeitos da fluoxetina e da olanzapina sobre os complexos respiratórios mitocondriais e verificaram que as duas drogas, isoladas ou em conjunto, alteram a atividade da cadeia tranportadora de elétrons no cérebro de ratos (AGOSTINHO; REUS; STRINGARI; RIBEIRO; FERREIRA; et al., 2011). Adicionalmente, o tratamento agudo com fluoxetina aumenta a atividade da enzima citrato sintase e tratamentos tanto agudos como crônicos diminuiu a atividade da enzima creatina quinase, enzimas importantes envolvidas no metabolismo energético celular (AGOSTINHO; REUS; STRINGARI; RIBEIRO; FERRARO; et al., 2011). Além disso, o tratamento neonatal com fluoxetina aumentou a capacidade respiratória mitocondrial e o potencial elétrico de membrana no tecido cardíaco (BRAZ; FREITAS; et al., 2016). Um estudo de Da Silva e colaboradores mostrou uma modulação positiva da respiração mitocondrial no hipotálamo e no músculo

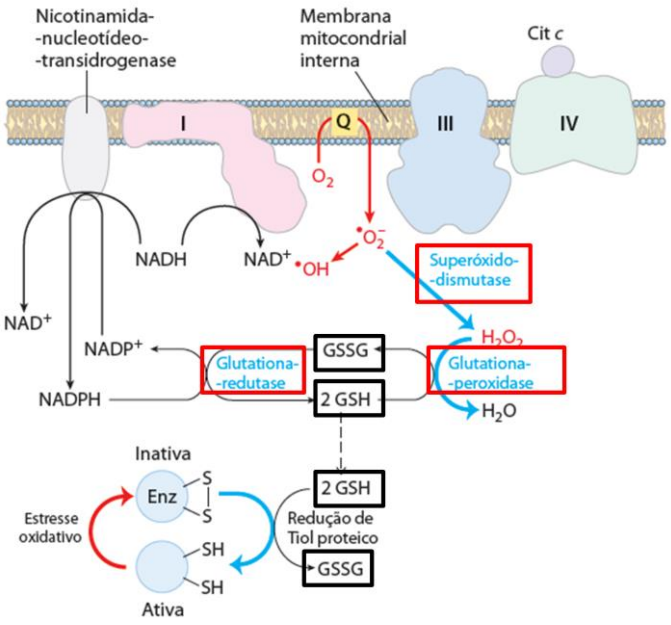
esquelético de ratos submetidos à manipulação neonatal persistindo até a idade adulta (DA SILVA; BRAZ; PEDROZA; et al., 2015).

Em contraste, estudos anteriores mostraram que altas doses (160-320 mol/L) de fluoxetina interferem no metabolismo energético em mitocôndrias de fígado de rato (SOUZA et al., 1994). Adicionalmente, doses elevadas (50-250 mol/L), mostraram que fluoxetina induz a inibição da capacidade oxidativa e diminui a atividade da ATP sintase em mitocôndrias de cérebro de ratos (CURTI et al., 1999). Além isso, fluoxetina *in vitro* inibiu a capacidade respiratória de mitocôndrias em doses maiores que 86 mol/L com substrato complexo I e maior que 266 mol/L com substrato complexo II no cérebro de porcos (HROUDOVA; FISAR, 2012).

2.4 Sistemas Antioxidantes e Fluoxetina

O termo "antioxidante" refere-se a qualquer substância que, em baixa concentração, comparada com a de um substrato oxidável, atrasa significativamente ou impede a oxidação do referido substrato. As células possuem sistemas antioxidantes complexos, constituído por várias enzimas antioxidantes abundantes nas mitocôndrias, como ilustrado na figura 7. (HALLIWELL; GUTTERIDGE, 1986).

Figura 8 - Sistemas antioxidantes enzimático destacado em vermelho e não enzimático destacado em preto.



Adaptado de (NELSON, 2011).

A superóxido dismustase dependente de manganês (Mn-SOD, SOD2), reduz O_2^- a H_2O_2, é uma primeira defesa antioxidante mitocondrial localizada na matriz mitocondrial. Existe ainda outra geradora de peróxido de hidrogênio, a superóxido dismutase dependente de cobre/zinco (Cu, Zn-SOD, SOD1) localizada no espaço intermembranar das mitocôndrias (INDO et al., 2015b). O H_2O_2 pode ser neutralizado por meio da glutationa peroxidase (GPx) dependente de selênio, e pela catalase convertendo-o a água (H_2O). A GPx foi a primeira a ser descrita e é considerada uma das principais enzimas que induzem a degradação de peróxido de hidrogênio. A atividade da GPx depende da incorporação de um resíduo de selenocisteína (SelCys) em cada uma das suas quatro cadeias polipeptídicas (KIELISZEK; BLAZEJAK, 2013). A GSH é o tiol não proteico mais abundante, com uma vasta gama de propriedades antioxidantes; além de ser um cofator para GPx, a GSH pode eliminar O_2^- e radical OH^- não enzimaticamente, regenerar outros antioxidantes para a sua forma ativa, manter grupos sulfidrilas (SH) de proteínas no seu estado reduzido, e pode ser conjugada e excretada com toxinas através da reação catalisada pela glutationaS-transferases (GST). A GST é uma família de enzimas de desintoxicação encontrada no citosol da maioria das células, alguns estudos indicam a importância dela não apenas na desintoxicação dos metabólitos, mas também na regulação do estresse oxidativo (ADACHI et al., 1981). A utilização da GSH resulta na formação de glutationa oxidada (GSSG)(GARCIA et al., 2010; FERREIRA et al., 2015).

Na literatura já há relatos da relação entre fármacos ISRS e os sistemas de defesas antioxidantes, entretanto com resultados bastante controversos. O tratamento crônico com fluoxetina, sertralina ou tioacetamida, pode aumentar significativamente os biomarcadores do estresse oxidativo no cérebro e no fígado (INKIELEWICZ-STEPNIAK, 2011, ZLATKOVIC et al. , 2014). Sob estresse crônico, a fluoxetina altera o sistema antioxidante enzimático com diminuição da atividade da SOD e promove a sinalização apoptótica incluindo diminuição da expressão de Bcl-2 e maior fragmentação do DNA em ratos *Wistar* (DJORDJEVIC et al., 2011). Em contraste, Aksu et al., estudando o potencial da fluoxetina como antioxidante no modelo de rim sob isquemia-reperfusão (IR), relataram que o pré-tratamento com fluoxetina restabeleceu significativamente o equilíbrio redox e diminuiu as medidas de inflamação no rim (AKSU et al., 2014). Novio et al. demonstraram efeito positivo

da fluoxetina contra o estresse na lesão celular oxidativa, com aumento de defesas antioxidantes endógenas (superóxido dismutase e catalase) e restauração de componentes não enzimáticos da cascata antioxidante das glutationas (NOVIO et al., 2011). Zafir e Banu também demonstraram o potencial antioxidante desta droga, com elevação de antioxidantes endógenos chaves como a superóxido dismutase, catalase, GST, glutationa redutase (GR) e níveis de GSH (ZAFIR; BANU, 2007).O tratamento com fluoxetina pode assim contribuir largamente para o aumento da resistência de ratos com estresse crônico ao dano oxidativo in vivo, em comparação com animais stressados que não recebem tratamento. Kolla et al. (KOLLA et al., 2005) demonstraram maior sobrevida de neurônios e redução de substâncias oxidativas como H_2O_2. Adicionalmente, o tratamento com fluoxetina durante o período crítico do desenvolvimento aumenta as defesas antioxidantes a a atividade enzimática metabólica no tronco encefálico e no coração de ratas adultas (BRAZ; PEDROZA; et al., 2016); também resulta em uma diminuição significativa na ansiedade, redução significativa da peroxidação lipídica e aumento da atividade da catalase e da glutationa-S-transferase no hipocampo (DA SILVA et al., 2014).

Diante do exposto nessa apresentação, é necessária uma maior compreenção a cerca dos efeitos do tratamento com fluoxetina no metabolismo oxidativo hepático a fim de gerar subsídios para ações intervencionistas que diminuam a incidência de doenças metabólicas na idade adulta.

3 HIPÓTESE

O tratamento farmacológico com fluoxetina em ratos machos durante o período crítico do desenvolvimento, compromete a capacidade respiratória mitocondrial e induz estresse oxidativo no fígado de ratos adultos.

4 OBJETIVOS

4.1 Objetivo Geral:

Avaliar em ratos machos aos 60 dias de vida, o efeito do tratamento com fluoxetina durante a lactação sobre a bioenergética mitocondrial e estresse oxidativo no fígado.

4.2 Objetivos Específicos:

- Avaliar *in vivo*, aos 21, 40 e 60 dias de vida o peso corporal;

- Avaliar *post mortem*, aos 60 dias de vida:

- O consumo de oxigênio mitocondrial, a produção mitocondrial de espécies reativas e o inchamento e integridade de mitocôndrias de fígado;
- Biomarcadores de estresse oxidativo no fígado;
- Atividade de enzimas antioxidantes no fígado;
- A concentração de glutationa reduzida no fígado.

5 MATERIAL E MÉTODOS

5.1 Animais

Os protocolos para este estudo foram aprovados pelo Comitê de Ética em Pesquisa Animal da Universidade Federal de Pernambuco de acordo com as diretrizes publicadas em "Principles of Laboratory Animal Care" (1 NIH, Bethesda, EUA) e as diretrizes do Canadian Councilon Animal Care (CCAC) (23076.015276/2012-56). Foram utilizados ratos da linhagem *Wistar*, 8 fêmeas e 8 machos provenientes da colônia do Departamento de Nutrição da Universidade Federal de Pernambuco. As fêmeas selecionadas entre 220-250g foram abrigadas em biotério sob condições padrão de temperatura, iluminação e umidade segundo Van Zutphen*et al.* (1993) com água e comida (dieta Labina – Purina S/A) *ad libitum* (VAN ZUTPHEN, 1993). A temperatura e a umidade controlada em 20-24°C e 60 +/- 10%, respectivamente. Foram promovidos períodos alternados e regulares de luz e escuridão (12/12 horas) e um período de adaptação de quinze dias para sincronizar o seu ciclo circadiano. Após a adaptação, as ratas quando em período estral, foram acasaladas na proporção de uma fêmea para um macho. Para isso foi acompanhado a tipagem das células do epitélio vaginal por método de esfregaço vaginal. A possível prenhez foi diagnosticada pela identificação de espermatozóides nas lâminas do esfregaço vaginal (MARCONDES; BIANCHI; TANNO, 2002). As ratas foram mantidas em gaiolas individuais (policarbonato cristal, 49 x 34 x 32) e em condições padrão de biotério durante todo período de gestação. No 1º dia após o nascimento, os filhotes foram selecionados de modo aleatório, no entanto considerando o peso entre 6 e 8 gramas. A ninhada foi formada de oito neonatos até o final da lactação. Os filhotes restantes da ninhada foram eutanasiados. Após o desmame, os filhotes machos foram alocados em gaiolas individuais produzida em policarbonato cristal transparente, autoclavável e resistente a ácidos, nas medidas de 30x20x19. A cama dos animais foi composta de maravalhade madeira de pinho autoclavada.

5.2 Tratamento

- *Farmacológico*

Foi utilizado durante a lactação (1º ao 21º dia de vida) o ISRS, a fluoxetina (Sigma), na concentração de 10 mg/Kg de peso corporal (p.c.), a qual já foi observada aumentar as concentrações plasmáticas da 5-HT e seu metabólito, 5-HTIIA(MILLER et al., 2008). A droga foi obtida na forma de cloridrato de fluoxetina e dissolvida em veículo controle, uma solução de Cloreto de Sódio (NaCl) a 0.9%.

- *Controle*

Foi utilizado 10ml/kg p.c. de solução de NaCl a 0,9%.

5.3 Via de Manipulação

O tratamento foi administrado por via Subcutânea (sc) e o horário de manipulação dos animais foi no início do ciclo escuro (8:00h). O horário de manipulação farmacológica foi mantido durante todo o experimento em concordância com o horário do segundo e maior pico de liberação da serotonina (SANCHEZ et al., 2008).

5.4 Grupos experimentais

No período de lactação foram formados dois grupos experimentais segundo o tratamento:

- **Grupo Controle** (Ct, n=6): os animais foram tratados diariamente com solução salina a 0,9%, 10ml/kg p.c., via subcutânea (s.c), do 1º ao 21º dia pós-natal;
- **Grupo Fluoxetina** (Fx, n=6): os animais foram tratados com fluoxetina na dose de 10mg/kg p.c.,s.c.; do 1º ao 21º dia pós-natal.

5.5 Medidas de peso corporal

O peso corporal dos filhotes foi mensurado diariamente (g) durante o período de lactação e também no 21º, 40º e 60º dias de vida. O peso foi registrado no início do ciclo claro/escuroatravés de balança eletrônica digital (Marte, modelo S-100 com sensibilidade de 0.01g) (DA SILVA; BRAZ; PEDROZA; et al., 2015).

5.6 Coleta e processamento do material biológico para análises bioquímicas

Aos 60 dias de vida, os animais foram decaptados por guilhotina e retirado o fígado para análises posteriores.

O fígado foi homogeneizados em tampão de extração (Tris base 100 mM, pH 7,5; EDTA 10 mM; ortovanadato de sódio 1 mM; PMSF 2 mM). Após a homogeneização, as amostras foram centrifugadas a 1180g, a 4° C, por 10 minutos e o sobrenadante submetido à quantificação de proteína.

5.7 Dosagem de proteína

A concentração de proteína foi determinada pelo método de Bradford (BRADFORD, 1976). O princípio do método baseia-se na determinação da concentração de ligações peptídicas através da medida da absorbância do complexo proteína-corante. Este complexo absorve em comprimento de onda de 595 nm. A absorbância é considerada diretamente proporcional à concentração de proteína na solução analisada, onde uma solução de BSA (2mg/ml) foi utilizada como padrão.

5.8 Isolamento das mitocôndrias hepáticas

As mitocôndrias foram isoladas de fígado de ratos jovens, controles e submetidos à restrição proteica, utilizando a técnica de centrifugação diferencial

(SCHNEIDER; HOGEBOOM, 1951). O fígado, retirado após a eutanasia do animal, foi lavado em solução de sacarose 250 mM contendo tampão 10 mM de HEPES (pH 7,2) e 0,5 mM de EGTA, picado com tesoura e homogeneizado em homogeneizador Potter-Elvehjem. O material foi centrifugado a 461g por 10 minutos. O sobrenadante resultante foi centrifugado durante 10 minutos a 4722g sendo a fase lipídica superior retirada com pipeta Pasteur. O sobrenadante foi descartado e o precipitado ressuspenso em 250 mM de sacarose, 5 mM de HEPES (pH 7,2) e 0,3 mM de EGTA, e novamente centrifugado como na condição anterior. A fração mitocondrial foi ressuspensa na mesma solução, porém isenta de EGTA.

5.9 Condições experimentais

Os experimentos com mitocôndrias isoladas de fígado foram realizados a 28 °C em meio de reação contendo 125 mM sacarose, 10 mM de HEPES (pH 7.2), 65 mM KCl, 2 mM K_2HPO_4, 1 mM $MgCl_2$. Como substrato respiratório foi utilizado substrato para o complexo II (5 mM de succinato e rotenona 2 µM). Foram adicionados aos experimentos de respiração celular: ADP (200 µM), oligomicina (1 µmg/mL) e CCCP (1 µM).

5.10 Medida do consumo de oxigênio mitocondrial

O consumo de oxigênio mitocondrial foi medido polarograficamente utilizando-se um eletrodo do tipo OXIGY conectado a um oxígrafo (HansatechInstrument), em uma câmara de vidro fechada (1 mL) e termostatizada (28 ^oC), equipada com agitador magnético (ROBINSON; COOPER, 1970). Utilizando mitocôndrias isoladas (0,5 mg de proteína/mL). Esse tipo de eletrodo compreende um cátodo de platina e um ânodo de prata, imersos numa solução eletrolítica (geralmente KCl). A superfície do cátodo é revestida por uma fina membrana de teflon ou polietileno, que são permeáveis ao oxigênio. Quando uma pequena voltagem é aplicada entre os eletrodos, a platina torna-se negativa em relação à prata, tornando-se polarizada. O oxigênio é então reduzido a peróxido de hidrogênio na superfície da platina, funcionando como aceptor de elétrons, segundo as reações:

$$O_2 + 2\,H_2O + 2e^- \rightarrow H_2O_2 + 2\,OH^-$$

$$H_2O_2 + 2e^- \rightarrow 2\,OH^-$$

Na superfície do ânodo a prata é oxidada, gerando cloreto de prata, segundo as reações:

$$4\,Ag \rightarrow Ag^+ + 2e^-$$

$$4\,Ag^+ + 4\,Cl^- \rightarrow 4\,AgCl$$

A corrente gerada pela diferença dos eletrodos é relacionada estequiometricamente à concentração de O_2 na superfície do cátodo. Os impulsos elétricos são transmitidos ao oxígrafo, onde foi realizada a leitura.

5.11 Produção mitocondrial de espécies reativas

A produção de espécies reativas de oxigênio e nitrogênio (ERO e ERN) pelas mitocôndrias isoladas foi determinada fluorimetricamente através da oxidação do H_2DCF-DA (diacetato de diclorodihidrofluoresceína, 5μM) (Molecular Probes, Invitrogen, Eugene, Oregon, USA). Utilizando mitocôndrias isoladas (0,5 mg de proteína/mL). A fluorescência foi monitorada ao longo do tempo em um espectrofluorímetro FluostarOmega usando comprimentos de onda de excitação e emissão de 488 e 525 nm, respectivamente, com largura da fenda de 5 nm (LEBEL *et al,* 1992; GARCIA-RUIZ *et al,* 1997). Os resultados foram expressos em unidades de fluorescência (U.F.).

5.12 Avaliação do inchamento e integridade da membrana mitocondrial

O acompanhamento espectrofotométrico da redução da absorbância a 520 nm (VERCESI et al., 1988) foi feito em um espectrofotômetro (SmartSpec Plus – BioRad) utilizando mitocôndrias isoladas (1 mg de proteína/mL). Essa técnica também pode ser utilizada para avaliar o fenômeno de TPM, o qual resulta em inchamento da organela. A confirmação do aumento do volume mitocondrial em decorrência da TPM foi feita através do uso de inibidores do poro de transição de permeabilidade (como ciclosporina A 0,1μM e EGTA).

5. 13 Avaliação da produção de malondealdeído (MDA)

Para avaliação da produção de MDA foi utilizada a técnica colorimétrica de Buege e Aust (BUEGE; AUST, 1978; COSTA et al., 2016a), uma técnica muito utilizada para avaliar a lipoperoxidação, pois o ácido tiobarbitúrico reage com os produtos da lipoperoxidação, entre eles o malondialdeído e outros aldeídos. Colocou-se uma alíquota do homogenizado (0,3 mg de proteína/mL), de ácido tricloroacético a 30% e de ácido tiobarbitúrico a 0.8% (v/v) que reage com os produtos da lipoperoxidação para formar um composto de coloração rosada. A mistura foi incubada por 15 minutos a 100ºC e em seguida resfriada. Na sequência, foi adicionado n-butanol e as amostras agitadas por 30 segundos, com o objetivo de extrair o pigmento formado. O material foi centrifugado a 1180g por 10 minutos, sendo então a fase com o n-butanol utilizada para a leitura da absorbância a 535nm, utilizando cubetas de quartzo. Os resultados foram expressos em nmoles de MDA por mg de proteína.

5. 14 Avaliação da oxidação proteica (Carbonilas)

As espécies de reativas de oxigênio podem induzir a oxidação de resíduos de aminoácidos de proteínas, produzindo assim carbonilas de proteína. O teor de carbonilas de proteína é o marcador mais amplamente utilizado de modificação oxidativa de proteínas. A oxidação das proteínas foi avaliada utilizando os procedimentos realçados por Reznick e Packer (REZNICK; PACKER, 1994). Adicionou-se ácido 2,2,2-tricloroacético (TCA) de 30% (p / v) à amostra (0,3 mg de proteína/mL) sobre gelo e, em seguida, esta mistura foi centrifugada durante 15 min a 1180 G. O sedimento foi suspenso em 2,4-dinitrofenilhidrazina 10 mM (DNPH) e imediatamente incubado num compartimento escuro durante 1 h. Em seguida, as amostras foram centrifugadas e lavadas três vezes com tampão etilo / acetato; posteriormente, o sedimento final suspenso em cloridrato de guanidina 6 M foi incubado durante 5 min num banho de água a 37 ° C e a absorvância foi medida a 370 nm. Os resultados foram expressos em µmol/mg de proteína.

5.15 Avaliação da concentração de sulfidrilas (SH)

A quantificação de SH foi baseada na redução do ácido 5,5'-ditiobis (2-nitrobenzóico) (DTNB) como descrit por Aksenov e Markesberk (AKSENOV; MARKESBERY, 2001). A alíquota do homogeneizado (0,45 mg de proteína) foi incubada no escuro com 30 µl de DTNB 10 mM e o volume final de 1 mL foi completado com tampão de extracção pH 7,4 e a leitura de absorvância foi efectuada num espectrofotómetro a 412 nm (LIBRARY S12 UV/VISIBLE). Os resultados foram expressos como mmol/mg de proteína.

5. 16 Atividade enzimatica: Superóxido dismutase (SOD)

A atividade da superóxido dismutase foi avaliada através do método de auto-oxidação da adrenalina, o qual compete com a SOD podendo ser medido em espectrofotometro a 420nm. Em uma cuteba de quartzo de 1mL, adicionou-se tampão fosfato (pH 10,2), amostra (0,1 mg de proteína) e adrenalina. A absorbância foi registrada por um período de aproximadamente 3 minutos, a 37 ^{o}C. Uma unidade de SOD foi definida como a quantidade de proteína necessária para inibir a autoxidação de 1 µmol de adrenalina por minuto. Os resultados foram expressos em U/mg de proteína.

5.17 Atividade enzimática: Catalase

A atividade da catalase (CAT) é diretamente proporcional a taxa de decomposição do peróxido de hidrogênio, sendo assim, a atividade da enzima pode ser medida através da avaliação do consumo de peróxido, a 30 ^{o}C, pelo decréscimo na absorção a 240 nm ([] máx do H_2O_2) de um meio de reação, contendo tampão fosfato (pH=7,4), amostra (0,08 mg de proteína) e 0,3 M de H_2O_2. Uma unidade de CAT foi definida como a quantidade de proteína necessária para converter 1 µmol H_2O_2 por minuto em H_2O. Os resultados foram expressos em U/mg de proteína (AEBI, 1984).

5.18 Atividade enzimática: Glutationa-S-Transferase

A atividade da glutationaS-transferase é diretamente proporcional a taxa de formação do composto DNP-SG (dinitrofenil S glutationa), podendo desta forma ser medida através do monitoramento da taxa de formação do composto. Em uma cuteba de quartzo de 1mL, adicionou-se 800 uL de tampão, amostra (0,4 mg de proteína), 50 uL de GSH (concentração final 1mM), 50uL de CDNB (concentração final de 1mM). A absorbância foi registrada por um período de aproximadamente 3 minutos com controle da temperatura (30°C). Os resultados foram expressos em U/mg de proteína (HABIG; JAKOBY, 1981).

5.19 Concentração de glutationa Reduzida (GSH)

O GSH é o principal antioxidante não enzimático presente nas células. No seu estado reduzido, o grupo tiol de cisteína é capaz de doar um equivalente de redução a moléculas instáveis tais como espécies reativas de oxigénio, diminuindo assim a toxicidade das moléculas instáveis. Adicionou-se um homogeneizado de 0,3 mg / ml a tampão fosfato 0,1 M (pH 8,0) contendo EDTA 5 mM e incubou-se com o-ftaldialdeído (OPT, 1 mg / ml) à temperatura ambiente durante 15 min. A intensidade de fluorescência foi medida a excitação de 350 nm e comprimentos de onda de emissão de 420 nm e comparada com uma curva GSH padrão conhecida (0,5-10 µM) (HISSIN; HILF, 1976). Os resultados forma expressos em µmol/mg de proteína.

5.20 Análise estatística

Todos os dados foram analisados segundo a normalidade da distribuição e expressos em média e erro padrão da média (EPM). Os resultados foram analisados pelo teste t student não pareado. Foi adotado o nível de significância de 5% em todos os casos. A construção do banco de dados e as análises estatísticas foram desenvolvidas no programa Excel (versão 2007, Microsoft, USA) e GraphPadPrism 6.0 (GraphPad Software Inc., La Jolla, CA, USA), respectivamente.

6 RESULTADOS

6.1 Artigo Original - TREATMENT WITH FLUOXETINE IN NEONATES RATS IMPROVES THE MITOCHONDRIAL RESPIRATORY CAPACITY AND REDUCES OXIDATIVE STRESS IN LIVER OF ADULT RATS

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Treatment with fluoxetine in neonates rats improves the mitochondrial respiratory capacity and reduces oxidative stress in liver of adult rats

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RunningTitle: Effect fluoxetine on the hepatic metabolism of male rats

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ABSTRACT

Recent studies have shown that exposure to fluoxetine treatment induces excessive production of ROS, and alters the antioxidant defense system in various tissues and cell types, mainly the liver. When fluoxetine is administered intraperitoneally, the drug rapidly reaches high concentrations in the liver, has potentially multiple toxic effects on energy metabolism in rat liver mitochondria. The aim of this study was to evaluate the effect of pharmacological treatment with fluoxetine during critical period for development on the mitochondrial bioenergetics and oxidative stress in liver of rat adult. To perform this study, we used rat pups from postnatal day 1 to postnatal day 21 (ie, during lactation period) with Fx or vehicle (control; Ct), and we evaluated mitochondrial oxygen consumption, respiratory control ratio, ROS production, mitochondrial swelling by pore opening, oxidative stress biomarkers, and antioxidant defense in liver of rats at 60 days of age. Our studies have shown, that treatment with Fx during the lactation period resulted in reduced body mass gain, improvement of the mitochondrial respiratory capacity, induced higher mitocondrial resistance to calcium ion preventing the mitochondrial permeability transition pore opening, as well as decreased oxidative stress biomarkers and increased the SH levels and enzymes antioxidant activities (SOD, CAT, GST) in liver of treated rats at 60 days of age. These findings suggest that pharmacological treatment with fluoxetine during critical period of development result in positive changes in liver of rats, as improvement of the mitochondrial bioenergetics and hepatic oxidative metabolism that persist in adulthood.

1 INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs) are a class of antidepressants such as fluoxetine, citalopram, sertraline among others, that are often prescribed to pregnant and lactating women with varying degrees of depression, thus exposing fetuses and infants to drug during critical periods of development (FLESCHLER; PESKIN, 2008). Some authors demonstrate that fluoxetine (Fx) can cross human placenta and its presence in breast milk could induce harmful effects on developing fetuses and newborns (DAVANZO et al., 2011; FRANCIS-OLIVEIRA et al., 2013). Despite the low concentration produced and actingin the brain, serotonin is associated with a variety of functions in central nervous system, since the control of energy to modulation of several behaviors (HALLIDAY; BAKER; HARPER, 1995).

The responsible organ for its activation is the liver, where fluoxetine undergoes to extensive metabolic conversion, leading to the formation of the active metabolite norfluoxetine among multiple other metabolites by cytochrome P450 (ALTAMURA; MORO; PERCUDANI, 1994; A et al., 2003). Due to inhibition of its own metabolism, elimination of fluoxetine and norfluoxetine from the body is extremely slow (CREWE et al., 1992). When fluoxetine is administered intraperitoneally, the drug rapidly reaches high concentrations in the liver. Fluoxetine and norfluoxetine showed potentially toxic effects on energy metabolism in rat liver mitochondria (SOUZA et al., 1994). This seems to be a consequence of the solubilization of the drug and/or its metabolites in the inner mitochondrial membrane. However, the molecular basis of fluoxetine-induced hepatotoxicity (FRIEDENBERG; ROTHSTEIN, 1996; JOHNSTON; WHEELER, 1997; CAI et al., 1999) is not yet well understood.

Mitochondria is the main organelle of cellular energy metabolism, responsible for the vast majority of adenosine-5-triphosphate (ATP) synthesis via oxidative phosphorylation. In recent years, in addition to its known function of generating energy for the cell, mitochondria have emerged as organelles equipped with sophisticated machinery to mediate the Ca^2 flow through the internal mitochondrial membrane, in addition are envolved insignaling pathways, injury and cell death (i.e. apoptosis) (FIGUEIRA et al., 2013; PERNAS; SCORRANO, 2016a). Mitochondrial energy metabolism is recognized as the main source of cellular ROS, such as superoxide anion (O_{2-}) (HALLIWELL; GUTTERIDGE, 1990). The superoxide anion, gives rise to other reactive species of oxygen and nitrogen, by different reactions. The dismutation of O_{2-} (Mn-SOD in the matrix and Cu/Zn-SOD in the intermembrane space), present in mitochondria and cytosol, producing hydrogen peroxide (H_2O_2) (NOHL; GILLE; STANIEK, 2005). H_2O_2 is permeable to membranes and can be converted into oxygen and water by the antioxidant enzymes, catalase (CAT), and glutathione peroxidase (HALLIWELL; GUTTERIDGE, 1990). In physiological conditions, the ROS production plays a primordial second messenger function, regulating the expression of genes sensitive to redox signals and alterations in cellular homeostasis through the synthesis of physiologically active molecules. However, in high concentrations, ROS may be important mediators of damage to the cellular structures of nucleic acids, lipids and proteins (CADENAS; DAVIES, 2000).

The effects of exposure to antidepressant drugs in the redox cellular state remain controversial. Numerous studies have shown that antidepressant drugs induce oxidative stress in various tissues and cell types (MORETTI et al., 2012; DE LONG et al., 2014; SONEI et al., 2016). Chronic treatment with fluoxetine, sertraline

or thioacetamide, selective serotonin reuptake inhibitors (SSRIs), may significantly to increase biomarkers of oxidative stress in the brain and liver (INKIELEWICZ-STEPNIAK, 2011; ABDEL SALAM et al., 2013; ZLATKOVIC et al., 2014). Under chronic stress, fluoxetine alters the antioxidant system and promotes apoptotic signaling in rats *Wistar* (DJORDJEVIC et al., 2011). In contrast, Aksu et al. (2014), studying a potential role for fluoxetine as an antioxidant in the ischemia-reperfusion (IR) kidney model, reported that pre-treatment with fluoxetine significantly restored redox balance and decreased measures of inflammation in kidney(AKSU et al., 2014); furthermore, chronic fluoxetine administration to stressed animals by restraint during 21 days prevented the stress-induced oxidative damage with an efficacy similar to curcuma, used as a standard since it integrates both antioxidant and antidepressant properties, as evidenced by significant enhancement of key antioxidant defense components in brain and liver (ZAFIR; BANU, 2007).

Taken together, the present study aimed to test the hypothesis that pharmacological treatment of male rats with fluoxetine during critical period for development in male rats may be associated with impairment of liver mitochondrial bioenergetics and induce oxidative stress in adulthood. To perform this study, we treated puppies from postnatal day 1 to portnatal day 21 (i.e., during the lactation period) with Fx or vehicle (control; Ct), and we evaluated mitochondrial oxygen consumption, respiratory control, ROS production, mitochondrial permeability transition pore opening, oxidative stress biomarkers, and antioxidant defeses in rats liver at 60 days of age.

2 MATERIAL AND METHODS

2.1 Animals

The animal protocols of this study have been approved by the Ethics Committee for Animal Research at the Federal University of Pernambuco in accordance with the guidelines published in “Principles of Laboratory Animal Care” (1 NIH, Bethesda, USA) and guidelines of the Canadian Council on Animal Care (CCAC) (Ethical Protocol 23076.015276/2012-56). Wistar rats (Rattus norvegicus) were maintained at a room temperature of 23 ± 1 °C in a 12-h alternating light–dark cycle (light 6:00 a.m.–6:00 p.m.). At ninety-days of age, rats were allowed to mate (1 female for 1 male), and six pregnant rats were transferred to individual cages from which at least four male offspring from each litter were selected for use in the present study. No significant difference in litter size among the mothers was observed. Treatment of pups with pharmacologic agents began 24 hours after birth. The dams received commercial chow *ad libitum*. After weaning, the pups received the same diet as their mothers, also *ad libidum*.

2.2 Pharmacological treatment and experimental groups

All male neonates received a subcutaneous injection of either fluoxetine (Fx) (10mg/kg, dissolved in saline solution, 10 ml/kg, bw; Fx group) or vehicle (NaCl 0.9%, 10ml/kg, bw; control-Ct group), once daily from the 1st to the 21st postnatal day (i.e.,during the suckling period) (SILVA et al., 2010; BRAZ; FREITAS; et al., 2016). To avoid a possible influence of circadian rhythm in these studies, injections were always administered between 7:00 a.m. and 8:00 a.m. (SANCHEZ et al., 2008; DA SILVA et al., 2014).

2.3 Body weight measurement

Body weights (in grams) were measured on the 21st postnatal day (weaning), 40 and 60 days after birth using a digital balance (Marte, model S-100 with a 0.001 gsensitivity) (MENDES-DA-SILVA et al., 2002; DA SILVA et al., 2014).

2.4 Biochemical analysis

For biochemical analyses, at 60-day-old rats were decapitated. The liver rapidly dissected and stored at –80 °C for later analysis. For the biochemical experiments, the tissues were homogenized in Tris-EDTA buffer (Tris 100 mM, pH 7.5; EDTA 10 mM, and protease inhibitors (orthovanadate 1mM and PMSF 2mM) on ice, and centrifuged for 10 min at 1180g at 4 °C. Aliquots of the supernatant were analyzed for total protein content using the Bradford protocol. A BSA solution (2mg / mL) was used as standard (BRADFORD, 1976).

2.5Mitochondria isolation

Liver mitochondria were prepared by homogenization followed by differential centrifugation (SCHNEIDER; HOGEBOOM, 1951). After decaptation, tissues were removed immediately and homogenized in a mixturecontaining 125 mM sucrose, 10 mM HEPES (pH 7,2), 65 mM potassium chloride, 2 mM potassium phosphate e 1 mM magnesium chloride. The homogenate was centrifuged at 461g for 10 min at 4°C, the resulting supernatant was carefully removed and centrifuged at 4722g for10 min at 4°C. The supernatant was discarded and the pellet resuspended in 250 mM sucrose, 5 mM HEPES (pH 7.2) and 0.3 mM EGTA, and centrifuged as in previous condition. The pellet containing isolated mitochondria was re-suspended in abuffer containing 250 mM sucrose and 5 mM HEPES (pH7.2). Mitochondrialprotein concentration was determined spectrophotometrically according to Bradford (BRADFORD, 1976).

2.6 Mitochondrial oxygen consumption

Measurement of mitochondrial respiration was performed at 28°C in a 600 SLchamber connected to a Clark-type oxygen electrode (Hansatech Instruments,

PentneyKing's Lynn, UK)asdescribed previously by Robinson and Cooper, 1970. Mitochondria were suspended at a concentration of 0.5 mgprotein/mL in respiration buffer containing contained 125 mM sucrose, 10 mM HEPES (pH 7.2), 65 mM KCL, 2 mM K_2HPO_4, 1 mM $MgCl_2$, 2 µM rotenone, 5 mM succinate and with 0.5 mM EGTA for the assays. Mitochondrial respiration was measured with Complex IIsubstrates. The following were added to the cell respiration experiments: ADP (200 µM), oligomycin (1 µmg/mL) and CCCP (1 µM) (ROBINSON; COOPER, 1970).

2.7 Mitochondrial ROS production

Mitochondrial ROS production in isolated mitochondria was performed at 28°C using a probe (5- (and 6)-chloromethyl-2',7'-dichlorodihydro fluoresce in diacetate, acetyl ester, [H_2DCF-DA]) that becomes fluorescent only after the removal of acetate groups in an oxidizing environment, and measuring emission as an indicator for reactive oxygen species (ROS) production in general. Briefly, mitochondrial suspensions (0.5 mg protein/mL) were incubated in the presence of 5µM H_2DCF-DA and fluorescence was monitored over 5 minutes of gentle shaking using temperature controlled spectrofluorimeter (FLUORstarOMEGA, USA) with excitation and emission wave lengths of 503 and 529 nm, respectively. ROS production was evaluated usingcomplex II substrate (5 mM succinate). Under these conditions, the linear increment in fluorescence in each reaction indicated the rate of ROS formation (LEBEL; ISCHIROPOULOS; BONDY, 1992; GARCIA-RUIZ et al., 1997).The results were expressed in fluorescence units (F.U.).

2.8 Mitochondrial permeability transition pore (MPTP) opening

MPTP was determined as described previously (VERCESI et al., 1988). Opening of the pore induces mitochondrial swelling, which is measured spectrophotometrically as a reduction in absorbance at 520 nm. Isolated

mitochondria were added in swelling buffer that contained (in mmol/l) 0.5 mg protein/mL in respiration buffer containing contained 125 mM sucrose, 10 mM HEPES (pH 7.2), 65 mM KCL, 2 mM K_2HPO_4, 1 mM $MgCl_2$, 2 µM rotenone, 5 mM succinate in the presence or no of 0.5 mM EGTA. The confirmation of mitochondrial volume increase as a consequence of MPTP was performed through the use of 0,1µM cyclosporin A (CsA), a classical inhibitor of the mitochondrial permeability transition pore and 0.5 mM EGTA, a calcium chelator (VERCESI et al., 1988).

2.9 Oxidative stress evaluation in liver

2.9.1 Evaluation of malondialdehyde (MDA) levels

A total of 0.3 mg/mL of tissue homogenate was used to measure MDA levels following reaction with thiobarbituric acid (TBA), at 100° C according to themethod of Draper (DRAPER et al., 1993; COSTA et al., 2016b). In this protocol, MDA or MDA-like substances react to produce a pink pigment with a maximumabsorption at 535 nm. The reaction was developed by the addition to the sample of 30% trichloroacetic acid and Tris-HCl (3 mmol/L) followed by thorough mixing and centrifugation at 1180gfor 10 min. Supernatant was transferred to another tube and 0.8% TBA (v/v) was added before mixing and boiling for 30 min. After cooling, the absorbance of the organic phase was measured at 535 nm in a spectrophotometer. Results were expressed as nmol per mg of protein.

2.9.2 Evaluation of protein oxidation

Reactive oxygen species can induce the oxidation of aminoacid residueson proteins, thus yielding protein carbonyls. The protein carbonylcontent is the most widely used marker of oxidative modification of proteins. The protein oxidation was assessed using the procedures highlighted by Reznick and Packer (REZNICK;

PACKER, 1994). 2,2,2-Trichloroacetic acid (TCA) of 30% (w/v) was added to the sample on ice, and then thismixturewas centrifuged for 15 min at 1180g. The pellet was suspended in 10 mM 2,4-dinitrophenylhydrazine (DNPH) and immediately incubated in a dark room for 1 h with shaking every 15 min. Thereafter, the samples were centrifuged and washed thrice with ethyl/acetate buffer; then, the final pellet suspended in 6 M guanidine hydrochloride was incubated for 5 min in a water bath, at 37 °C and the absorbance was measured at 370 nm. Results were expressed as µmol/mg protein.

2.9.3 Evaluation of sulfhydryls (SH) groups

The quantification of sulfhydryls will be based on the reduction of 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) by thios described by Aksenov e Markesberkv(AKSENOV; MARKESBERY, 2001). The aliquot of the homogenate (200 µg protein) was incubated in the dark with 30 µL of DTNB 10mM and the final volume of 1mL was completed with extraction buffer pH 7.4 and the absorbance reading was made in a spectrophotometer at 412 nm LIBRA S12 UV / VISIBLE . The results were expressed as mol/mg protein.

2.9.4 Superoxide dismutase (SOD) assay

The determination of total superoxide dismutase enzyme activity (t-SOD) was performed according to the method of Misra and Fridovich (MISRA; FRIDOVICH, 1972). Supernatants (0.3 mg/mL) collected from homogenized liver following centrifugation were incubated with 0.880 mL of sodium carbonate (0.05%, pH 10.2, 0.1 mmol/L EDTA) at 37° C. Thirty millimoles per liter of epinephrine (in 0.05% acetic acid) was added and SOD activityat 37ºC was measured by the kinetics of inhibition of 1 epinephrine auto oxidation at 480 nm (MISRA; FRIDOVICH, 1972). One unit of

SOD was defined as the amount of protein required to inhibit the autoxidation of 1 µmol de epinephrine per minute. The results were expressed in U/mg protein.

2.9.5 Catalase (CAT) assay

A total of 0.3 mg/mL of tissue homogenate was used to measure CAT activity according to the method described by Aebi (AEBI, 1984). The principle of the assay is based on the determination of the rate constant (k) of H_2O_2 decomposition, which in our conditions of temperature and pH was defined as 4.6×10^7. The rate constant of the enzyme was determined by measuring the change in absorbance (at 240 nm) per minute over a 4-min period at 30ºC (AEBI, 1984). One unit of CAT was defined as the amount of protein required to convert 1 µmol de H2O2 per minute to H2O. The results were expressed in U/mg protein.

2.9.6 Glutathione S-Transferase (GST) assay

A total of 0.3 mg/mL of liver homogenate was used to measure GST activity according to the method of Habig et al. by determination of absorbance at 340 nm after addition of 1 mmol/L of 1-chloro-2,4-dinitrobenzene (CDNB) (HABIG et al., 1974). GST activity was calculated using a 2,4-dinitrophenyl-S-glutathione (DNP-SG) substrate. GST activity was expressed as U/mg protein. Based on its molecular absorbance, 1 enzymatic unit was defined as the amount of protein required to the form of 1 µmol/L DNP-SG per minute (HABIG et al., 1974).

2.9.7 Reduced Glutathione (GSH) levels

GSH is the major non-enzymatic antioxidant present inmammalian cells; in its reduced state, the thiol group of cysteine is able to donate a reducing equivalent to unstable molecules such as reactive oxygen species, thereby decreasing the toxicity of the unstable molecules. A homogenate of 0.3 mg/mL was added to 0.1 M

phosphate buffer (pH 8.0) containing 5 mM EDTA and incubated with o-phthaldialdehyde (OPT, 1 mg/ml) at room temperature for 15 min. Fluorescence intensity was measured at 350 nm excitation and 420 nm emission wavelengths and compared with a known standard GSH curve (0.5–10 μM) (HISSIN; HILF, 1976). Results were expressed as μmol/mg protein.

2.10 Statistical analysis

All results are expressed as mean ± SEM. A student's t-test was performed to assess significant differences between the two groups. The data were considered to be statistically significant when $p \leq 0.05$. The statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software Inc., La Jolla, CA, USA).

3 RESULTS

3.1 Effects of fluoxetine treatment on body weight

Fx treatment during lactation resulted in a small, but significant decrease in body weight which maintained until at least 60 days old (21 days of age Ct: 45.50± 1.5; Fx: 30.20 ± 0.80 g; ***p<0.0001; 40 days of age Ct: 147.8 ± 4.27; Fx: 116.4 ± 1.50 g; ***p<0.0001; 60 days of age Ct: 179.0 ± 7.40; Fx: 150.6 ± 5.41 g; *p<0.05) (Fig.1).

3.2 Effects of fluoxetine treatment on liver mitochondrial bioenergetics

To assess mitochondrial function in liver after fluoxetine treatment, we evaluated the mitochondrial oxygen consumption in Fx-treated and Ct groups and observed that liver mitochondria from the Fx group had a significantly higher coupling state under many conditions: basal (Ct: 5.73 ± 0.66; Fx: 8.69 ± 1.06 nmolO_2/min/mg prot.; *p<0.05), ADP-stimulated phosphorylation (Ct:18.25 ± 2.32; Fx: 38.94 ± 0.63 nmolO_2/min/mg prot.; ***p<0.001), resting (Ct:3.80 ± 0.48; Fx: 7.06 ± 0.42

nmolO_2/min/mg prot.; **p<0.01) and after uncoupling agent, CCCP (Ct: 21.77 ± 3.75; Fx: 35.36 ± 2.25 nmolO_2/min/mg prot.; *p<0.05) (Fig. 2A). However, Fx did not induce a significant difference in the respiratory control ratio (Ct: 5.00 ± 0.82; Fx: 5.54 ± 0.25; p=0.64) (Fig. 2B).

In addition, we observed that mitochondrial ROS production no showed significant difference between the Fx-treated and control groups (Ct: 391.4 ± 68.97; Fx: 402.8 ± 76.14 F.U.; p= 0.91) (Fig. 3). Consistent with the above results, on the assessment of mitochondrial swelling, visualized through decay of absorbance at 520 nm, liver mitochondria from the Fx-treated group are more resistant mitochondrial pore opening than the Ct group (Ct: 0.697 ± 0.07; Fx: 0.914 ± 0.02; *p<0.05); in the presence of the classical transition pore inhibitor of mitochondrial permeability, cyclosporine A, this resistance is potentiated (Fx: 0.914 ± 0.02; Fx + CsA: 1.164 ± 0.04; ***p<0.001), the same happens, in the presence of EGTA, calcium chelator (Fx: 0.914 ± 0.02; Fx + EGTA: 1.202 ± 0.03; ***p<0.001) (Figure 4).

3.3Effects of fluoxetine treatment on oxidative stress biomarkers

Oxidative stress biomarkers were analised in liver, evaluating MDA levels, carbonyl content and SH groups. The MDA levels showed decrease in Fx-treated group (C: 4.05 ± 0.84; Fx: 1.00 ± 0.18 nmol/mg de prot.; *p<0.05 Fig. 5A). The carbonyl contents, did not presente a significant difference between the Fx-treated and control group (C: 23.71 ± 2.57; Fx: 27.67 ± 3.09 µmol/mg de prot.; p=0.346; Fig. 5B). However, it was observed an increase in SH Fx-treated group (C: 58.96 ± 2.09; Fx: 67.55 ± 2.68 mol/mg de prot.; *p<0.05; Fig. 5C).

3.4 Effects of fluoxetine treatment in antioxidant defenses

The decrease observed in MDA levels could be explained, in part, due to the increase of SOD, CAT and GST activities observed in liver of Fx-treated rats (SOD = Ct: 134.7± 29.58.; Fx: 290.2 ± 50.12 U/mg de prot.; *p<0.05; CAT = Ct: 8.40 ± 2.10; Fx: 16.34 ± 0.98 U/mg de prot.; **p<0.01; GST = Ct: 27.45 ± 0.93; Fx: 31.29 ± 1.35 µmol/mg de prot.; *p<0.05; Fig. 6A–C). In addition to the evaluation of antioxidant enzymatic system, we also measured as non-enzymatic defense, the reduced glutathione (GSH) levels; however, we did not observe difference in liver (Ct: 111.3 ± 7.69; Fx: 115.0 ± 11.29 µmol/mg de prot.; p=0.78; Fig. 7).

2 DISCUSSION

Fluoxetine is the drug of choice for the treatment of depression because of its safer profile, fewer side effects and greater tolerability (WILDE; BENFIELD, 1998). In the present study, we investigated the hypothesis that o pharmacological treatment of male rats with fluoxetine during critical periods of development may be associated with impairment of the liver mitochondrial capacity and induce oxidative stress in adulthood. However, our studies have shown, on the contrary, that treatment with Fx during the lactation period reduced body mass gain and improvement of the mitochondrial respiratory capacity.

The significant difference in the body weights of control and Fx-treated rats in the present study corroborates with previous studies conducted in adult animals showing that fluoxetine treatment results in decreased body weight, an effect apparently mediated by fluoxetine’s impact on the serotonin (5-HT) signaling pathways (BLUNDELL; LATHAM, 1979; MCGUIRK; MUSCAT; WILLNER, 1992; LEIBOWITZ; ALEXANDER, 1998; DA SILVA et al., 2014). The pharmacological treatment with antidepressant fenfluramine (reuptake inhibitor and 5—HT release stimulator) increased proopiomelanocortin (POMC) expression (HEISLER et al.,

2002). The neuropeptide POMC is synthesized in hypothalamic nuclei and emits preganglionic neuron projections in the mediolateral spinal cord; they communicate with skeletal muscle by sympathetic postganglionic fibers (CECHETTO; SAPER, 1988; BROBERGER, 2005), which may activate also UCP in skeletal muscle; changes in skeletal muscle energy metabolism can occur resulting in increased energy expenditure and decreased body weight (ANGIOLINI et al., 2006). Corroborating with our hypothesis, previous studies of our laboratory showed that the pharmacological treatment with Fx, asselective serotonin reuptake inhibitor resulted in positive modulation of UCP and mitochondrial bioenergetics in brown fat tissue (DA SILVA; BRAZ; PEDROZA; et al., 2015).

In regard to mitochondrial permeability transition pore opening, we observed that fluoxetine-treated animals are more resistant to pore opening, as well as decreases oxidative stress biomarkers, and increases antioxidant defense (SOD, CAT, GST activity and SH levels) in liver of treated rats at 60 days of age. These findings suggest that pharmacological treatment with fluoxetine during critical periods of development can change mitochondrial bioenergetics and result on persistent changes in liver energy metabolism lasting into later life.

Mammalian cells from different tissues, including the liver, have a system that regulates the redox state of cellular thiols and protects proteins containing sulfhydryl groups (SH) of excessive oxidation. Proteins extrate containing SH in amino acid residues are susceptible to a variety of oxidative damages. It includes low molecular weight donors of SH groups and enzymes, which can catalyze the reduction of SH groups in proteins and deoxidation of prooxidants by conjugation (AKSENOV; MARKESBERY, 2001). Antioxidant enzymes are part this complex cellular defense system, superoxide dismutase (SOD1 and SOD2), reduce O_2^- to H_2O_2, is the first

antioxidant defense located in the cytoplasm and mitochondrial matrix. H_2O_2 is neutralized by the action of catalase by converting it to water (H_2O) (HALLIWELL; GUTTERIDGE, 1990). GSH, a non-protein thiol with a wide range of antioxidant properties, can eliminate O_2^- and OH^- radical non-enzymatically, regenerate other antioxidants to its active form, and can be conjugated and excreted with toxins through the reaction catalyzed by glutathione S- Transferases (GST) (GARCIA et al., 2010, FERREIRA et al., 2015). The GST is a family enzymes found in the cytosol of most cells whit importance not only in detoxification of metabolites but also on regulation of oxidative stress (MODEN; MANNERVIK, 2014). GST activity is an accurate index of early stage liver damage in rats (ADACHI et al., 1981).

Studies have related important role of fluoxetine in anti-inflammatory mechanisms, cell survival and neuronal trophic (anti-apoptotic properties), as well as its role on enzymes of the antioxidant system. Zhang et al. have discovered a neuroprotective function of this drug against microglial activation due to neurotoxicity in neurons (ZHANG et al., 2012). Using rats submitted to carrageenan, Abdel-Salam et al. also demonstrated the anti-inflammatory action of fluoxetine and found a response similar to that of standard drugs used to treat inflammatory processes (ABDEL-SALAM; BAIUOMY; ARBID, 2004). Novio et al. demonstrated a positive effect of fluoxetine against stress induced by oxidative cellular injury, with protective augmentation of endogenous antioxidant defenses (superoxide dismutase, diaphorase and catalase) and restoration of non-enzymatic components of the antioxidant cascade (glutathione) (NOVIO et al., 2011). Zafir and Banu also demonstrated the antioxidant potential of this drug, startingsimultaneous elevation of key endogenous antioxidants, SOD, CAT, GST, glutathione reductase (GR) and GSH levels by fluoxetine treatment may thus largely contribute to the increased

resistance of chronically stressed rats to *in vivo* oxidative damage, in comparison to stressed animals without treatment (ZAFIR; BANU, 2007). By restoring the activity of glutathione reductase, fluoxetine may act to increase cellular levels of GSH, the predominant thiol antioxidant in the brain, which is controlled in part by glutathione reductase. Kolla et al. demonstrated higher survival and reduction in oxidative substances such hydrogen peroxide (H_2O_2) in neurons (KOLLA et al., 2005).

Agostinho et al. evaluated the effects of fluoxetine and olanzapine on mitochondrial respiratory chains and found that the two drugs, either alone or in conjunction, alter the activity of these chains in the brain of rats (AGOSTINHO; REUS; STRINGARI; RIBEIRO; FERREIRA; et al., 2011). Moreover, acute treatment with fluoxetine alters the activity of the enzyme citrate synthase and both; acute and chronic treatments modify the activity of the enzyme creatine kinase (AGOSTINHO et al., 2009; AGOSTINHO; REUS; STRINGARI; RIBEIRO; FERRARO; et al., 2011). These enzymes are involved in cell metabolism and the relationship between fluoxetine and energy metabolism has been clearly demonstrated, which is correlated with neuropsychiatric disorders (BEN-SHACHAR; KARRY, 2008).

In contrast, previous study showed that high doses (160–320 mol/L) of Fx interfere in energy metabolism in rat liver mitochondria (SOUZA et al., 1994). Additionally, high doses (50–250 mol/L), showed that Fx induces inhibition of oxphox capacity and decreases the activity of ATP synthase in rat brain mitochondria (CURTI et al., 1999). When this, Fx inhibited mitochondria respiration capacity at doses higher than 86 mol/L with complex I substrate and higher than 266 mol/L with complex II substrate (HROUDOVA; FISAR, 2012). It is important to highlight that this discrepancy in the findings could be explained for differences in the drug concentration or by difference in the age at which the treatment was conducted. In

our model, we performed the treatment during period critical development, while in the previous studys; animals were treated when they reached adulthood.

In our *in vivo* experimental model we observed that fluoxetine during the nursing period increased mitochondrial respiratory activity throughout several mitochondrial respiration stages, in regard to mitochondrial permeability transition pore opening, we observed that fluoxetine-treated animals are mare resistant to pore opening in liver of adult rats. A number of prior studies have shown that increased oxygen consumption and electron transport chain (ETC) activity prevent mitochondrial ROS production in several different tissues (KORSHUNOV; SKULACHEV; STARKOV, 1997; SKULACHEV, 1998; SANGLE et al., 2010; DA SILVA; BRAZ; PEDROZA; et al., 2015; DA SILVA; BRAZ; SILVA-FILHO; et al., 2015). Some studies have suggested that the mechanism involved in decreasing ROS production is related to the prevention of anion superoxide (O_2^-) formation through a decrease oxygen tension in the mitochondrial milieu (SKULACHEV, 1998; MURPHY, 2009b).

Another possible mechanism involves the capacity of the ETC to maintain NADH at lower levels, which prevents ROS formation by mitochondrial matrix flavoenzymes (STARKOV et al., 2004; TRETTER; ADAM-VIZI, 2004). A different possibility is that increased electron transport rates are often accompanied by lower mitochondrial membrane potential (ΔΨm), a condition that thermodynamically disfavors the reverse flow of electrons from Complex II to Complex I, thereby decreasing electron leak and O_2^- formation (TURRENS, 2003). Previous studies have shown that inhibition of oxidative phosphorylation causes a reversal of electron transport via the ETC, resulting in increased ROS production, increased oxidative stress, a decline in energy production and an induction of mitochondrial permeability

transition pore (MPTP) opening (KOWALTOWSKI; CASTILHO; VERCESI, 2001; RASHEED; TABASSUM; PARVEZ, 2016). Mitochondrial permeability transition (MPT) represents an abrupt increase in the permeability of the inner mitochondrial membrane to low molecular weight molecules due to the opening of the MPTP, with subsequent osmotic changes leading to mitochondrial swelling and cell death mediated by necrosis or apoptosis (HALESTRAP; PASDOIS, 2009; KOWALTOWSKI et al., 2009; CIRCU; AW, 2010). Our present observations, however, suggest that in our model Fx would not induce MPTP, since we observed the opposite effect of increased mitochondrial respiration and decreased ROS production with Fx treatment. In our evaluation of MPTP opening, we indeed did observe that with fluoxetine treatment mitochondria are more resistant to pore opening in liver, suggesting that fluoxetine does not impair mitochondrial bioenergetics.

The mitochondrial oxygen consumption significantly high in the Fx group suggest that the mitochondria progressively increase the rate of proton leak, which partially dissipates the mitochondrial membrane potential, suggesting the action of an uncoupling agent. Uncoupling protein-2 (UCP2), modulates the coupling between substrates oxidation and ATP synthesis, acting as mitochondrial proton carrier (SKULACHEV, 1991; BOSS; MUZZIN; GIACOBINO, 1998). It has been proposed that nonphosphorylating (uncoupled or noncoupled) mitochondrial respiration allows the maintenance of low levels of both O_2 and ROS when phosphorylating respiration fails to do so due to a lack of ADP. An increase state 4 respiration in isolated mitochondria, which serves as an indicator of inner membrane proton leak would thus stimulate O_2 consumption and decrease the formation of ROS(VIDAL-PUIG et al., 2000). This hypothesis is based upon the observation that mitochondrial membrane potential regulates the production of reactive oxygen species (ROS)

(BRAND et al., 2002). According to this hypothesis, mild mitochondrial uncoupling could markedly decrease superoxide production by decreasing the mitochondrial membrane potential below a critical level. An increase in mitochondrial membrane potential slows electron transport through the respiratory chain, resulting in an increase in the ubiquinone free-radical half-life. As a result, electrons have an increased probability of interacting with oxygen to form ROS. Thus, mild uncoupling of the mitochondria could be a mechanism to prevent the formation of oxygen free radicals (FANG et al., 2013).

This corroborates with previous studies that demonstrated that Fx during developmental age increases the antioxidant defense and metabolic enzymes activity in brainstem and heart in adult female rats (BRAZ; PEDROZA; et al., 2016); increased mitochondrial respiratory capacity, mitochondrial membrane potential, decreased ROS production and increased the antioxidant capacity in the cardiac tissue from male rats (BRAZ; FREITAS; et al., 2016). Positive modulation of the mitochondrial respiration was also observed in the hypothalamus and skeletal muscle persisting into adulthood, that may to contribute to permanent changes in energy balance in the Fx treated from male rats (DA SILVA; BRAZ; PEDROZA; et al., 2015). It also results in significant decrease in anxiety, reduction of lipid peroxidation and increase in catalase and glutathione-S-transferase activities on the hippocampus of female rats (DA SILVA et al., 2014).

Taking into account the latest available evidence, we believe that the potentially favourable antioxidant effect of the fluoxetine could be mediated by the four previously commented mechanisms. First, it has been suggested that *in vitro* neuroprotective actions of some antidepressants include the upregulation of superoxide dismutase activity, with superoxide dismutase1 gene expression as a

potential target of antidepressant regulation (LI et al., 2000; KOLLA et al., 2005). Secondly, monoamines inhibit lipid peroxidation, eliminate free radicals and chelate iron ions, which are important elements of free radical reactions. It has been noted that fluoxetine restores not only normal metabolism of monoamines but also their physiological levels in synaptic clefts. Considering the reactive oxygen species–scavenging potential of monoamines, this effect of fluoxetine imposes a limitation on free radical reactions and concentration of their products (LIU; MORI, 1993). Thirdly, increased glutaminergic transmission is characteristic of depression (MULLER; SCHWARZ, 2007). Pathologically high levels of glutamate can cause excitotoxicity by allowing high levels of calcium ions to enter the cell, which, if present in excess, stimulate the production of reactive oxygen species. Fluoxetine has a cytoprotective effect involving limitation of overproduction of calcium ions (LI et al., 2003). Fourthly, fluoxetine is capable of reducing the immune and inflammatory components (YARON et al., 1999; MAES, 2001; STRUMPER et al., 2003) that favour the generation of reactive oxygen species (WINTERBOURN, 2002; GALECKI et al., 2009). This antidepressant drug has been shown to inhibit the expression of pro-inflammatory cytokines (e.g. tumour necrosis factor-alpha) (MAES, 2001) and prostaglandin E2 (YARON et al., 1999) that are involved in enhancing reactive oxygen species (GALECKI et al., 2009). Its inhibitory effects have been suggested to be mediated, in part, by the protein kinase A (MAES, 2001). Additionally, the reduction in neutrophil counts by fluoxetine (STRUMPER et al., 2003) limits the production of hypochlorus acid, which by reacting with reduced glutathione, decreases the amount of its form (WINTERBOURN, 2002).

Taking our current data together with the literature, the hypothesis that pharmacological treatment with fluoxetine during critical periods of development may

alter mitochondrial bioenergetics and result in persistent changes in liver energy metabolism lasting later in life.

3 CONCLUSION

Our results suggest that chronic treatment with fluoxetine during critical periods of development could help to decrease the incidence of metabolic diseases in liver, in part by improving mitochondrial function and reducing the hepatic oxidative stress in adulthood.

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5 REFERENCES

A, L.L., Dorado, P., Berecz, R., Gonzalez, A., Jesus Norberto, M., de la Rubia, A., Caceres, M., 2003. Determination of fluoxetine and norfluoxetine in human plasma by high-performance liquid chromatography with ultraviolet detection in psychiatric patients. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences 783, 25-31.

Abdel-Salam, O.M., Baiuomy, A.R., Arbid, M.S., 2004. Studies on the anti-inflammatory effect of fluoxetine in the rat. Pharmacological research 49, 119-131.

Abdel Salam, O.M., Mohammed, N.A., Sleem, A.A., Farrag, A.R., 2013. The effect of antidepressant drugs on thioacetamide-induced oxidative stress. European review for medical and pharmacological sciences 17, 735-744.

Adachi, Y., Horii, K., Suwa, M., Tanihata, M., Ohba, Y., Yamamoto, T., 1981. Serum glutathione S-transferase in experimental liver damage in rats. Gastroenterologia Japonica 16, 129-133.

Aebi, H., 1984. Catalase in vitro. Methods in enzymology 105, 121-126.

Agostinho, F.R., Reus, G.Z., Stringari, R.B., Ribeiro, K.F., Ferraro, A.K., Benedet, J., Rochi, N., Scaini, G., Streck, E.L., Quevedo, J., 2011a. Treatment with olanzapine, fluoxetine and olanzapine/fluoxetine alters citrate synthase activity in rat brain. Neuroscience letters 487, 278-281.

Agostinho, F.R., Reus, G.Z., Stringari, R.B., Ribeiro, K.F., Ferreira, G.K., Jeremias, I.C., Scaini, G., Rezin, G.T., Streck, E.L., Quevedo, J., 2011b. Olanzapine plus fluoxetine treatment alters mitochondrial respiratory chain activity in the rat brain. Acta neuropsychiatrica 23, 282-291.

Agostinho, F.R., Scaini, G., Ferreira, G.K., Jeremias, I.C., Reus, G.Z., Rezin, G.T., Castro, A.A., Zugno, A.I., Quevedo, J., Streck, E.L., 2009. Effects of olanzapine, fluoxetine and olanzapine/fluoxetine on creatine kinase activity in rat brain. Brain research bulletin 80, 337-340.

Aksenov, M.Y., Markesbery, W.R., 2001. Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. Neuroscience letters 302, 141-145.

Aksu, U., Guner, I., Yaman, O.M., Erman, H., Uzun, D., Sengezer-Inceli, M., Sahin, A., Yelmen, N., Gelisgen, R., Uzun, H., Sahin, G., 2014. Fluoxetine ameliorates imbalance of redox homeostasis and inflammation in an acute kidney injury model. Journal of physiology and biochemistry 70, 925-934.

Altamura, A.C., Moro, A.R., Percudani, M., 1994. Clinical pharmacokinetics of fluoxetine. Clinical pharmacokinetics 26, 201-214.

Ben-Shachar, D., Karry, R., 2008. Neuroanatomical pattern of mitochondrial complex I pathology varies between schizophrenia, bipolar disorder and major depression. PloS one 3, e3676.

Boss, O., Muzzin, P., Giacobino, J.P., 1998. The uncoupling proteins, a review. European journal of endocrinology 139, 1-9.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry 72, 248-254.

Brand, M.D., Pamplona, R., Portero-Otin, M., Requena, J.R., Roebuck, S.J., Buckingham, J.A., Clapham, J.C., Cadenas, S., 2002. Oxidative damage and phospholipid fatty acyl composition in skeletal muscle mitochondria from mice underexpressing or overexpressing uncoupling protein 3. The Biochemical journal 368, 597-603.

Braz, G.R., Freitas, C.M., Nascimento, L., Pedroza, A.A., da Silva, A.I., Lagranha, C., 2016a. Neonatal SSRI exposure improves mitochondrial function and antioxidant defense in rat heart. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme 41, 362-369.

Braz, G.R., Pedroza, A.A., Nogueira, V.O., de Vasconcelos Barros, M.A., de Moura Freitas, C., de Brito Alves, J.L., da Silva, A.I., Costa-Silva, J.H., Lagranha, C.J., 2016b. Serotonin modulation in neonatal age does not impair cardiovascular physiology in adult female rats: Hemodynamics and oxidative stress analysis. Life sciences 145, 42-50.

Cadenas, E., Davies, K.J., 2000. Mitochondrial free radical generation, oxidative stress, and aging. Free radical biology & medicine 29, 222-230.

Cai, Q., Benson, M.A., Talbot, T.J., Devadas, G., Swanson, H.J., Olson, J.L., Kirchner, J.P., 1999. Acute hepatitis due to fluoxetine therapy. Mayo Clinic proceedings 74, 692-694.

Circu, M.L., Aw, T.Y., 2010. Reactive oxygen species, cellular redox systems, and apoptosis. Free radical biology & medicine 48, 749-762.

Costa, L.R., Macedo, P.C., de Melo, J.S., Freitas, C.M., Alves, A.S., Barbosa, H.M., Lira, E., Fernandes, M.P., Batista-de-Oliveira-Hornsby, M., Lagranha, C., 2016. Safflower (Catharmus tinctorius L.) oil supplementation in overnourished rats during early neonatal development: effects on heart and liver function in the adult. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme 41, 1271-1277.

Crewe, H.K., Lennard, M.S., Tucker, G.T., Woods, F.R., Haddock, R.E., 1992. The effect of selective serotonin re-uptake inhibitors on cytochrome P4502D6 (CYP2D6) activity in human liver microsomes. British journal of clinical pharmacology 34, 262-265.

Curti, C., Mingatto, F.E., Polizello, A.C., Galastri, L.O., Uyemura, S.A., Santos, A.C., 1999. Fluoxetine interacts with the lipid bilayer of the inner membrane in isolated rat brain mitochondria, inhibiting electron transport and F1F0-ATPase activity. Molecular and cellular biochemistry 199, 103-109.

da Silva, A.I., Braz, G.R., Pedroza, A.A., Nascimento, L., Freitas, C.M., Ferreira, D.J., Manhaes de Castro, R., Lagranha, C.J., 2015a. Fluoxetine induces lean phenotype in rat by increasing the brown/white adipose tissue ratio and UCP1 expression. Journal of bioenergetics and biomembranes 47, 309-318.

da Silva, A.I., Braz, G.R., Silva-Filho, R., Pedroza, A.A., Ferreira, D.S., Manhaes de Castro, R., Lagranha, C., 2015b. Effect of fluoxetine treatment on mitochondrial bioenergetics in central and peripheral rat tissues. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme 40, 565-574.

da Silva, A.I., Monteiro Galindo, L.C., Nascimento, L., Moura Freitas, C., Manhaes-de-Castro, R., Lagranha, C.J., Lopes de Souza, S., 2014. Fluoxetine treatment of rat neonates significantly reduces oxidative stress in the hippocampus and in behavioral indicators of anxiety later in postnatal life. Canadian journal of physiology and pharmacology 92, 330-337.

Davanzo, R., Copertino, M., De Cunto, A., Minen, F., Amaddeo, A., 2011. Antidepressant drugs and breastfeeding: a review of the literature. Breastfeeding medicine : the official journal of the Academy of Breastfeeding Medicine 6, 89-98.

De Long, N.E., Hyslop, J.R., Raha, S., Hardy, D.B., Holloway, A.C., 2014. Fluoxetine-induced pancreatic beta cell dysfunction: New insight into the benefits of folic acid in the treatment of depression. Journal of affective disorders 166, 6-13.

Djordjevic, J., Djordjevic, A., Adzic, M., Elakovic, I., Matic, G., Radojcic, M.B., 2011. Fluoxetine affects antioxidant system and promotes apoptotic signaling in Wistar rat liver. European journal of pharmacology 659, 61-66.

Draper, H.H., Squires, E.J., Mahmoodi, H., Wu, J., Agarwal, S., Hadley, M., 1993. A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. Free radical biology & medicine 15, 353-363.

Fang, X.L., Shu, G., Yu, J.J., Wang, L.N., Yang, J., Zeng, Q.J., Cheng, X., Zhang, Z.Q., Wang, S.B., Gao, P., Zhu, X.T., Xi, Q.Y., Zhang, Y.L., Jiang, Q.Y., 2013. The anorexigenic effect of serotonin is mediated by the generation of NADPH oxidase-dependent ROS. PloS one 8, e53142.

Figueira, T.R., Barros, M.H., Camargo, A.A., Castilho, R.F., Ferreira, J.C., Kowaltowski, A.J., Sluse, F.E., Souza-Pinto, N.C., Vercesi, A.E., 2013. Mitochondria as a source of reactive oxygen and nitrogen species: from molecular mechanisms to human health. Antioxidants & redox signaling 18, 2029-2074.

Fleschler, R., Peskin, M.F., 2008. Selective serotonin reuptake inhibitors (SSRIs) in pregnancy: a review. MCN. The American journal of maternal child nursing 33, 355-361; quiz 362-353.

Francis-Oliveira, J., Ponte, B., Barbosa, A.P., Verissimo, L.F., Gomes, M.V., Pelosi, G.G., Britto, L.R., Moreira, E.G., 2013. Fluoxetine exposure during pregnancy and lactation: Effects on acute stress response and behavior in the novelty-suppressed feeding are age and gender-dependent in rats. Behavioural brain research 252, 195-203.

Friedenberg, F.K., Rothstein, K.D., 1996. Hepatitis secondary to fluoxetine treatment. The American journal of psychiatry 153, 580.

Galecki, P., Szemraj, J., Bienkiewicz, M., Florkowski, A., Galecka, E., 2009. Lipid peroxidation and antioxidant protection in patients during acute depressive episodes and in remission after fluoxetine treatment. Pharmacological reports : PR 61, 436-447.

Garcia-Ruiz, C., Colell, A., Mari, M., Morales, A., Fernandez-Checa, J.C., 1997. Direct effect of ceramide on the mitochondrial electron transport chain leads to generation of reactive oxygen species. Role of mitochondrial glutathione. The Journal of biological chemistry 272, 11369-11377.

Habig, W.H., Pabst, M.J., Fleischner, G., Gatmaitan, Z., Arias, I.M., Jakoby, W.B., 1974. The identity of glutathione S-transferase B with ligandin, a major binding protein of liver. Proceedings of the National Academy of Sciences of the United States of America 71, 3879-3882.

Halestrap, A.P., Pasdois, P., 2009. The role of the mitochondrial permeability transition pore in heart disease. Biochimica et biophysica acta 11, 8.

Halliday, G., Baker, K., Harper, C., 1995. Serotonin and alcohol-related brain damage. Metabolic brain disease 10, 25-30.

Halliwell, B., Gutteridge, J.M., 1990. The antioxidants of human extracellular fluids. Archives of biochemistry and biophysics 280, 1-8.

Hissin, P.J., Hilf, R., 1976. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Analytical biochemistry 74, 214-226.

Hroudova, J., Fisar, Z., 2012. In vitro inhibition of mitochondrial respiratory rate by antidepressants. Toxicology letters 213, 345-352.

Inkielewicz-Stepniak, I., 2011. Impact of fluoxetine on liver damage in rats. Pharmacological reports : PR 63, 441-447.

Johnston, D.E., Wheeler, D.E., 1997. Chronic hepatitis related to use of fluoxetine. The American journal of gastroenterology 92, 1225-1226.

Kolla, N., Wei, Z., Richardson, J.S., Li, X.M., 2005. Amitriptyline and fluoxetine protect PC12 cells from cell death induced by hydrogen peroxide. Journal of psychiatry & neuroscience : JPN 30, 196-201.

Korshunov, S.S., Skulachev, V.P., Starkov, A.A., 1997. High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. FEBS letters 416, 15-18.

Kowaltowski, A.J., Castilho, R.F., Vercesi, A.E., 2001. Mitochondrial permeability transition and oxidative stress. FEBS letters 495, 12-15.

Kowaltowski, A.J., de Souza-Pinto, N.C., Castilho, R.F., Vercesi, A.E., 2009. Mitochondria and reactive oxygen species. Free radical biology & medicine 47, 333-343.

LeBel, C.P., Ischiropoulos, H., Bondy, S.C., 1992. Evaluation of the probe 2',7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chemical research in toxicology 5, 227-231.

Li, X.M., Chlan-Fourney, J., Juorio, A.V., Bennett, V.L., Shrikhande, S., Bowen, R.C., 2000. Antidepressants upregulate messenger RNA levels of the neuroprotective enzyme superoxide dismutase (SOD1). Journal of psychiatry & neuroscience : JPN 25, 43-47.

Li, Y.F., Liu, Y.Q., Huang, W.C., Luo, Z.P., 2003. Cytoprotective effect is one of common action pathways for antidepressants. Acta Pharmacol Sin 24, 996-1000.

Liu, J., Mori, A., 1993. Monoamine metabolism provides an antioxidant defense in the brain against oxidant- and free radical-induced damage. Archives of biochemistry and biophysics 302, 118-127.

Maes, M., 2001. The immunoregulatory effects of antidepressants. Human psychopharmacology 16, 95-103.

Mendes-da-Silva, C., de Souza, S.L., Barreto-Medeiros, J.M., de Freitas-Silva, S.R., Antunes, D.E., Cunha, A.D., Ribas, V.R., de Franca, M.F., Nogueira, M.I., Manhaes-de-Castro, R., 2002. Neonatal treatment with fluoxetine reduces depressive behavior induced by forced swim in adult rats. Arquivos de neuro-psiquiatria 60, 928-931.

Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. The Journal of biological chemistry 247, 3170-3175.

Moden, O., Mannervik, B., 2014. Glutathione transferases in the bioactivation of azathioprine. Advances in cancer research 122, 199-244.

Moretti, M., Colla, A., de Oliveira Balen, G., dos Santos, D.B., Budni, J., de Freitas, A.E., Farina, M., Severo Rodrigues, A.L., 2012. Ascorbic acid treatment, similarly to fluoxetine, reverses depressive-like behavior and brain oxidative damage induced by chronic unpredictable stress. Journal of psychiatric research 46, 331-340.

Muller, N., Schwarz, M.J., 2007. The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression. Molecular psychiatry 12, 988-1000.

Murphy, M.P., 2009. How mitochondria produce reactive oxygen species. The Biochemical journal 417, 1-13.

Nohl, H., Gille, L., Staniek, K., 2005. Intracellular generation of reactive oxygen species by mitochondria. Biochemical pharmacology 69, 719-723.

Novio, S., Nunez, M.J., Amigo, G., Freire-Garabal, M., 2011. Effects of fluoxetine on the oxidative status of peripheral blood leucocytes of restraint-stressed mice. Basic & clinical pharmacology & toxicology 109, 365-371.

Pernas, L., Scorrano, L., 2016. Mito-Morphosis: Mitochondrial Fusion, Fission, and Cristae Remodeling as Key Mediators of Cellular Function. Annual review of physiology 78, 505-531.

Rasheed, M.Z., Tabassum, H., Parvez, S., 2016. Mitochondrial permeability transition pore: a promising target for the treatment of Parkinson's disease. Protoplasma.

Reznick, A.Z., Packer, L., 1994. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods in enzymology 233, 357-363.

Robinson, J., Cooper, J.M., 1970. Method of determining oxygen concentrations in biological media, suitable for calibration of the oxygen electrode. Analytical biochemistry 33, 390-399.

Sanchez, S., Sanchez, C., Paredes, S.D., Cubero, J., Rodriguez, A.B., Barriga, C., 2008. Circadian variations of serotonin in plasma and different brain regions of rats. Molecular and cellular biochemistry 317, 105-111.

Sangle, G.V., Chowdhury, S.K., Xie, X., Stelmack, G.L., Halayko, A.J., Shen, G.X., 2010. Impairment of mitochondrial respiratory chain activity in aortic endothelial cells induced by glycated low-density lipoprotein. Free radical biology & medicine 48, 781-790.

Schneider, W.C., Hogeboom, G.H., 1951. Cytochemical studies of mammalian tissues; the isolation of cell components by differential centrifugation: a review. Cancer research 11, 1-22.

Silva, C.M., Goncalves, L., Manhaes-de-Castro, R., Nogueira, M.I., 2010. Postnatal fluoxetine treatment affects the development of serotonergic neurons in rats. Neuroscience letters 483, 179-183.

Skulachev, V.P., 1991. Fatty acid circuit as a physiological mechanism of uncoupling of oxidative phosphorylation. FEBS letters 294, 158-162.

Skulachev, V.P., 1998. Uncoupling: new approaches to an old problem of bioenergetics. Biochimica et biophysica acta 1363, 100-124.

Sonei, N., Amiri, S., Jafarian, I., Anoush, M., Rahimi-Balaei, M., Bergen, H., Haj-Mirzaian, A., Hosseini, M.J., 2016. Mitochondrial dysfunction bridges negative affective disorders and cardiomyopathy in socially isolated rats: Pros and cons of

fluoxetine. The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry, 1-15.

Souza, M.E., Polizello, A.C., Uyemura, S.A., Castro-Silva, O., Curti, C., 1994. Effect of fluoxetine on rat liver mitochondria. Biochemical pharmacology 48, 535-541.

Starkov, A.A., Fiskum, G., Chinopoulos, C., Lorenzo, B.J., Browne, S.E., Patel, M.S., Beal, M.F., 2004. Mitochondrial alpha-ketoglutarate dehydrogenase complex generates reactive oxygen species. The Journal of neuroscience : the official journal of the Society for Neuroscience 24, 7779-7788.

Strumper, D., Durieux, M.E., Hollmann, M.W., Troster, B., den Bakker, C.G., Marcus, M.A., 2003. Effects of antidepressants on function and viability of human neutrophils. Anesthesiology 98, 1356-1362.

Tretter, L., Adam-Vizi, V., 2004. Generation of reactive oxygen species in the reaction catalyzed by alpha-ketoglutarate dehydrogenase. The Journal of neuroscience : the official journal of the Society for Neuroscience 24, 7771-7778.

Turrens, J.F., 2003. Mitochondrial formation of reactive oxygen species. The Journal of physiology 552, 335-344.

Vercesi, A.E., Ferraz, V.L., Macedo, D.V., Fiskum, G., 1988. Ca2+-dependent NAD(P)+-induced alterations of rat liver and hepatoma mitochondrial membrane permeability. Biochemical and biophysical research communications 154, 934-941.

Vidal-Puig, A.J., Grujic, D., Zhang, C.Y., Hagen, T., Boss, O., Ido, Y., Szczepanik, A., Wade, J., Mootha, V., Cortright, R., Muoio, D.M., Lowell, B.B., 2000. Energy metabolism in uncoupling protein 3 gene knockout mice. The Journal of biological chemistry 275, 16258-16266.

Wilde, M.I., Benfield, P., 1998. Fluoxetine. A pharmacoeconomic review of its use in depression. Pharmacoeconomics 13, 543-561.

Winterbourn, C.C., 2002. Biological reactivity and biomarkers of the neutrophil oxidant, hypochlorous acid. Toxicology 181-182, 223-227.

Yaron, I., Shirazi, I., Judovich, R., Levartovsky, D., Caspi, D., Yaron, M., 1999. Fluoxetine and amitriptyline inhibit nitric oxide, prostaglandin E2, and hyaluronic acid production in human synovial cells and synovial tissue cultures. Arthritis and rheumatism 42, 2561-2568.

Zafir, A., Banu, N., 2007. Antioxidant potential of fluoxetine in comparison to Curcuma longa in restraint-stressed rats. European journal of pharmacology 572, 23-31.

Zhang, F., Zhou, H., Wilson, B.C., Shi, J.S., Hong, J.S., Gao, H.M., 2012. Fluoxetine protects neurons against microglial activation-mediated neurotoxicity. Parkinsonism & related disorders 18 Suppl 1, S213-217.

Zlatkovic, J., Todorovic, N., Tomanovic, N., Boskovic, M., Djordjevic, S., Lazarevic-Pasti, T., Bernardi, R.E., Djurdjevic, A., Filipovic, D., 2014. Chronic administration of fluoxetine or clozapine induces oxidative stress in rat liver: a histopathological study. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 59, 20-30.

FIGURE LEGENDS:

Figure 1 – Effect of chronic treatment with fluoxetine on body weight of male rats at 21, 40 and 60 days of life. The pups received daily fluoxetine (Fx = 10 mg / kg bw, black bar) or vehicle (C= 0.9% NaCl 10 mL / kg bw, white bar) from the 1st to 21th day of life. C n= 4 and Fx n= 5. Data are presented as mean ± SEM, *p<0.05.Groups were compared by unpaired Student's t-test.

Figure 2 - Effect of chronic treatment with fluoxetine on themitochondrial oxygen consumption (A) and respiratory control ratio-RCR (state 3/state 4) (B) in liver of male rats at 60 days of life. The pups received daily fluoxetine (Fx = 10 mg / kg bw, black bar) or vehicle (C= 0.9% NaCl 10 mL / kg bw, white bar) from the 1st to 21th day of life.Were added to the experiments: 200 μM ADP (ADP stimulation or state 3); 1 μmg/mL oligomycin (resting or state 4) and 1μM CCCP (Carbonyl cyanide m-chlorophenyl hydrazone) (uncoupling state). C n= 5 and Fx n= 3. Data are presented as mean ± SEM, *p<0.05; **p<0.001; ***p<0.0001. Groups werecompared by unpaired Student's t-test.

Figure 3 - Effect of chronic treatment with fluoxetine on the RS production in liver of male rats at 60 days of life. The pups received daily fluoxetine (Fx = 10 mg / kg bw, black bar) or vehicle (C= 0.9% NaCl 10 mL / kg bw, white bar) from the 1st to 21th day of life. C n= 4 and Fx n= 5. Data are presented as mean ± SEM, *p<0.05. Groups were compared by unpaired Student's t-test.

Figure 4– Effect of chronic treatment with fluoxetine on the mitochondrial pore opening in liver of male rats at 60 days of life. The pups received daily fluoxetine (Fx = 10 mg / kg bw, black bar) or vehicle (C= 0.9% NaCl 10 mL / kg bw, white bar) from the 1st to 21th day of life. Bar chart with mean values of the groups, control and

fluoxetine; in addition, control and fluoxetine in the presence of 0,1 μM cyclosporin A (CsA), a classical inhibitor of the transition pore of mitochondrial permeability and 0,5 μM EGTA, a calcium chelator. C n= 5 and Fx n= 6. Data are presented as mean ± SEM, *p<0.05. Groups were compared by unpaired Student's t-test.

Figure 5 - Effect of chronic treatment with fluoxetine on oxidative stress biomarkers (MDA levels, carbonyl content, SH grops oxidation) in liver of male rats at 60 days of life. The pups received daily fluoxetine (Fx = 10 mg / kg bw, black bar) or vehicle (C= 0.9% NaCl 10 mL/ kg bw, white bar) from the 1st to 21th day of life. A) MDA levels; B) Carbonyl content; C) SH grops oxidation. C n= 6 and Fx n= 5. Data are presented as mean ± SEM, *p<0.05. Groups were compared by unpaired Student's t-test.

Figure 6 - Effect of chronic treatment with fluoxetine on the antioxidant ezymatic defense in liver of male rats at 60 days of life. The pups received daily fluoxetine (Fx = 10 mg / kg bw, black bar) or vehicle (C= 0.9% NaCl 10 mL / kg bw, white bar) from the 1st to 21th day of life. A) Superoxide dismutase (SOD) activity; B) Catalase (CAT) activity; C) glutathione S-transferase (GST) activity. C n= 5 and Fx n= 5. Data are presented as mean ± SEM, *p<0.05. Groups were compared by unpaired Student's t-test.

Figure 7 - Effect of chronic treatment with fluoxetine on the levels of reduced glutathione in liver of male rats at 60 days of life. The pups received daily fluoxetine (Fx = 10 mg / kg bw, black bar) or vehicle (C= 0.9% NaCl 10 mL / kg bw, white bar) from the 1st to 21th day of life. C n= 6 and Fx n= 5. Data are presented as mean ± SEM, *p<0.05. Groups were compared by unpaired Student's t-test.

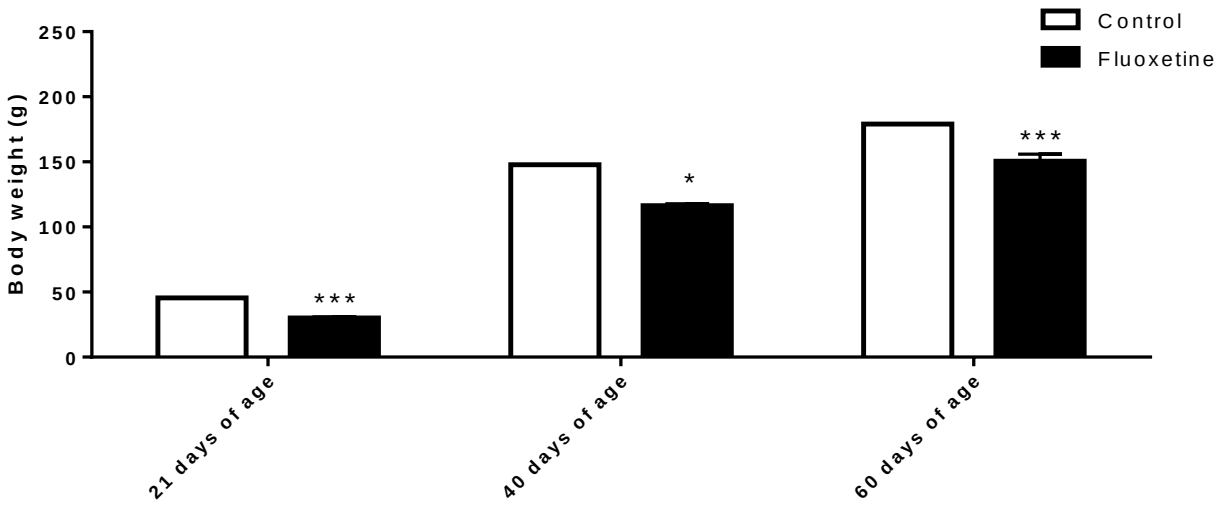
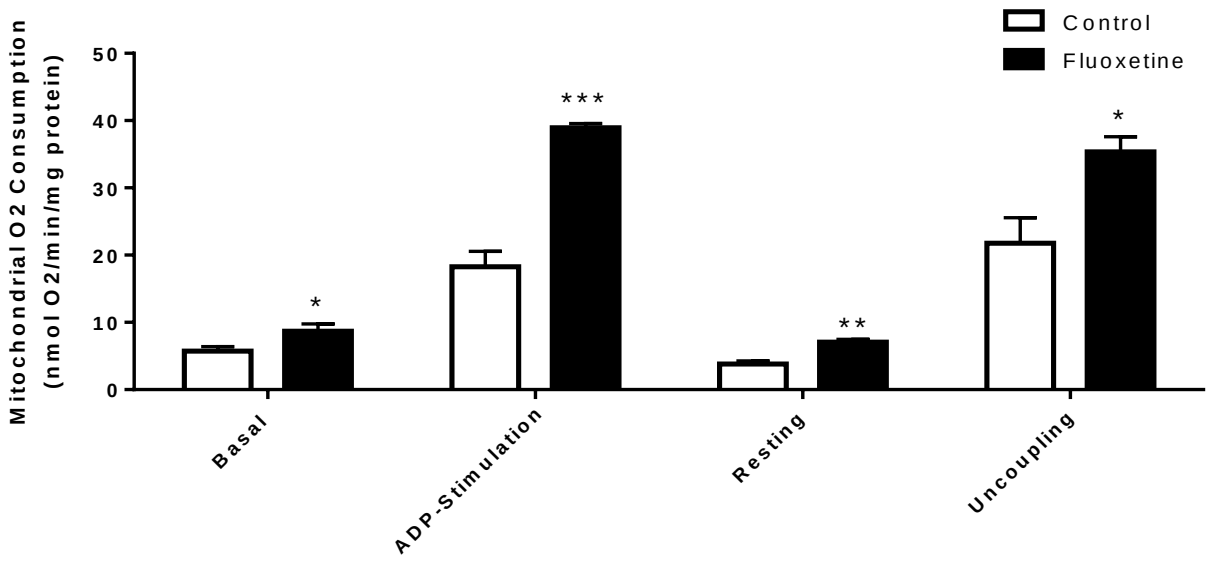

Control
Fluoxetine
Body weight (g)
250
200
150
100
50
0
***
*
***
21 days of age
40 days of age
60 days of age

Figure 1

A


Mitochondrial O2 Consumption
(nmol O2/min/mg protein)
50
40
30
20
10
0
Control
Fluoxetine
*
***
**
*
Basal
ADP-Stimulation
Resting
Uncoupling

B

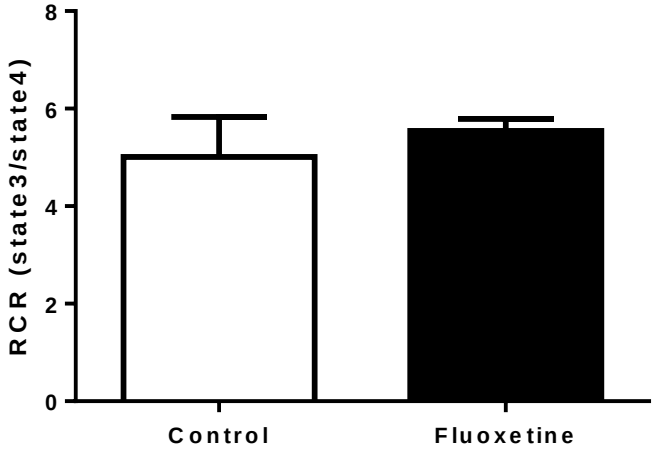

RCR (state3/state4)
8
6
4
2
0
Control
Fluoxetine

Figure 2

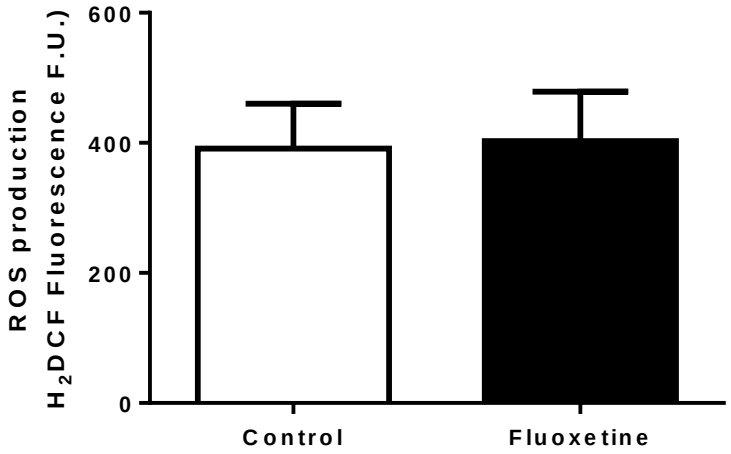

ROS production
H2DCF Fluorescence F.U.)
600
400
200
0
Control
Fluoxetine

Figure 3

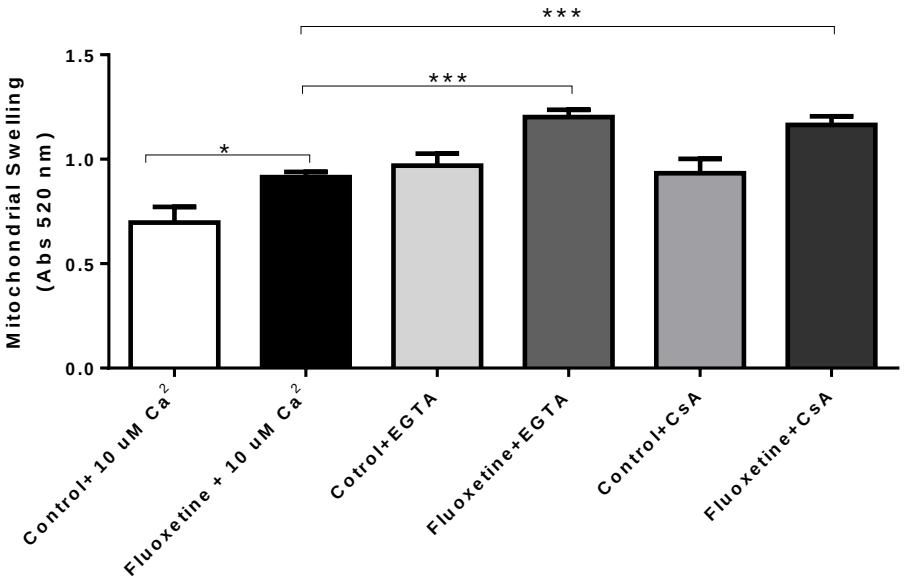
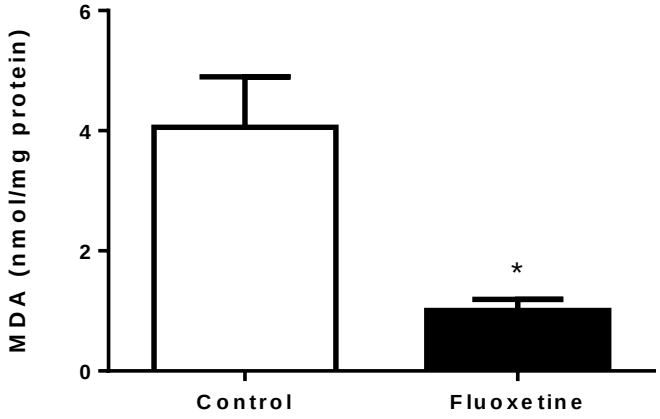
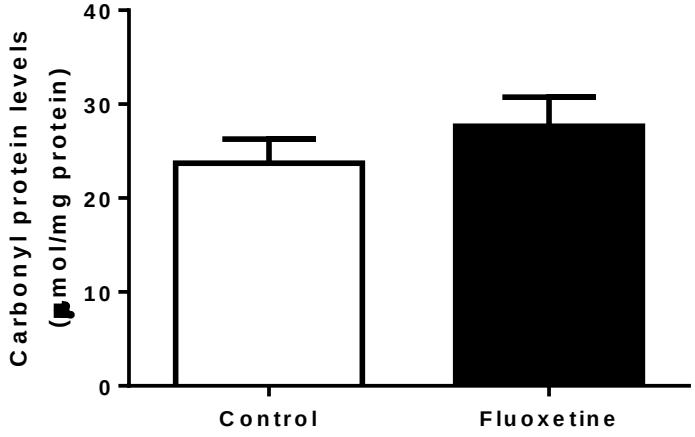

***
***
*
Mitochondrial Swelling (Abs 520 nm)
1.5
1.0
0.5
0.0
Control+ 10 uM Ca2
Fluoxetine + 10 uM Ca2
Cotrol+EGTA
Fluoxetine+EGTA
Control+CsA
Fluoxetine+CsA

Figure 4

A


MDA (nmol/mg protein)
6
4
2
0
*
Control
Fluoxetine

B


Carbonyl protein levels
(nmol/mg protein)
40
30
20
10
0
Control
Fluoxetine

C

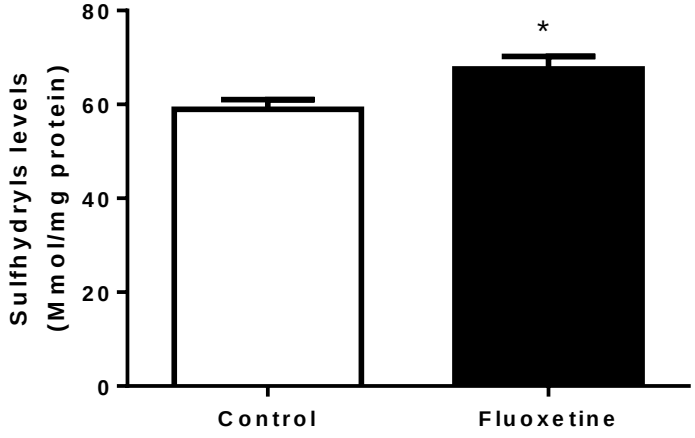
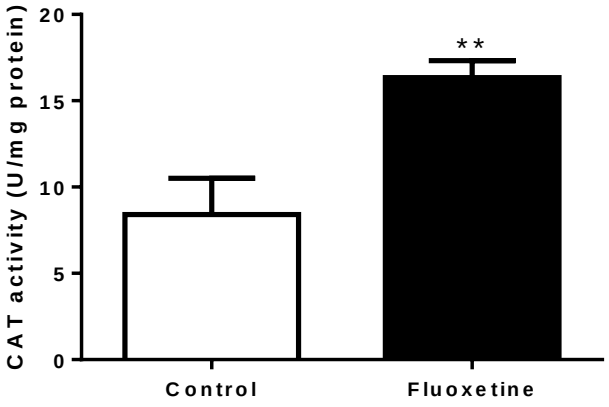
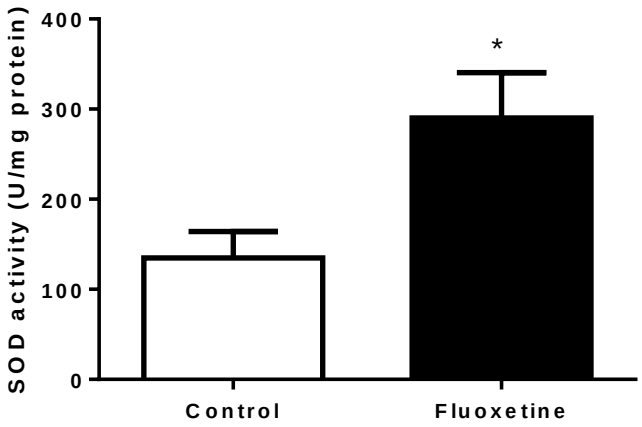

Sulfhydryls levels
(Mmol/mg protein)
80
60
40
20
0
*
Control
Fluoxetine

Figure 5

A


CAT activity (U/mg protein)
20
15
10
5
0
**
Control
Fluoxetine

B


SOD activity (U/mg protein)
400
300
200
100
0
*
Control
Fluoxetine

C

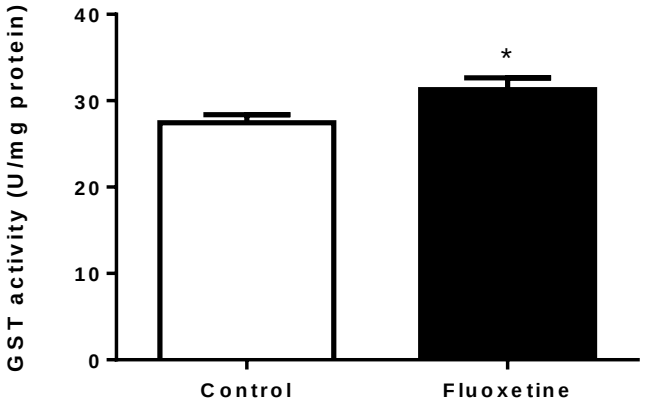

GST activity (U/mg protein)
40
30
20
10
0
*
Control
Fluoxetine

Figure 6

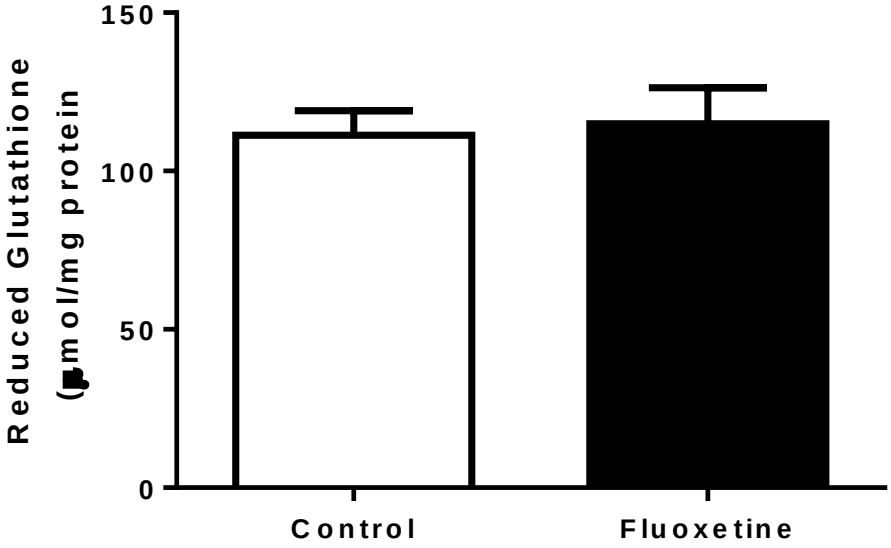

Reduced Glutathione
(μmol/mg protein
150
100
50
0
Control
Fluoxetine

Figure 7

7 CONSIDERAÇÕES FINAIS

A fluoxetina, um ISRS, é um fármaco largamente prescrito para o tratamento de distúrbios neurológicos, como depressão e ansiedade, entretanto seus efeitos sobre o metabolismo oxidativo hepático é controverso. A depender da concentração do fármaco e do período do desenvolvimento em que o tratamento foi conduzido, os efeitos podem ser benéficos ou danosos ao metabolismo oxidativo hepático. Em nosso modelo, realizado durante um período crítico do desenvolvimento, observamos que o tratamento com fluoxetina resultou em peso corporal reduzido, melhora da capacidade respiratória mitocondrial, membrana mitocondrial integra e resistente ao íon Ca^{2+}, diminuição de biomarcadores de estresse oxidativo, associado a um aumento nos níveis de SH, além de aumento na atividade de defesas antioxidantes enzimáticas (atividade da SOD, CAT, GST) no fígado de ratos adultos, refutando nossa hipótese. Podemos concluir com nossos achados que o tratamento farmacológico com fluoxetina durante períodos críticos do desenvolvimento não compromete a capacidade respiratória mitocondrial e o metabolismo oxidativo do fígado de ratos que persiste na vida adulta.

REFERÊNCIAS

A, L. L. et al. Determination of fluoxetine and norfluoxetine in human plasma by high-performance liquid chromatography with ultraviolet detection in psychiatric patients. **J Chromatogr B Analyt Technol Biomed Life Sci,** v. 783, n. 1, p. 25-31, Jan 05 2003.

ABDEL-SALAM, O. M.; BAIUOMY, A. R.; ARBID, M. S. Studies on the anti-inflammatory effect of fluoxetine in the rat. **Pharmacol Res,** v. 49, n. 2, p. 119-31, Feb 2004.

ABDEL SALAM, O. M. et al. The effect of antidepressant drugs on thioacetamide-induced oxidative stress. **Eur Rev Med Pharmacol Sci,** v. 17, n. 6, p. 735-44, Mar 2013.

ADACHI, Y. et al. Serum glutathione S-transferase in experimental liver damage in rats. **Gastroenterol Jpn,** v. 16, n. 2, p. 129-33, 1981.

ADAM-VIZI, V.; CHINOPOULOS, C. Bioenergetics and the formation of mitochondrial reactive oxygen species. **Trends Pharmacol Sci,** v. 27, n. 12, p. 639-45, Dec 2006.

ADAM-VIZI, V.; STARKOV, A. A. Calcium and mitochondrial reactive oxygen species generation: how to read the facts. **J Alzheimers Dis,** v. 20 Suppl 2, p. S413-26, 2010.

AEBI, H. Catalase in vitro. **Methods Enzymol,** v. 105, p. 121-6, 1984.

AGOSTINHO, F. R. et al. Treatment with olanzapine, fluoxetine and olanzapine/fluoxetine alters citrate synthase activity in rat brain. **Neurosci Lett,** v. 487, n. 3, p. 278-81, Jan 10 2011.

AGOSTINHO, F. R. et al. Olanzapine plus fluoxetine treatment alters mitochondrial respiratory chain activity in the rat brain. **Acta Neuropsychiatr,** v. 23, n. 6, p. 282-91, Dec 2011.

AGOSTINHO, F. R. et al. Effects of olanzapine, fluoxetine and olanzapine/fluoxetine on creatine kinase activity in rat brain. **Brain Res Bull,** v. 80, n. 6, p. 337-40, Dec 16 2009.

AKSENOV, M. Y.; MARKESBERY, W. R. Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. **Neurosci Lett,** v. 302, n. 2-3, p. 141-5, Apr 20 2001.

AKSU, U. et al. Fluoxetine ameliorates imbalance of redox homeostasis and inflammation in an acute kidney injury model. **J Physiol Biochem,** v. 70, n. 4, p. 925-34, Dec 2014.

ALENINA, N. et al. Growth retardation and altered autonomic control in mice lacking brain serotonin. **Proc Natl Acad Sci U S A,** v. 106, n. 25, p. 10332-7, Jun 23 2009.

ALTAMURA, A. C.; MORO, A. R.; PERCUDANI, M. Clinical pharmacokinetics of fluoxetine. **Clin Pharmacokinet,** v. 26, n. 3, p. 201-14, Mar 1994.

ANGIOLINI, E. et al. Regulation of placental efficiency for nutrient transport by imprinted genes. **Placenta,** v. 27 Suppl A, p. S98-102, Apr 2006.

BAINES, C. P. The mitochondrial permeability transition pore and ischemia-reperfusion injury. **Basic Res Cardiol,** v. 104, n. 2, p. 181-8, 2009.

BAO, L. et al. Mitochondria are the source of hydrogen peroxide for dynamic brain-cell signaling. **J Neurosci,** v. 29, n. 28, p. 9002-10, Jul 15 2009.

BARTOSZ, G. Reactive oxygen species: destroyers or messengers? **Biochem Pharmacol,** v. 77, n. 8, p. 1303-15, Apr 15 2009.

BEASLEY, C. M., JR. et al. Efficacy, adverse events, and treatment discontinuations in fluoxetine clinical studies of major depression: a meta-analysis of the 20-mg/day dose. **J Clin Psychiatry,** v. 61, n. 10, p. 722-8, Oct 2000.

BELLO, N. T.; LIANG, N. C. The use of serotonergic drugs to treat obesity--is there any hope? **Drug Des Devel Ther,** v. 5, p. 95-109, Feb 10 2011.

BEN-SHACHAR, D.; KARRY, R. Neuroanatomical pattern of mitochondrial complex I pathology varies between schizophrenia, bipolar disorder and major depression. **PLoS One,** v. 3, n. 11, p. e3676, 2008.

BERRIDGE, M. J.; BOOTMAN, M. D.; RODERICK, H. L. Calcium signalling: dynamics, homeostasis and remodelling. **Nat Rev Mol Cell Biol,** v. 4, n. 7, p. 517-29, 2003.

BLUNDELL, J. E.; LATHAM, C. J. Serotonergic influences on food intake: effect of 5-hydroxytryptophan on parameters of feeding behaviour in deprived and free-feeding rats. **Pharmacol Biochem Behav,** v. 11, n. 4, p. 431-7, Oct 1979.

BOSS, O.; MUZZIN, P.; GIACOBINO, J. P. The uncoupling proteins, a review. **Eur J Endocrinol,** v. 139, n. 1, p. 1-9, Jul 1998.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Anal Biochem,** v. 72, p. 248-54, May 7 1976.

BRAND, M. D. et al. Oxidative damage and phospholipid fatty acyl composition in skeletal muscle mitochondria from mice underexpressing or overexpressing uncoupling protein 3. **Biochem J,** v. 368, n. Pt 2, p. 597-603, Dec 01 2002.

BRAZ, G. R. et al. Neonatal SSRI exposure improves mitochondrial function and antioxidant defense in rat heart. **Appl Physiol Nutr Metab,** v. 41, n. 4, p. 362-9, Apr 2016.

BRAZ, G. R. et al. Serotonin modulation in neonatal age does not impair cardiovascular physiology in adult female rats: Hemodynamics and oxidative stress analysis. **Life Sci,** v. 145, p. 42-50, Jan 15 2016.

BROBERGER, C. Brain regulation of food intake and appetite: molecules and networks. **J Intern Med,** v. 258, n. 4, p. 301-27, Oct 2005.

BROOKES, P. S. et al. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. **Am J Physiol Cell Physiol,** v. 287, n. 4, p. C817-33, Oct 2004.

BRUNI, F.; LIGHTOWLERS, R. N.; CHRZANOWSKA-LIGHTOWLERS, Z. M. Human mitochondrial nucleases. **FEBS J**, Dec 07 2016.

BUEGE, J. A.; AUST, S. D. Microsomal lipid peroxidation. **Methods Enzymol,** v. 52, p. 302-10, 1978.

CADENAS, E.; DAVIES, K. J. Mitochondrial free radical generation, oxidative stress, and aging. **Free Radic Biol Med,** v. 29, n. 3-4, p. 222-30, 2000.

CAI, Q. et al. Acute hepatitis due to fluoxetine therapy. **Mayo Clin Proc,** v. 74, n. 7, p. 692-4, Jul 1999.

CAPE, J. L.; BOWMAN, M. K.; KRAMER, D. M. A semiquinone intermediate generated at the Qo site of the cytochrome bc1 complex: importance for the Q-cycle and superoxide production. **Proc Natl Acad Sci U S A,** v. 104, n. 19, p. 7887-92, 2007.

CARAFOLI, E. The fateful encounter of mitochondria with calcium: how did it happen? **Biochim Biophys Acta,** v. 1797, n. 6-7, p. 595-606, Jun-Jul 2010.

CARDOSO, A. R. et al. Mitochondrial compartmentalization of redox processes. **Free Radic Biol Med,** v. 52, n. 11-12, p. 2201-8, 2012.

CASES, O. et al. Lack of barrels in the somatosensory cortex of monoamine oxidase A-deficient mice: role of a serotonin excess during the critical period. **Neuron,** v. 16, n. 2, p. 297-307, Feb 1996.

CECHETTO, D. F.; SAPER, C. B. Neurochemical organization of the hypothalamic projection to the spinal cord in the rat. **J Comp Neurol,** v. 272, n. 4, p. 579-604, Jun 22 1988.

CHAN, D. C. Fusion and fission: interlinked processes critical for mitochondrial health. **Annu Rev Genet,** v. 46, p. 265-87, 2012.

CIRCU, M. L.; AW, T. Y. Reactive oxygen species, cellular redox systems, and apoptosis. **Free Radic Biol Med,** v. 48, n. 6, p. 749-62, Mar 15 2010.

CLARK, C. T.; WEISSBACH, H.; UDENFRIEND, S. 5-Hydroxytryptophan decarboxylase: preparation and properties. **J Biol Chem,** v. 210, n. 1, p. 139-48, Sep 1954.

COGLIATI, S. et al. Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. **Cell,** v. 155, n. 1, p. 160-71, Sep 26 2013.

COSTA, L. R. et al. Safflower (Catharmus tinctorius L.) oil supplementation in overnourished rats during early neonatal development: effects on heart and liver function in the adult. **Appl Physiol Nutr Metab,** v. 41, n. 12, p. 1271-1277, Dec 2016a.

_____. Safflower (Catharmus tinctorius L.) oil supplementation in overnourished rats during early neonatal development: effects on heart and liver function in the adult. **Appl Physiol Nutr Metab,** v. 41, n. 12, p. 1271-1277, Dec 2016b.

COTE, F. et al. Maternal serotonin is crucial for murine embryonic development. **Proc Natl Acad Sci U S A,** v. 104, n. 1, p. 329-34, Jan 2 2007.

CREWE, H. K. et al. The effect of selective serotonin re-uptake inhibitors on cytochrome P4502D6 (CYP2D6) activity in human liver microsomes. **Br J Clin Pharmacol,** v. 34, n. 3, p. 262-5, Sep 1992.

CURTI, C. et al. Fluoxetine interacts with the lipid bilayer of the inner membrane in isolated rat brain mitochondria, inhibiting electron transport and F1F0-ATPase activity. **Mol Cell Biochem,** v. 199, n. 1-2, p. 103-9, Sep 1999.

DA SILVA, A. I. et al. Fluoxetine induces lean phenotype in rat by increasing the brown/white adipose tissue ratio and UCP1 expression. **J Bioenerg Biomembr,** v. 47, n. 4, p. 309-18, Aug 2015.

DA SILVA, A. I. et al. Effect of fluoxetine treatment on mitochondrial bioenergetics in central and peripheral rat tissues. **Appl Physiol Nutr Metab,** v. 40, n. 6, p. 565-74, Jun 2015.

DA SILVA, A. I. et al. Fluoxetine treatment of rat neonates significantly reduces oxidative stress in the hippocampus and in behavioral indicators of anxiety later in postnatal life. **Can J Physiol Pharmacol,** v. 92, n. 4, p. 330-7, Apr 2014.

DAVANZO, R. et al. Antidepressant drugs and breastfeeding: a review of the literature. **Breastfeed Med,** v. 6, n. 2, p. 89-98, Apr 2011.

DE LONG, N. E. et al. Fluoxetine-induced pancreatic beta cell dysfunction: New insight into the benefits of folic acid in the treatment of depression. **J Affect Disord,** v. 166, p. 6-13, Sep 2014.

DEIRÓ, T. C. B. J. et al. Neonatal administration of citalopram delays somatic maturation in rats. **Brazilian Journal of Medical and Biological Research,** v. 37, p. 1503-1509, 2004.

DJORDJEVIC, J. et al. Fluoxetine affects antioxidant system and promotes apoptotic signaling in Wistar rat liver. **Eur J Pharmacol,** v. 659, n. 1, p. 61-6, May 20 2011.

DOBBING, J. Undernutrition and the developing brain. The relevance of animal models to the human problem. **Am J Dis Child,** v. 120, n. 5, p. 411-5, Nov 1970.

DORIGHELLO, G. G. et al. Correlation between Mitochondrial Reactive Oxygen and Severity of Atherosclerosis. **Oxid Med Cell Longev,** v. 2016, p. 7843685, 2016.

DRAPER, H. H. et al. A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. **Free Radic Biol Med,** v. 15, n. 4, p. 353-63, Oct 1993.

ERSPAMER, V. Observations of the metabolism of endogenous 5-hydroxytryptamine (enteramine) in the rat. **Experientia,** v. 10, n. 11, p. 471-2, Nov 15 1954.

_____. Historical introduction: the Italian contribution to the discovery of 5-hydroxytryptamine (enteramine, serotonin). **J Hypertens Suppl,** v. 4, n. 1, p. S3-5, Apr 1986.

FANG, X. L. et al. The anorexigenic effect of serotonin is mediated by the generation of NADPH oxidase-dependent ROS. **PLoS One,** v. 8, n. 1, p. e53142, 2013.

FENG, S.; HE, X. Mechanism-based inhibition of CYP450: an indicator of drug-induced hepatotoxicity. **Curr Drug Metab,** v. 14, n. 9, p. 921-45, Nov 2013.

FENTON, H. J. H. LXXIII.-Oxidation of tartaric acid in presence of iron. **Journal of the Chemical Society, Transactions,** v. 65, n. 0, p. 899-910, 1894.

FERREIRA, D. J. et al. Perinatal low-protein diet alters brainstem antioxidant metabolism in adult offspring. **Nutr Neurosci,** v. 19, n. 8, p. 369-375, Jun 2 2015.

FIGUEIRA, T. R. et al. Mitochondria as a source of reactive oxygen and nitrogen species: from molecular mechanisms to human health. **Antioxid Redox Signal,** v. 18, n. 16, p. 2029-74, Jun 1 2013.

FLESCHLER, R.; PESKIN, M. F. Selective serotonin reuptake inhibitors (SSRIs) in pregnancy: a review. **MCN Am J Matern Child Nurs,** v. 33, n. 6, p. 355-61; quiz 362-3, Nov-Dec 2008.

FRANCIS-OLIVEIRA, J. et al. Fluoxetine exposure during pregnancy and lactation: Effects on acute stress response and behavior in the novelty-suppressed feeding are age and gender-dependent in rats. **Behav Brain Res,** v. 252, p. 195-203, Sep 01 2013.

FRIEDENBERG, F. K.; ROTHSTEIN, K. D. Hepatitis secondary to fluoxetine treatment. **Am J Psychiatry,** v. 153, n. 4, p. 580, Apr 1996.

FUKUI, H.; MORAES, C. T. The mitochondrial impairment, oxidative stress and neurodegeneration connection: reality or just an attractive hypothesis? **Trends Neurosci,** v. 31, n. 5, p. 251-6, May 2008.

GALECKI, P. et al. Lipid peroxidation and antioxidant protection in patients during acute depressive episodes and in remission after fluoxetine treatment. **Pharmacol Rep,** v. 61, n. 3, p. 436-47, May-Jun 2009.

GARAU, C. et al. Age-related changes in circadian rhythm of serotonin synthesis in ring doves: effects of increased tryptophan ingestion. **Exp Gerontol,** v. 41, n. 1, p. 40-8, Jan 2006.

GARCIA-RUIZ, C. et al. Direct effect of ceramide on the mitochondrial electron transport chain leads to generation of reactive oxygen species. Role of mitochondrial glutathione. **J Biol Chem,** v. 272, n. 17, p. 11369-77, Apr 25 1997.

GARCIA, J. et al. Regulation of mitochondrial glutathione redox status and protein glutathionylation by respiratory substrates. **J Biol Chem,** v. 285, n. 51, p. 39646-54, Dec 17 2010.

GARLID, K. D. et al. On the mechanism of fatty acid-induced proton transport by mitochondrial uncoupling protein. **J Biol Chem,** v. 271, n. 5, p. 2615-20, 1996.

GEBHARDT, R. Metabolic zonation of the liver: regulation and implications for liver function. **Pharmacol Ther,** v. 53, n. 3, p. 275-354, 1992.

GLANCY, B.; BALABAN, R. S. Role of mitochondrial Ca2+ in the regulation of cellular energetics. **Biochemistry,** v. 51, n. 14, p. 2959-73, 2012a.

_____. Role of mitochondrial Ca2+ in the regulation of cellular energetics. **Biochemistry,** v. 51, n. 14, p. 2959-73, Apr 10 2012b.

HABER, F.; WEISS, J. The Catalytic Decomposition of Hydrogen Peroxide by Iron Salts. **Proceedings of the Royal Society of London. Series A, Mathematical and Physical Sciences,** v. 147, n. 861, p. 332-351, 1934.

HABIG, W. H.; JAKOBY, W. B. Glutathione S-transferases (rat and human). **Methods Enzymol,** v. 77, p. 218-31, 1981.

HABIG, W. H. et al. The identity of glutathione S-transferase B with ligandin, a major binding protein of liver. **Proc Natl Acad Sci U S A,** v. 71, n. 10, p. 3879-82, Oct 1974.

HALESTRAP, A. P.; PASDOIS, P. The role of the mitochondrial permeability transition pore in heart disease. **Biochim Biophys Acta,** v. 11, n. 15, p. 8, 2009.

HALLIDAY, G.; BAKER, K.; HARPER, C. Serotonin and alcohol-related brain damage. **Metab Brain Dis,** v. 10, n. 1, p. 25-30, Mar 1995.

HALLIWELL, B.; GUTTERIDGE, J. M. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. **Arch Biochem Biophys,** v. 246, n. 2, p. 501-14, May 1 1986.

_____. The antioxidants of human extracellular fluids. **Arch Biochem Biophys,** v. 280, n. 1, p. 1-8, Jul 1990.

HEISLER, L. K. et al. Activation of central melanocortin pathways by fenfluramine. **Science,** v. 297, n. 5581, p. 609-11, Jul 26 2002.

HEMERYCK, A.; BELPAIRE, F. M. Selective serotonin reuptake inhibitors and cytochrome P-450 mediated drug-drug interactions: an update. **Curr Drug Metab,** v. 3, n. 1, p. 13-37, Feb 2002.

HENDRICKS, T. J. et al. Pet-1 ETS gene plays a critical role in 5-HT neuron development and is required for normal anxiety-like and aggressive behavior. **Neuron,** v. 37, n. 2, p. 233-47, Jan 23 2003.

HIDALGO, C.; DONOSO, P. Crosstalk between calcium and redox signaling: from molecular mechanisms to health implications. **Antioxid Redox Signal,** v. 10, n. 7, p. 1275-312, Jul 2008.

HIEMKE, C.; HARTTER, S. Pharmacokinetics of selective serotonin reuptake inhibitors. **Pharmacol Ther,** v. 85, n. 1, p. 11-28, Jan 2000.

HISSIN, P. J.; HILF, R. A fluorometric method for determination of oxidized and reduced glutathione in tissues. **Anal Biochem,** v. 74, n. 1, p. 214-26, Jul 1976.

HJORTH, S. et al. Serotonin autoreceptor function and antidepressant drug action. **J Psychopharmacol,** v. 14, n. 2, p. 177-85, Jun 2000.

HOYER, D.; HANNON, J. P.; MARTIN, G. R. Molecular, pharmacological and functional diversity of 5-HT receptors. **Pharmacol Biochem Behav,** v. 71, n. 4, p. 533-54, Apr 2002.

HROUDOVA, J.; FISAR, Z. In vitro inhibition of mitochondrial respiratory rate by antidepressants. **Toxicol Lett,** v. 213, n. 3, p. 345-52, Sep 18 2012.

HUCH, M.; DOLLE, L. The plastic cellular states of liver cells: Are EpCAM and Lgr5 fit for purpose? **Hepatology**, Jan 22 2016.

INDO, H. P. et al. A mitochondrial superoxide theory for oxidative stress diseases and aging. **J Clin Biochem Nutr,** v. 56, n. 1, p. 1-7, Jan 2015a.

_____. A mitochondrial superoxide theory for oxidative stress diseases and aging. **J Clin Biochem Nutr,** v. 56, n. 1, p. 1-7, Jan 2015b.

INKIELEWICZ-STEPNIAK, I. Impact of fluoxetine on liver damage in rats. **Pharmacol Rep,** v. 63, n. 2, p. 441-7, 2011.

JEZEK, P.; HLAVATA, L. Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism. **Int J Biochem Cell Biol,** v. 37, n. 12, p. 2478-503, Dec 2005.

JOHNSTON, D. E.; WHEELER, D. E. Chronic hepatitis related to use of fluoxetine. **Am J Gastroenterol,** v. 92, n. 7, p. 1225-6, Jul 1997.

JORNAYVAZ, F. R.; SHULMAN, G. I. Regulation of mitochondrial biogenesis. **Essays Biochem,** v. 47, p. 69-84, 2010.

KALGUTKAR, A. S.; DIDIUK, M. T. Structural alerts, reactive metabolites, and protein covalent binding: how reliable are these attributes as predictors of drug toxicity? **Chem Biodivers,** v. 6, n. 11, p. 2115-37, Nov 2009.

KALUDERCIC, N.; GIORGIO, V. The Dual Function of Reactive Oxygen/Nitrogen Species in Bioenergetics and Cell Death: The Role of ATP Synthase. **Oxid Med Cell Longev,** v. 2016, p. 3869610, 2016.

KATO, S.; FUJIWARA, I.; YOSHIDA, N. Nitrogen-containing heteroalicycles with serotonin receptor binding affinity: development of gastroprokinetic and antiemetic agents. **Med Res Rev,** v. 19, n. 1, p. 25-73, Jan 1999.

KEVIN FOSKETT, J.; MADESH, M. Regulation of the mitochondrial Ca(2+) uniporter by MICU1 and MICU2. **Biochem Biophys Res Commun,** v. 449, n. 4, p. 377-83, Jul 11 2014.

KIELISZEK, M.; BLAZEJAK, S. Selenium: Significance, and outlook for supplementation. **Nutrition,** v. 29, n. 5, p. 713-8, May 2013.

KOLLA, N. et al. Amitriptyline and fluoxetine protect PC12 cells from cell death induced by hydrogen peroxide. **J Psychiatry Neurosci,** v. 30, n. 3, p. 196-201, May 2005.

KOPP, J. L.; GROMPE, M.; SANDER, M. Stem cells versus plasticity in liver and pancreas regeneration. **Nat Cell Biol,** v. 18, n. 3, p. 238-45, Feb 25 2016.

KORSHUNOV, S. S.; SKULACHEV, V. P.; STARKOV, A. A. High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. **FEBS Lett,** v. 416, n. 1, p. 15-8, Oct 13 1997.

KOWALTOWSKI, A. J.; CASTILHO, R. F.; VERCESI, A. E. Mitochondrial permeability transition and oxidative stress. **FEBS Lett,** v. 495, n. 1-2, p. 12-5, 2001.

KOWALTOWSKI, A. J. et al. Mitochondria and reactive oxygen species. **Free Radic Biol Med,** v. 47, n. 4, p. 333-43, Aug 15 2009.

KOWALTOWSKI, A. J.; VERCESI, A. E. Mitochondrial damage induced by conditions of oxidative stress. **Free Radic Biol Med,** v. 26, n. 3-4, p. 463-71, 1999.

LEBEL, C. P.; ISCHIROPOULOS, H.; BONDY, S. C. Evaluation of the probe 2',7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. **Chem Res Toxicol,** v. 5, n. 2, p. 227-31, Mar-Apr 1992.

LEIBOWITZ, S. F.; ALEXANDER, J. T. Hypothalamic serotonin in control of eating behavior, meal size, and body weight. **Biol Psychiatry,** v. 44, n. 9, p. 851-64, Nov 01 1998.

LEMASTERS, J. J. et al. Mitochondrial calcium and the permeability transition in cell death. **Biochim Biophys Acta,** v. 11, n. 401, p. 1, 2009.

LENAZ, G. Mitochondria and reactive oxygen species. Which role in physiology and pathology? **Adv Exp Med Biol,** v. 942, p. 93-136, 2012.

LESCH, K. P.; WAIDER, J. Serotonin in the modulation of neural plasticity and networks: implications for neurodevelopmental disorders. **Neuron,** v. 76, n. 1, p. 175-91, Oct 04 2012.

LI, X. M. et al. Antidepressants upregulate messenger RNA levels of the neuroprotective enzyme superoxide dismutase (SOD1). **J Psychiatry Neurosci,** v. 25, n. 1, p. 43-7, Jan 2000.

LI, Y. F. et al. Cytoprotective effect is one of common action pathways for antidepressants. **Acta Pharmacol Sin,** v. 24, n. 10, p. 996-1000, 2003.

LIU, J.; MORI, A. Monoamine metabolism provides an antioxidant defense in the brain against oxidant- and free radical-induced damage. **Arch Biochem Biophys,** v. 302, n. 1, p. 118-27, Apr 1993.

MAERTENS, C. et al. Block by fluoxetine of volume-regulated anion channels. **Br J Pharmacol,** v. 126, n. 2, p. 508-14, Jan 1999.

MAES, M. The immunoregulatory effects of antidepressants. **Hum Psychopharmacol,** v. 16, n. 1, p. 95-103, Jan 2001.

MANHÃES DE CASTRO, R. et al. Reduction of intraspecific aggression in adult rats by neonatal treatment with a selective serotonin reuptake inhibitor. **Brazilian Journal of Medical and Biological Research,** v. 34, p. 121-124, 2001.

MANNI, M. E. et al. Monoamine Oxidase Is Overactivated in Left and Right Ventricles from Ischemic Hearts: An Intriguing Therapeutic Target. **Oxid Med Cell Longev,** v. 2016, p. 4375418, 2016.

MARCONDES, F. K.; BIANCHI, F. J.; TANNO, A. P. Determination of the estrous cycle phases of rats: some helpful considerations. **Braz J Biol,** v. 62, n. 4A, p. 609-14, Nov 2002.

MASAND, P. S.; GUPTA, S. Selective serotonin-reuptake inhibitors: an update. **Harv Rev Psychiatry,** v. 7, n. 2, p. 69-84, Jul-Aug 1999.

MAZER, C. et al. Serotonin depletion during synaptogenesis leads to decreased synaptic density and learning deficits in the adult rat: a possible model of neurodevelopmental disorders with cognitive deficits. **Brain Res,** v. 760, n. 1-2, p. 68-73, Jun 20 1997.

MBYE, L. H. et al. Comparative neuroprotective effects of cyclosporin A and NIM811, a nonimmunosuppressive cyclosporin A analog, following traumatic brain injury. **J Cereb Blood Flow Metab,** v. 29, n. 1, p. 87-97, 2009.

MCCONN, D. J.; ZHAO, Z. Integrating in vitro kinetic data from compounds exhibiting induction, reversible inhibition and mechanism-based inactivation: in vitro study design. **Curr Drug Metab,** v. 5, n. 2, p. 141-6, Apr 2004.

MCGUIRK, J.; MUSCAT, R.; WILLNER, P. Effects of chronically administered fluoxetine and fenfluramine on food intake, body weight and the behavioural satiety sequence. **Psychopharmacology (Berl),** v. 106, n. 3, p. 401-7, 1992.

MENDES-DA-SILVA, C. et al. Neonatal treatment with fluoxetine reduces depressive behavior induced by forced swim in adult rats. **Arq Neuropsiquiatr,** v. 60, n. 4, p. 928-31, Dec 2002.

MILLAN, M. J. et al. Signaling at G-protein-coupled serotonin receptors: recent advances and future research directions. **Trends Pharmacol Sci,** v. 29, n. 9, p. 454-64, Sep 2008.

MILLER, B. H. et al. Genetic regulation of behavioral and neuronal responses to fluoxetine. **Neuropsychopharmacology,** v. 33, n. 6, p. 1312-22, May 2008.

MISRA, H. P.; FRIDOVICH, I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. **J Biol Chem,** v. 247, n. 10, p. 3170-5, May 25 1972.

MIYAJIMA, A.; TANAKA, M.; ITOH, T. Stem/progenitor cells in liver development, homeostasis, regeneration, and reprogramming. **Cell Stem Cell,** v. 14, n. 5, p. 561-74, May 1 2014.

MIYATA, M. et al. Development of monocrotaline-induced pulmonary hypertension is attenuated by a serotonin receptor antagonist. **Lung,** v. 178, n. 2, p. 63-73, 2000.

MODEN, O.; MANNERVIK, B. Glutathione transferases in the bioactivation of azathioprine. **Adv Cancer Res,** v. 122, p. 199-244, 2014.

MORETTI, M. et al. Ascorbic acid treatment, similarly to fluoxetine, reverses depressive-like behavior and brain oxidative damage induced by chronic unpredictable stress. **J Psychiatr Res,** v. 46, n. 3, p. 331-40, Mar 2012.

MORGANE, P. J. et al. The effects of protein malnutrition on the developing central nervous system in the rat. **Neuroscience & Biobehavioral Reviews,** v. 2, n. 3, p. 137-230, 1978.

MORGANE, P. J.; MOKLER, D. J.; GALLER, J. R. Effects of prenatal protein malnutrition on the hippocampal formation. **Neurosci Biobehav Rev,** v. 26, n. 4, p. 471-83, Jun 2002.

MULLER, N.; SCHWARZ, M. J. The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression. **Mol Psychiatry,** v. 12, n. 11, p. 988-1000, Nov 2007.

MURPHY, M. P. How mitochondria produce reactive oxygen species. **Biochem J,** v. 417, n. 1, p. 1-13, 2009a.

_____. How mitochondria produce reactive oxygen species. **Biochem J,** v. 417, n. 1, p. 1-13, Jan 01 2009b.

NAKAYAMA, S. et al. Combination of GSH trapping and time-dependent inhibition assays as a predictive method of drugs generating highly reactive metabolites. **Drug Metab Dispos,** v. 39, n. 7, p. 1247-54, Jul 2011.

NASYROVA, D. I. et al. [Development of central and peripheral serotonin-producing systems in rats in ontogenesis]. **Zh Evol Biokhim Fiziol,** v. 45, n. 1, p. 68-74, Jan-Feb 2009.

NEBIGIL, C. G. et al. Serotonin is a novel survival factor of cardiomyocytes: mitochondria as a target of 5-HT2B receptor signaling. **FASEB J,** v. 17, n. 10, p. 1373-5, 2003.

NELSON, D. L. C., M. M. **Princípios de Bioquímica de Lehninger**. 5º Edition. 2011.

NICHOLLS, D. G. Mitochondrial calcium function and dysfunction in the central nervous system. **Biochim Biophys Acta,** v. 11, n. 24, p. 17, 2009.

NOHL, H.; GILLE, L.; STANIEK, K. Intracellular generation of reactive oxygen species by mitochondria. **Biochem Pharmacol,** v. 69, n. 5, p. 719-23, Mar 1 2005.

NOVIO, S. et al. Effects of fluoxetine on the oxidative status of peripheral blood leucocytes of restraint-stressed mice. **Basic Clin Pharmacol Toxicol,** v. 109, n. 5, p. 365-71, Nov 2011.

NUNNARI, J. et al. Mitochondrial transmission during mating in Saccharomyces cerevisiae is determined by mitochondrial fusion and fission and the intramitochondrial segregation of mitochondrial DNA. **Mol Biol Cell,** v. 8, n. 7, p. 1233-42, Jul 1997.

OZAWA, T. Mitochondrial genome mutation in cell death and aging. **J Bioenerg Biomembr,** v. 31, n. 4, p. 377-90, 1999.

PERNAS, L.; SCORRANO, L. Mito-Morphosis: Mitochondrial Fusion, Fission, and Cristae Remodeling as Key Mediators of Cellular Function. **Annu Rev Physiol,** v. 78, p. 505-31, 2016a.

_____. Mito-Morphosis: Mitochondrial Fusion, Fission, and Cristae Remodeling as Key Mediators of Cellular Function. **Annu Rev Physiol,** v. 78, p. 505-31, 2016b.

PERSICO, A. M. et al. Barrel pattern formation requires serotonin uptake by thalamocortical afferents, and not vesicular monoamine release. **J Neurosci,** v. 21, n. 17, p. 6862-73, Sep 01 2001.

PRESKORN, S. H. Clinically relevant pharmacology of selective serotonin reuptake inhibitors. An overview with emphasis on pharmacokinetics and effects on oxidative drug metabolism. **Clin Pharmacokinet,** v. 32 Suppl 1, p. 1-21, 1997.

QUIJANO, C. et al. Interplay between oxidant species and energy metabolism. **Redox Biol,** v. 8, p. 28-42, Nov 30 2015.

RAPPORT, M. M.; GREEN, A. A.; PAGE, I. H. Serum vasoconstrictor, serotonin; chemical inactivation. **J Biol Chem,** v. 176, n. 3, p. 1237-41, Dec 1948.

RASHEED, M. Z.; TABASSUM, H.; PARVEZ, S. Mitochondrial permeability transition pore: a promising target for the treatment of Parkinson's disease. **Protoplasma**, Jan 29 2016.

REZNICK, A. Z.; PACKER, L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. **Methods Enzymol,** v. 233, p. 357-63, 1994.

ROBINSON, J.; COOPER, J. M. Method of determining oxygen concentrations in biological media, suitable for calibration of the oxygen electrode. **Anal Biochem,** v. 33, n. 2, p. 390-9, Feb 1970.

RONCHI, J. A. et al. Increased Susceptibility of Gracilinanus microtarsus Liver Mitochondria to Ca(2)(+)-Induced Permeability Transition Is Associated with a More Oxidized State of NAD(P). **Oxid Med Cell Longev,** v. 2015, p. 940627, 2015.

RUI, L. Energy metabolism in the liver. **Compr Physiol,** v. 4, n. 1, p. 177-97, Jan 2014.

RUSSMANN, S.; KULLAK-UBLICK, G. A.; GRATTAGLIANO, I. Current concepts of mechanisms in drug-induced hepatotoxicity. **Curr Med Chem,** v. 16, n. 23, p. 3041-53, 2009.

SANCHEZ, S. et al. Circadian variations of serotonin in plasma and different brain regions of rats. **Mol Cell Biochem,** v. 317, n. 1-2, p. 105-11, Oct 2008.

SANDLER, M.; REVELEY, M. A.; GLOVER, V. Human platelet monoamine oxidase activity in health and disease: a review. **J Clin Pathol,** v. 34, n. 3, p. 292-302, Mar 1981.

SANGLE, G. V. et al. Impairment of mitochondrial respiratory chain activity in aortic endothelial cells induced by glycated low-density lipoprotein. **Free Radic Biol Med,** v. 48, n. 6, p. 781-90, Mar 15 2010.

SANTO-DOMINGO, J.; WIEDERKEHR, A.; DE MARCHI, U. Modulation of the matrix redox signaling by mitochondrial Ca(2.). **World J Biol Chem,** v. 6, n. 4, p. 310-23, Nov 26 2015.

SAVELIEVA, K. V. et al. Genetic disruption of both tryptophan hydroxylase genes dramatically reduces serotonin and affects behavior in models sensitive to antidepressants. **PLoS One,** v. 3, n. 10, p. e3301, 2008.

SAWYER, E. K.; HOWELL, L. L. Pharmacokinetics of fluoxetine in rhesus macaques following multiple routes of administration. **Pharmacology,** v. 88, n. 1-2, p. 44-9, 2011.

SCHNEIDER, W. C.; HOGEBOOM, G. H. Cytochemical studies of mammalian tissues; the isolation of cell components by differential centrifugation: a review. **Cancer Res,** v. 11, n. 1, p. 1-22, Jan 1951.

SILVA, C. M. et al. Postnatal fluoxetine treatment affects the development of serotonergic neurons in rats. **Neurosci Lett,** v. 483, n. 3, p. 179-83, Oct 15 2010.

SINHA, K. et al. Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis. **Arch Toxicol,** v. 87, n. 7, p. 1157-80, Jul 2013.

SKULACHEV, V. P. Fatty acid circuit as a physiological mechanism of uncoupling of oxidative phosphorylation. **FEBS Lett,** v. 294, n. 3, p. 158-62, Dec 09 1991.

_____. Uncoupling: new approaches to an old problem of bioenergetics. **Biochim Biophys Acta,** v. 1363, n. 2, p. 100-24, Feb 25 1998.

SLUSE, F. E.; JARMUSZKIEWICZ, W. Uncoupling proteins outside the animal and plant kingdoms: functional and evolutionary aspects. **FEBS Lett,** v. 510, n. 3, p. 117-20, 2002.

SLUSE, F. E. et al. Mitochondrial UCPs: new insights into regulation and impact. **Biochim Biophys Acta**, p. 6, 2006.

SMITH, E. M. et al. In vitro inhibition of cytochrome P450-mediated reactions by gemfibrozil, erythromycin, ciprofloxacin and fluoxetine in fish liver microsomes. **Aquat Toxicol,** v. 109, p. 259-66, Mar 2012.

SONEI, N. et al. Mitochondrial dysfunction bridges negative affective disorders and cardiomyopathy in socially isolated rats: Pros and cons of fluoxetine. **World J Biol Psychiatry**, p. 1-15, Mar 31 2016.

SOUZA, M. E. et al. Effect of fluoxetine on rat liver mitochondria. **Biochem Pharmacol,** v. 48, n. 3, p. 535-41, Aug 03 1994.

SPITELLER, G. Are changes of the cell membrane structure causally involved in the aging process? **Ann N Y Acad Sci,** v. 959, p. 30-44, 2002.

STANIEK, K.; NOHL, H. Are mitochondria a permanent source of reactive oxygen species? **Biochim Biophys Acta,** v. 1460, n. 2-3, p. 268-75, Nov 20 2000.

STARKOV, A. A. et al. Mitochondrial alpha-ketoglutarate dehydrogenase complex generates reactive oxygen species. **J Neurosci,** v. 24, n. 36, p. 7779-88, Sep 08 2004.

STRUMPER, D. et al. Effects of antidepressants on function and viability of human neutrophils. **Anesthesiology,** v. 98, n. 6, p. 1356-62, Jun 2003.

SULLIVAN, E. C.; MENDOZA, S. P.; CAPITANIO, J. P. Similarity in temperament between mother and offspring rhesus monkeys: sex differences and the role of monoamine oxidase-a and serotonin transporter promoter polymorphism genotypes. **Dev Psychobiol,** v. 53, n. 6, p. 549-63, Sep 2011.

SUN, X. et al. Circadian 5-HT production regulated by adrenergic signaling. **Proc Natl Acad Sci U S A,** v. 99, n. 7, p. 4686-91, Apr 02 2002.

TAHARA, E. B.; NAVARETE, F. D.; KOWALTOWSKI, A. J. Tissue-, substrate-, and site-specific characteristics of mitochondrial reactive oxygen species generation. **Free Radic Biol Med,** v. 46, n. 9, p. 1283-97, May 1 2009.

TANG, W.; LU, A. Y. Metabolic bioactivation and drug-related adverse effects: current status and future directions from a pharmaceutical research perspective. **Drug Metab Rev,** v. 42, n. 2, p. 225-49, May 2010.

TRETTER, L.; ADAM-VIZI, V. Generation of reactive oxygen species in the reaction catalyzed by alpha-ketoglutarate dehydrogenase. **J Neurosci,** v. 24, n. 36, p. 7771-8, Sep 08 2004.

TURRENS, J. F. Mitochondrial formation of reactive oxygen species. **J Physiol,** v. 552, n. Pt 2, p. 335-44, Oct 15 2003.

VALKO, M. et al. Free radicals and antioxidants in normal physiological functions and human disease. **Int J Biochem Cell Biol,** v. 39, n. 1, p. 44-84, 2007.

VAN DER LAAN, M.; HORVATH, S. E.; PFANNER, N. Mitochondrial contact site and cristae organizing system. **Curr Opin Cell Biol,** v. 41, p. 33-42, 2016.

VAN ZUTPHEN, L. F. Toxicity testing and genetic quality control. **J Exp Anim Sci,** v. 35, n. 5-6, p. 202-9, Sep 1993.

VASWANI, M.; LINDA, F. K.; RAMESH, S. Role of selective serotonin reuptake inhibitors in psychiatric disorders: a comprehensive review. **Prog Neuropsychopharmacol Biol Psychiatry,** v. 27, n. 1, p. 85-102, Feb 2003.

VAZQUEZ-ACEVEDO, M. et al. Dissecting the peripheral stalk of the mitochondrial ATP synthase of chlorophycean algae. **Biochim Biophys Acta,** v. 10, n. 16, p. 30022-6, 2016.

VERCESI, A. E. et al. Ca2+-dependent NAD(P)+-induced alterations of rat liver and hepatoma mitochondrial membrane permeability. **Biochem Biophys Res Commun,** v. 154, n. 3, p. 934-41, Aug 15 1988.

VERCESI, A. E. et al. ATP and Ca2+ homeostasis in Trypanosoma cruzi. **Braz J Med Biol Res,** v. 26, n. 4, p. 355-63, Apr 1993.

VERCESI, A. E. et al. Thapsigargin causes Ca2+ release and collapse of the membrane potential of Trypanosoma brucei mitochondria in situ and of isolated rat liver mitochondria. **J Biol Chem,** v. 268, n. 12, p. 8564-8, Apr 25 1993.

VIDAL-PUIG, A. J. et al. Energy metabolism in uncoupling protein 3 gene knockout mice. **J Biol Chem,** v. 275, n. 21, p. 16258-66, May 26 2000.

WANG, H. et al. Visualizing liver anatomy, physiology and pharmacology using multiphoton microscopy. **J Biophotonics,** v. 10, n. 1, p. 46-60, Jan 2017.

WILDE, M. I.; BENFIELD, P. Fluoxetine. A pharmacoeconomic review of its use in depression. **Pharmacoeconomics,** v. 13, n. 5 Pt 1, p. 543-61, 1998.

WINTERBOURN, C. C. Biological reactivity and biomarkers of the neutrophil oxidant, hypochlorous acid. **Toxicology,** v. 181-182, p. 223-7, Dec 27 2002.

YARON, I. et al. Fluoxetine and amitriptyline inhibit nitric oxide, prostaglandin E2, and hyaluronic acid production in human synovial cells and synovial tissue cultures. **Arthritis Rheum,** v. 42, n. 12, p. 2561-8, Dec 1999.

YOUDIM, M. B.; EDMONDSON, D.; TIPTON, K. F. The therapeutic potential of monoamine oxidase inhibitors. **Nat Rev Neurosci,** v. 7, n. 4, p. 295-309, Apr 2006.

ZAFIR, A.; BANU, N. Antioxidant potential of fluoxetine in comparison to Curcuma longa in restraint-stressed rats. **Eur J Pharmacol,** v. 572, n. 1, p. 23-31, Oct 15 2007.

ZHANG, F. et al. Fluoxetine protects neurons against microglial activation-mediated neurotoxicity. **Parkinsonism Relat Disord,** v. 18 Suppl 1, p. S213-7, Jan 2012.

ZLATKOVIC, J. et al. Chronic administration of fluoxetine or clozapine induces oxidative stress in rat liver: a histopathological study. **Eur J Pharm Sci,** v. 59, p. 20-30, Aug 01 2014.

ZOROV, D. B. et al. Regulation and pharmacology of the mitochondrial permeability transition pore. **Cardiovasc Res,** v. 83, n. 2, p. 213-25, 2009.

ANEXO A – Parecer do Comitê de Ética em Pesquisa

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Recife, 02 de maio de 2012.

Ofício nº 434/12

Da Comissão de Ética no Uso de Animais (CEUA) da UFPE
Para: **Prof.Raul Manhães de Castro**
Departamento de Nutrição
Universidade Federal de Pernambuco
Processo nº 23076.015276/2012-56

Os membros da Comissão de Ética no Uso de Animais do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (CEUA-UFPE) avaliaram seu projeto de pesquisa intitulado, **"INIBIÇÃO DA RECAPTAÇÃO DE SEROTONINA DURANTE O DESENVOLVIMENTO: UM ESTUDO DO BALANÇO ENERGÉTICO E DA FUNÇÃO MITOCONDRIAL".**

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

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

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

Origem dos animais: Departamento de Nutrição-UFPE; Animais: Ratos; Linhagem: Wistar; Sexo: Machos e Fêmeas; número de animais previsto no protocolo:8 ratas lactantes e 64 filhotes; Peso: Ratas 240-260g e filhotes 6-7g; Idade: Ratas adultas e seus filhotes.

Atenciosamente,

Profa. Maria Teresa Jansem
Prosidente do CEEA

CCB: Integrar para desenvolver

ANEXO B – Artigo de coautoria

Developmental Origins of Cardiometabolic Diseases: Role of the Maternal Diet

João H. Costa-Silva*, **Aiany C. Simões-Alves** and Mariana P. Fernandes

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Developmental Origins of Cardiometabolic Diseases: Role of the Maternal Diet

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Developmental origins of cardiometabolic diseases have been related to maternal nutritional conditions. In this context, the rising incidence of arterial hypertension, diabetes type II, and dyslipidemia has been attributed to genetic programming. Besides, environmental conditions during perinatal development such as maternal undernutrition or overnutrition can program changes in the integration among physiological systems leading to cardiometabolic diseases. This phenomenon can be understood in the context of the phenotypic plasticity and refers to the adjustment of a phenotype in response to environmental input without genetic change, following a novel, or unusual input during development. Experimental studies indicate that fetal exposure to an adverse maternal environment may alter the morphology and physiology that contribute to the development of cardiometabolic diseases. It has been shown that both maternal protein restriction and overnutrition alter the central and peripheral control of arterial pressure and metabolism. This review will address the new concepts on the maternal diet induced-cardiometabolic diseases that include the potential role of the perinatal malnutrition.

Keywords: developmental plasticity, perinatal nutrition, cardiometabolic control, protein restriction

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INTRODUCTION

Cardiovascular and metabolic diseases, such as hypertension, type II diabetes, and dyslipidemia are highly prevalent in the world and have important effects on the public health, increasing risk factors for the development of other diseases, including coronary heart disease, stroke, and heart failure (Landsberg et al., 2013). The etiology of these cardiometabolic diseases includes a complex phenotype that arises from numerous genetic, environmental, nutritional, behavioral, and ethnic origins (Landsberg et al., 2013; Ng et al., 2014). In this regard, it has been observed that the eating habits and behaviors and nutritional condition in early phases of life may play a key role on the etiology of these diseases by inducing physiological dysfunctions (Lucas, 1998; Victora et al., 2008; Wells, 2012). This phenomenon can be understood in the context of phenotypic plasticity and it refers to the ability of an organism to react to both an internal and external environmental inputs with a change in the form, state, physiology, or rate of activity without genetic changes (West-Eberhard, 2005b). Indeed the nutritional factors rise as important element in this theme and it has been highlighted since Barker (Barker, 1990, 1994, 1995, 1998, 1999a,b, 2000; Barker and Martyn, 1992; Fall and Barker, 1997; Osmond and Barker, 2000). In this context, new evidence from epidemiological and clinical studies have showed the association of the maternal under- and

overnutrition with development of cardiometabolic dysfuntions (Ashton, 2000; Hemachandra et al., 2006; Antony and Laxmaiah, 2008; Conde and Monteiro, 2014; Costa-Silva et al., 2015; Parra et al., 2015). Thus, this review will address the new concepts about the involvement of the maternal protein malnutrition and overnutrition on the development of the cardiometabolic diseases.

PERINATAL ORIGIN OF CARDIOMETABOLIC DISEASES: THE ROLE OF PHENOTYPIC PLASTICITY

Biological and medical consequences of perinatal nutritional factors have been extensively studied in the field of the "developmental origins of health and diseases" proposed by Barker and colleagues since 1986 (Barker and Osmond, 1986; Barker et al., 1989, 1993; Barker, 2007). This field of research proposes that cardiometabolic diseases can be "programmed" by the "adaptative" effects of both under- and overnutrition during early phases of growth and development on the cell physiology (Barker and Osmond, 1986; Hales and Barker, 1992; Alfaradhi and Ozanne, 2011; Chavatte-Palmer et al., 2016). As stated before, it aims to study how an organism reacts to a different environmental input, such as malnutrition, and induces changes in the phenotype, but without altering the genotype (Barker et al., 2005; West-Eberhard, 2005a; Labayen et al., 2006; Andersen et al., 2009; Biosca et al., 2011). In this context, epigenetic alterations, such as DNA methylation, histone acetylation, and microRNA expression are considered the molecular basis of the phenotypic plasticity (Wells, 2011). These modifications termed as "epigenetic" were firstly described by Conrad Waddigton in 1940 and it studies the relationship between cause and effect in the genes to produce a phenotype (Jablonka and Lamb, 2002). Nowadays, this concept is employed to describe the process of the gene expression and its linking to modifications in the cromatin structure without altering DNA sequence (Chong and Whitelaw, 2004; Egger et al., 2004). Among all epigenetic modifications, the DNA methylation is one that has been best studied and is related to addition of methyl groups on DNA cytosine residues, normally on the cytosine followed by guanine residue (CpG dinucleotides), which can produce inhibition of the gene expression by impairing transcriptional factor binding (Waterland and Michels, 2007; Mansego et al., 2013; Chango and Pogribny, 2015; Mitchell et al., 2016). In this context, it has been investigated how nutritional aspect may induce these epigenetic modifications.

Macro- and micro-nutrient compositions have been identified as important nutritional factors inducing epigenetic processes, such as DNA methylation (Mazzio and Soliman, 2014; Szarc vel Szic et al., 2015). It is considered at least three ways by which nutrients can induce DNA methylation, alter gene expression, and modify cellular phenotype: (i) by providing methyl group supply for inducing S- adenosyl-L-methionine formation (genomic DNA methylation), modifying the methyltransferase activity, or impairing DNA demethylation process; (ii) by modifying chromatin remodeling, or lysine and arginine residues in the N-terminal histone tails; and (iii) by altering microRNA expression (Chong and Whitelaw, 2004; Egger et al., 2004; Hardy and Tollefsbol, 2011; Stone et al., 2011). In this context, altered contents of amino acids, such as methionine and cysteine, as well as reduced choline and folate diet amount can modify the process of the DNA methylation leading to both DNA hyper- and hypomethylation (Fiorito et al., 2014). For example, deficiency of choline can precipitate DNA hypermethylation associated with organ dysfunction, mainly in liver metabolism (Karlic and Varga, 2011; Wei, 2013).

High fat diet (HFD) during perinatal period has been identified as risk factor to predispose and induce epigenetic processes in the parents and their offspring (Mazzio and Soliman, 2014; Szarc vel Szic et al., 2015). Both hypo- and hypermethylation processes participate in this dysregulation attributed to HFD consumption (Ng et al., 2010; Milagro et al., 2013). In adipose tissue, for example, it was observed that gene promoter of the fatty acid synthase enzyme suffered methylation (Lomba et al., 2010) and that important obesity-related genes such as leptin have disruption on their methylation status (Milagro et al., 2009).

MATERNAL PROTEIN UNDERNUTRITION: EARLY- AND LONG-TERM OUTCOMES

Maternal malnutrition is associated with the risk of developing cardiovascular disease and co-morbidities in offspring's later life including hypertension, metabolic syndrome, and type-II diabetes (Barker et al., 2007; Nuyt, 2008; Nuyt and Alexander, 2009). In humans, studies have provided support for the positive association between low birth weight and increased incidence of hypertension (Ravelli et al., 1976; Hales et al., 1991; Sawaya and Roberts, 2003; Sawaya et al., 2004).

Maternal low-protein diet model during both gestation and lactation is one of the most extensively studied animal models of phenotypic plasticity (Ozanne and Hales, 2004; Costa-Silva et al., 2009; Falcão-Tebas et al., 2012; Fidalgo et al., 2013; de Brito Alves et al., 2014; Barros et al., 2015). Feeding a low-protein diet (8% protein) during gestation and lactation is associated with growth restriction, asymmetric reduction in organ growth, elevated systolic blood pressure, dyslipidemia, and increased fasting plasma insulin concentrations in the most of studies in rodents (Ozanne and Hales, 2004; Costa-Silva et al., 2009; Falcão-Tebas et al., 2012; Fidalgo et al., 2013; Leandro et al., 2012; de Brito Alves et al., 2014, 2016; Ferreira et al., 2015; Paulino-Silva and Costa-Silva, 2016). However, it is known that the magnitude of the cardiovascular and metabolic outcomes are dependent on the both time exposure to protein restricted-diet (Zohdi et al., 2012, 2015) and growth trajectory throughout the postnatal period (Wells, 2007, 2011). A rapid and increased catch-up

Abbreviations: AKT/PKB, Protein kinase B; CB, Carotid body; CNS, Central nervous system; CRP, C-reactive protein; ERK, Extracellular signal-regulated kinase; GSH, Glutathione reduced; HFD, High fat diet; HIF-1α, Hypoxic inducible factor 1 alpha; IGF2, Insulin-like growth factor 2; IL-6, Interleukin-6; IR, Insulin receptor; IRS, Insulin receptor substrate; mTOR, Mammalian target of rapamycin; PI3K, Phosphatidylinositol 3-kinase; RAS, Renin-angiotensin system; ROS, Reactive oxygen species; TNF-α, Tumor necrosis factor alpha.

growth and childhood weight gain appear to augment metabolic disruption in end organs, for example liver (Tarry-Adkins et al., 2016; Wang et al., 2016).

Although, the relationship between maternal protein restriction, sympathetic overactivity and hypertension have been suggested (Johansson et al., 2007; Franco et al., 2008; Barros et al., 2015), few studies have described the physiological dysfunctions responsible for producing these effects. Nowadays, it is well accepted that perinatal protein malnutrition raise risks of hypertension by mechanisms that include abnormal vascular function (Franco Mdo et al., 2002; Brawley et al., 2003; Franco et al., 2008), altered nephron morphology and function, and stimulation of the renin-angiotensin system (RAS) (Nuyt and Alexander, 2009; Siddique et al., 2014). Recently, studies have highlighted contribution of the sympathetic overactivity associated to enhanced respiratory rhythm and O_2/CO_2 sensitivity on the development of the maternal low-protein diet-induced hypertension by mechanisms independent of the baroreflex function (Chen et al., 2010; Barros et al., 2015; Costa-Silva et al., 2015; de Brito Alves et al., 2015; Paulino-Silva and Costa-Silva, 2016). Offspring from dams subjected to perinatal protein restriction had relevant short-term effects on the carotid body (CB) sensitivity and respiratory control. With enhanced baseline sympathetic activity and amplified ventilatory and sympathetic responses to peripheral chemoreflex activation, prior to the establishment of hypertension (de Brito Alves et al., 2014, 2015). The underlying mechanism involved in these effects seems to be linked with up-regulation of hypoxic inducible factor (HIF-1α) in CB peripheral chemoreceptors (Ito et al., 2011, 2012; de Brito Alves et al., 2015). However, the epigenetic mechanisms in these effects are still unclear. It is hypothesized that epigenetic mechanism produced by DNA methylation could be involved (Altobelli et al., 2013; Prabhakar, 2013; Nanduri and Prabhakar, 2015).

The central nervous system (CNS) compared to other organ systems has increased vulnerability to reactive oxygen species (ROS). ROS are known to modulate the sympathetic activity and their increased production in key brainstem sites is involved in the etiology of several cardiovascular diseases, for example, diseases caused by sympathetic overexcitation, such as neurogenic hypertension (Chan et al., 2006; Essick and Sam, 2010). Ferreira and colleagues showed that perinatal protein undernutrition increased lipid peroxidation and decreased the activity of several antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activities) as well as elements of the GSH system, in adult brainstem. Dysfunction in the brainstem oxidative metabolism, using the same experimental model, were observed in rats immediately after weaning associated to the increase in ROS production, with a decrease in antioxidant defense and redox status (Ferreira et al., 2015, 2016). Related to the metabolic effects on the heart, it was observed that these animals showed decreased mitochondrial oxidative phosphorylation capacity and increased ROS in the myocardium. In addition, maternal low-protein diet induced a significant decrease in enzymatic antioxidant capacity (superoxide dismutase, catalase, glutathione-S-transferase, and glutathione reductase activities) and glutathione level when compared with normoprotein group (Nascimento et al., 2014).

Regarding hepatic metabolism, studies showed that protein restricted rats had suppressed gluconeogenesis by a mechanism primarily mediated by decrease on the mRNA level of hepatic phosphoenolpyruvate carboxykinase, a key gluconeogenic enzyme, and enhancement of the insulin signals through the insulin receptor (IR)/IR substrate (IRS)/phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin complex 1 (mTOR) pathway in the liver (Toyoshima et al., 2010). In relation to lipid metabolism, there was decreased liver triglyceride content in adult rats exposed to protein restriction during gestation and lactation. It was suggested that this effect could be due to increased fatty-acid transport into the mitochondrial matrix or alterations in triglyceride biosynthesis (Qasem et al., 2015). A maternal protein restriction was shown to reduce the lean and increase the fat contents of 6-month old offspring with a tendency for reduced number of muscle myofibers associated with reduced expression of mRNA of Insulin-like growth factor 2 gene (IGF2 mRNA) in pigs (Chavatte-Palmer et al., 2016).

MATERNAL OVERNUTRITION AND RISK FACTOR FOR THE CARDIOMETABOLIC DYSFUNTIONS

Nutritional transition is a phenomenon well documented in developing countries in the twentieth and twenty-first centuries, and has induced high incidence of the chronic diseases and high prevalence of the obesity (Batista Filho and Rissin, 2003; Batista Filho and Batista, 2010; Ribeiro et al., 2015). It is evident that protein malnutrition was an health problem in the first half of the twentieth century. Now, it was replaced by a diet enriched in saturated fat or other HFDs, predisposing to overweight, and obesity (Batista et al., 2013). Nowadays, it suggested that two billion people in the world are overweight and obese individuals, with major prevalence is related to diet induced-obesity, which have been associated to cardiovascular and endocrine dysfunctions (Hotamisligil, 2006; Aubin et al., 2008; Zhang et al., 2012; Ng et al., 2014; Wensveen et al., 2015).

Recently, the obesity has been considered a physiological state of chronic inflammation, characterized by elevated levels of inflammatory markers including C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) (Wensveen et al., 2015; Erikci Ertunc and Hotamisligil, 2016; Lyons et al., 2016). Maternal HFD chronic consumption enhances the circulating free fatty acids and induce the activation of inflammatory pathways, enhancing chronic inflammation in offspring (Gruber et al., 2015). Studies of Roberts et al. (2015) found that cardiometabolic dysfunction was associated with changes such as elevated serum triglycerides, elevated oxidative stress levels, insulin resistance, vascular disorders, and development of hypertension (Roberts et al., 2015).

In animals on a HFD the hormone leptin has been considered one of the most important physiological mediators of the cardiometabolic dysfunction (Correia and Rahmouni, 2006; Harlan et al., 2013; Harlan and Rahmouni, 2013). Since

hyperleptinemia, common in overweight and obesity conditions, produce a misbalance in autonomic system, with sympathetic overactivation (Machleidt et al., 2013; Kurajoh et al., 2015; Manna and Jain, 2015), and reduced sensitivity of vagal afferent neurons (de Lartigue, 2016). This disorder of vagal afferent signaling can activate orexigenic pathways in the CNS and drive hyperphagia, obesity, and cardiometabolic diseases at long-term (de Lartigue, 2016). Some authors have described that, at least in part, cardiovascular dysfuntion elicited by HFD or obesity may be due to changes in the neural control of respiratory and autonomic systems (Bassi et al., 2012, 2015; Hall et al., 2015; Chaar et al., 2016). Part of these effects were suggested to be influenced by atrial natriuretric peptide and renin-angiotensin pathways (Bassi et al., 2012; Gusmão, 2012).

Interestingly, it has been shown that offspring from mothers fed HFD have high risk to develop pathologic cardiac hypertrophy. This condition would be linked to re-expression of cardiac fetal genes, systolic, and diastolic dysfunction and sympathetic overactivity on the heart. These effects lead to reduced cardioprotective signaling that would predispose them to cardiac dysfunctions in adulthood (Taylor et al., 2005; Wang et al., 2010; Fernandez-Twinn et al., 2012; Blackmore et al., 2014). Regarding arterial blood pressure control, it has been described that maternal HFD induces early and persistent alterations in offspring renal and adipose RAS components (Armitage et al., 2005). These changes seem to be dependent upon the period of exposure to the maternal HFD, and contribute to increased adiposity and hypertension in offspring (Samuelsson et al., 2008; Elahi et al., 2009; Guberman et al., 2013; Mazzio and Soliman, 2014; Tan et al., 2015). Studies in baboons subjected to HFD showed that microRNA expression and putative gene targets involved in developmental disorders and cardiovascular diseases were up-regulated and others were down-regulated. The authors suggested that the epigenetic modifications caused by HFD may be involved in the developmental origins of cardiometabolic diseases (Maloyan et al., 2013).

Other metabolic outcomes induced by HFD have been pointed out in the last years and it has demonstrated that HFD displayed a drastic modification on metabolic control of the glucose metabolism and lead to increased insulin level in serum (Fan et al., 2013) and enhanced insulin action through AKT/PKB (protein kinase B) and ERK (extracellular signal-regulated kinase), and activation of mammalian target of rapamycin (mTOR) pathways in cardiac tissue (Fernandez-Twinn et al., 2012; Fan et al., 2013). Offspring from HFD mothers showed alterations in blood glucose and insulin levels, with high predisposition to insulin resistance and cardiac dysfunction (Taylor et al., 2005; Wang et al., 2010). Part of these effects are associated with enhanced production of ROS and reduction in the levels of the anti-oxidant enzymes, such as superoxide dismutase, suggesting a misbalance in the control of the oxidative stress (Fernandez-Twinn et al., 2012).

Altogether, this review addressed the new concept on the maternal diet induced-cardiometabolic diseases that include the potential role of the perinatal malnutrition. It showed that the etiology of these diseases is multifactorial involving genetic and environmental influences and their physiological integration. It is well recognized that both perinatal undernutrition and overnutrition are related with the risk of developing metabolic syndrome and hypertension in adult life (**Figure 1**). The underlying mechanism can be explained in the context of phenotypic plasticity during development that includes adaptive change on the CNS, heart, kidney, liver, muscle, and adipose tissue metabolisms with consequent physiology dysfunction and

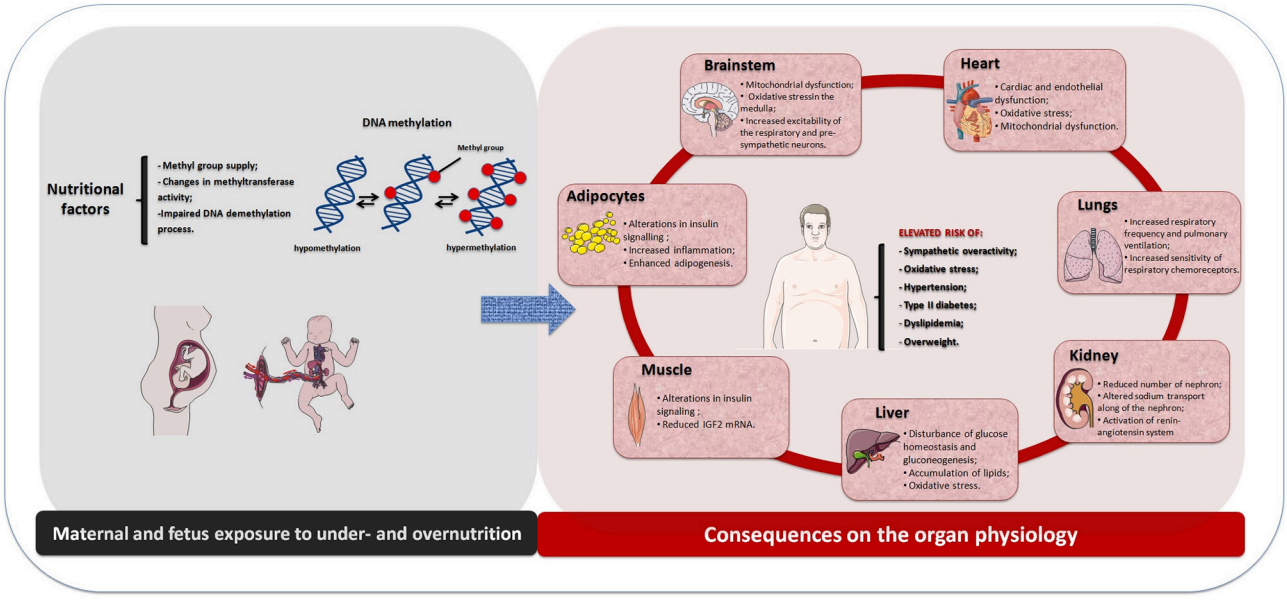

FIGURE 1 | Schematic drawing showing the physiological effects induced by maternal and fetus exposure to under- or overnutrition through DNA methylation and their consequences on the organ physiology and increased risk of the cardiometabolic diseases in the offspring.

with subsequent cardiometabolic diseases. Moreover, maternal undernutrition or overnutrition may predispose epigenetic modifications in dams and their offspring, with predominance of DNA methylation, leading to altered gene expression during development and growth. Further, it can provide a different physiological condition which may contribute to the developmental origins of the cardiometabolic diseases. These physiological dysfunctions seem to be linked to the impaired central and peripheral control of both metabolic and cardiovascular functions by mechanisms that include enhanced sympathetic-respiratory activities and disruption in metabolism of end organs at early life. It is suggested that those effects could be associated to inflammatory conditions and impaired oxidative balance, which may contribute to adult cardiometabolic diseases.

AUTHOR CONTRIBUTIONS

JC, AS, and MF drafted and revised critically the work for important intellectual content and final review of the manuscript.

REFERENCES

Alfaradhi, M. Z., and Ozanne, S. E. (2011). Developmental programming in response to maternal overnutrition. *Front. Genet.* 2:27. doi: 10.3389/fgene.2011.00027

Altobelli, G., Bogdarina, I. G., Stupka, E., Clark, A. J., and Langley-Evans, S. (2013). Genome-wide methylation and gene expression changes in newborn rats following maternal protein restriction and reversal by folic acid. *PLoS ONE* 8:e82989. doi: 10.1371/journal.pone.0082989

Andersen, L. G., Angquist, L., Gamborg, M., Byberg, L., Bengtsson, C., Canoy, D., et al. (2009). Birth weight in relation to leisure time physical activity in adolescence and adulthood: meta-analysis of results from 13 nordic cohorts. *PLoS ONE* 4:e8192. doi: 10.1371/journal.pone.0008192

Antony, G. M., and Laxmaiah, A. (2008). Human development, poverty, health & nutrition situation in India. *Indian J. Med. Res.* 128, 198–205.

Armitage, J. A., Lakasing, L., Taylor, P. D., Balachandran, A. A., Jensen, R. I., Dekou, V., et al. (2005). Developmental programming of aortic and renal structure in offspring of rats fed fat-rich diets in pregnancy. *J. Physiol.* 565(Pt 1), 171–184. doi: 10.1113/jphysiol.2005.084947

Ashton, N. (2000). Perinatal development and adult blood pressure. *Braz. J. Med. Biol. Res.* 33, 731–740. doi: 10.1590/S0100-879X2000000700002

Aubin, M. C., Lajoie, C., Clément, R., Gosselin, H., Calderone, A., and Perrault, L. P. (2008). Female rats fed a high-fat diet were associated with vascular dysfunction and cardiac fibrosis in the absence of overt obesity and hyperlipidemia: therapeutic potential of resveratrol. *J. Pharmacol. Exp. Ther.* 325, 961–968. doi: 10.1124/jpet.107.135061

Barker, D. J. (1990). The fetal and infant origins of adult disease. *BMJ* 301:1111. doi: 10.1136/bmj.301.6761.1111

Barker, D. J. (1994). Maternal and fetal origins of coronary heart disease. *J. R. Coll. Physicians Lond.* 28, 544–551.

Barker, D. J. (1995). The wellcome foundation lecture, 1994. The fetal origins of adult disease. *Proc. Biol. Sci.* 262, 37–43. doi: 10.1098/rspb.1995.0173

Barker, D. J. (1998). *In utero* programming of chronic disease. *Clin. Sci.* 95, 115–128. doi: 10.1042/cs0950115

Barker, D. J. (1999a). Fetal origins of cardiovascular disease. *Ann. Med.* 31(Suppl. 1), 3–6.

Barker, D. J. (1999b). The long-term outcome of retarded fetal growth. *Schweiz. Med. Wochenschr.* 129, 189–196.

Barker, D. J. (2000). *In utero* programming of cardiovascular disease. *Theriogenology* 53, 555–574. doi: 10.1016/S0093-691X(99)00258-7

Barker, D. J. (2007). The origins of the developmental origins theory. *J. Intern. Med.* 261, 412–417. doi: 10.1111/j.1365-2796.2007.01809.x

Barker, D. J., Gluckman, P. D., Godfrey, K. M., Harding, J. E., Owens, J. A., and Robinson, J. S. (1993). Fetal nutrition and cardiovascular disease in adult life. *Lancet* 341, 938–941. doi: 10.1016/0140-6736(93)91224-A

Barker, D. J., and Martyn, C. N. (1992). The maternal and fetal origins of cardiovascular disease. *J. Epidemiol. Commun. Health* 46, 8–11. doi: 10.1136/jech.46.1.8

Barker, D. J., and Osmond, C. (1986). Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* 1, 1077–1081. doi: 10.1016/S0140-6736(86)91340-1

Barker, D. J., Osmond, C., Forsén, T. J., Kajantie, E., and Eriksson, J. G. (2005). Trajectories of growth among children who have coronary events as adults. *N. Engl. J. Med.* 353, 1802–1809. doi: 10.1056/NEJMoa044160

Barker, D. J., Osmond, C., Forsen, T. J., Kajantie, E., and Eriksson, J. G. (2007). Maternal and social origins of hypertension. *Hypertension.* 50, 565–571. doi: 10.1161/HYPERTENSIONAHA.107.091512

Barker, D. J., Winter, P. D., Osmond, C., Margetts, B., and Simmonds, S. J. (1989). Weight in infancy and death from ischaemic heart disease. *Lancet* 2, 577–580. doi: 10.1016/S0140-6736(89)90710-1

Barros, M. A., De Brito Alves, J. L., Nogueira, V. O., Wanderley, A. G., and Costa-Silva, J. H. (2015). Maternal low-protein diet induces changes in the cardiovascular autonomic modulation in male rat offspring. *Nutr. Metab. Cardiovasc. Dis.* 25, 123–130. doi: 10.1016/j.numecd.2014.07.011

Bassi, M., Giusti, H., Leite, C. M., Anselmo-Franci, J. A., do Carmo, J. M., da Silva, A. A., et al. (2012). Central leptin replacement enhances chemorespiratory responses in leptin-deficient mice independent of changes in body weight. *Pflugers Arch.* 464, 145–153. doi: 10.1007/s00424-012-1111-1

Bassi, M., Nakamura, N. B., Furuya, W. I., Colombari, D. S., Menani, J. V., do Carmo, J. M., et al. (2015). Activation of the brain melanocortin system is required for leptin-induced modulation of chemorespiratory function. *Acta Physiol. (Oxf).* 213, 893–901. doi: 10.1111/apha.12394

Batista, T. M., Ribeiro, R. A., da Silva, P. M., Camargo, R. L., Lollo, P. C., Boschero, A. C., et al. (2013). Taurine supplementation improves liver glucose control in normal protein and malnourished mice fed a high-fat diet. *Mol. Nutr. Food Res.* 57, 423–434. doi: 10.1002/mnfr.201200345

Batista Filho, M., and Batista, L. V. (2010). Transição alimentar/nutricional ou mutação antropológica? *Ciênc. Cult.* 62, 26–30.

Batista Filho, M., and Rissin, A. (2003). A transição nutricional no Brasil: tendências regionais e temporais. *Cad. Saúde Pública* 19, S181–S191. doi: 10.1590/S0102-311X2003000700019

Biosca, M., Rodríguez, G., Ventura, P., Samper, M. P., Labayen, I., Collado, M. P., et al. (2011). Central adiposity in children born small and large for gestational age. *Nutr. Hosp.* 26, 971–976. doi: 10.1590/S0212-16112011000500008

Blackmore, H. L., Niu, Y., Fernandez-Twinn, D. S., Tarry-Adkins, J. L., Giussani, D. A., and Ozanne, S. E. (2014). Maternal diet-induced obesity programs cardiovascular dysfunction in adult male mouse offspring independent of current body weight. *Endocrinology* 155, 3970–3980. doi: 10.1210/en.2014-1383

Brawley, L., Itoh, S., Torrens, C., Barker, A., Bertram, C., Poston, L., et al. (2003). Dietary protein restriction in pregnancy induces hypertension and vascular defects in rat male offspring. *Pediatr. Res.* 54, 83–90. doi: 10.1203/01.PDR.0000065731.00639.02

Chaar, L. J., Coelho, A., Silva, N. M., Festuccia, W. L., and Antunes, V. R. (2016). High-fat diet-induced hypertension and autonomic imbalance are associated with an upregulation of CART in the dorsomedial hypothalamus of mice. *Physiol. Rep.* 4:e12811. doi: 10.14814/phy2.12811

Chan, S. H., Tai, M. H., Li, C. Y., and Chan, J. Y. (2006). Reduction in molecular synthesis or enzyme activity of superoxide dismutases and catalase contributes to oxidative stress and neurogenic hypertension in spontaneously hypertensive rats. *Free Radic. Biol. Med.* 40, 2028–2039. doi: 10.1016/j.freeradbiomed.2006.01.032

Chango, A., and Pogribny, I. P. (2015). Considering maternal dietary modulators for epigenetic regulation and programming of the fetal epigenome. *Nutrients* 7, 2748–2770. doi: 10.3390/nu7042748

Chavatte-Palmer, P., Tarrade, A., and Rousseau-Ralliard, D. (2016). Diet before and during pregnancy and offspring health: the importance of animal models and what can be learned from them. *Int. J. Environ. Res. Public Health* 13:E586. doi: 10.3390/ijerph13060586

Chen, J. H., Tarry-Adkins, J. L., Matharu, K., Yeo, G. S., and Ozanne, S. E. (2010). Maternal protein restriction affects gene expression profiles in the kidney at weaning with implications for the regulation of renal function and lifespan. *Clin. Sci.* 119, 373–384. doi: 10.1042/CS20100230

Chong, S., and Whitelaw, E. (2004). Epigenetic germline inheritance. *Curr. Opin. Genet. Dev.* 14, 692–696. doi: 10.1016/j.gde.2004.09.001

Conde, W. L., and Monteiro, C. A. (2014). Nutrition transition and double burden of undernutrition and excess of weight in Brazil. *Am. J. Clin. Nutr.* 100, 1617S–1622S. doi: 10.3945/ajcn.114.084764

Correia, M. L., and Rahmouni, K. (2006). Role of leptin in the cardiovascular and endocrine complications of metabolic syndrome. *Diabetes Obes. Metab.* 8, 603–610. doi: 10.1111/j.1463-1326.2005.00562.x

Costa-Silva, J. H., Silva, P. A., Pedi, N., Luzardo, R., Einicker-Lamas, M., Lara, L. S., et al. (2009). Chronic undernutrition alters renal active Na+ transport in young rats: potential hidden basis for pathophysiological alterations in adulthood? *Eur. J. Nutr.* 48, 437–445. doi: 10.1007/s00394-009-0032-z

Costa-Silva, J. H., de Brito-Alves, J. L., Barros, M. A., Nogueira, V. O., Paulino-Silva, K. M., de Oliveira-Lira, A., et al. (2015). New insights on the maternal diet induced-hypertension: potential role of the phenotypic plasticity and sympathetic-respiratory overactivity. *Front. Physiol.* 6:345. doi: 10.3389/fphys.2015.00345

de Brito Alves, J. L., de Oliveira, J. M., Ferreira, D. J., de Barros, M. A., Nogueira, V. O., Alves, D. S., et al. (2016). Maternal protein restriction induced-hypertension is associated to oxidative disruption at transcriptional and functional levels in the medulla oblongata. *Clin. Exp. Pharmacol. Physiol.* doi: 10.1111/1440-1681.12667. [Epub ahead of print].

de Brito Alves, J. L., Nogueira, V. O., Cavalcanti Neto, M. P., Leopoldino, A. M., Curti, C., Colombari, D. S., et al. (2015). Maternal protein restriction increases respiratory and sympathetic activities and sensitizes peripheral chemoreflex in male rat offspring. *J. Nutr.* 145, 907–914. doi: 10.3945/jn.114.202804

de Brito Alves, J. L., Nogueira, V. O., de Oliveira, G. B., da Silva, G. S., Wanderley, A. G., Leandro, C. G., et al. (2014). Short- and long-term effects of a maternal low-protein diet on ventilation, O_2/CO_2 chemoreception and arterial blood pressure in male rat offspring. *Br. J. Nutr.* 111, 606–615. doi: 10.1017/S0007114513002833

de Lartigue, G. (2016). Role of the vagus nerve in the development and treatment of diet-induced obesity. *J. Physiol.* 594, 5791–5815. doi: 10.1113/JP271538

Egger, G., Liang, G., Aparicio, A., and Jones, P. A. (2004). Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 429, 457–463. doi: 10.1038/nature02625

Elahi, M. M., Cagampang, F. R., Mukhtar, D., Anthony, F. W., Ohri, S. K., and Hanson, M. A. (2009). Long-term maternal high-fat feeding from weaning through pregnancy and lactation predisposes offspring to hypertension, raised plasma lipids and fatty liver in mice. *Br. J. Nutr.* 102, 514–519. doi: 10.1017/S000711450820749X

Erikci Ertunc, M., and Hotamisligil, G. S. (2016). Lipid signaling and lipotoxicity in metabolic inflammation: indications for metabolic disease pathogenesis and treatment. *J. Lipid Res.* doi: 10.1194/jlr.r066514. [Epub ahead of print].

Essick, E. E., and Sam, F. (2010). Oxidative stress and autophagy in cardiac disease, neurological disorders, aging and cancer. *Oxid. Med. Cell Longev.* 3, 168–177. doi: 10.4161/oxim.3.3.12106

Falcão-Tebas, F., Bento-Santos, A., Fidalgo, M. A., de Almeida, M. B., dos Santos, J. A., Lopes de Souza, S., et al. (2012). Maternal low-protein diet-induced delayed reflex ontogeny is attenuated by moderate physical training during gestation in rats. *Br. J. Nutr.* 107, 372–377. doi: 10.1017/S0007114511002947

Fall, C. H., and Barker, D. J. (1997). The fetal origins of coronary heart disease and non-insulin dependent diabetes in India. *Indian Pediatr.* 34, 5–8.

Fan, L., Lindsley, S. R., Comstock, S. M., Takahashi, D. L., Evans, A. E., He, G. W., et al. (2013). Maternal high-fat diet impacts endothelial function in nonhuman primate offspring. *Int. J. Obes. (Lond).* 37, 254–262. doi: 10.1038/ijo.2012.42

Fernandez-Twinn, D. S., Blackmore, H. L., Siggens, L., Giussani, D. A., Cross, C. M., Foo, R., et al. (2012). The programming of cardiac hypertrophy in the offspring by maternal obesity is associated with hyperinsulinemia, AKT, ERK, and mTOR activation. *Endocrinology* 153, 5961–5971. doi: 10.1210/en.2012-1508

Ferreira, D. J., da Silva Pedroza, A. A., Braz, G. R., da Silva-Filho, R. C., Lima, T. A., Fernandes, M. P., et al. (2016). Mitochondrial bioenergetics and oxidative status disruption in brainstem of weaned rats: immediate response to maternal protein restriction. *Brain Res.* 1642, 553–561. doi: 10.1016/j.brainres.2016.04.049

Ferreira, D. S., Liu, Y., Fernandes, M. P., and Lagranha, C. J. (2015). Perinatal low-protein diet alters brainstem antioxidant metabolism in adult offspring. *Nutr. Neurosci.* 19, 369–375. doi: 10.1179/1476830515Y.0000000030

Fidalgo, M., Falcão-Tebas, F., Bento-Santos, A., de Oliveira, E., Nogueira-Neto, J. F., de Moura, E. G., et al. (2013). Programmed changes in the adult rat offspring caused by maternal protein restriction during gestation and lactation are attenuated by maternal moderate-low physical training. *Br. J. Nutr.* 109, 449–456. doi: 10.1017/S0007114512001316

Fiorito, G., Guarrera, S., Valle, C., Ricceri, F., Russo, A., Grioni, S., et al. (2014). B-vitamins intake, DNA-methylation of One Carbon Metabolism and homocysteine pathway genes and myocardial infarction risk: the EPICOR study. *Nutr. Metab. Cardiovasc. Dis.* 24, 483–488. doi: 10.1016/j.numecd.2013.10.026

Franco, M. C., Casarini, D. E., Carneiro-Ramos, M. S., Sawaya, A. L., Barreto-Chaves, M. L., and Sesso, R. (2008). Circulating renin-angiotensin system and catecholamines in childhood: is there a role for birthweight? *Clin. Sci.* 114, 375–380. doi: 10.1042/CS20070284

Franco Mdo, C., Arruda, R. M., Dantas, A. P., Kawamoto, E. M., Fortes, Z. B., Scavone, C., et al. (2002). Intrauterine undernutrition: expression and activity of the endothelial nitric oxide synthase in male and female adult offspring. *Cardiovasc. Res.* 56, 145–153. doi: 10.1016/S0008-6363(02)00508-4

Gruber, L., Hemmerling, J., Schüppel, V., Müller, M., Boekschoten, M. V., and Haller, D. (2015). Maternal high-fat diet accelerates development of crohn's disease-like ileitis in TNFDeltaARE/WT offspring. *Inflamm. Bowel Dis.* 21, 2016–2025. doi: 10.1097/MIB.0000000000000465

Guberman, C., Jellyman, J. K., Han, G., Ross, M. G., and Desai, M. (2013). Maternal high-fat diet programs rat offspring hypertension and activates the adipose renin-angiotensin system. *Am. J. Obstet. Gynecol.* 209, 262.e1–262.e18. doi: 10.1016/j.ajog.2013.05.023

Gusmão, D. O. (2012). *Relação entre Leptina, Peptídeo Natriurético Atrial e Estrógeno em um Modelo Animal de Hipertensão Associada a Obesidade.* Universidade Federal da Bahia.

Hales, C. N., and Barker, D. J. (1992). Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 35, 595–601. doi: 10.1007/BF00400248

Hales, C. N., Barker, D. J., Clark, P. M., Cox, L. J., Fall, C., Osmond, C., et al. (1991). Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ* 303, 1019–1022. doi: 10.1136/bmj.303.6809.1019

Hall, J. E., do Carmo, J. M., da Silva, A. A., Wang, Z., and Hall, M. E. (2015). Obesity-induced hypertension: interaction of neurohumoral and renal mechanisms. *Circ. Res.* 116, 991–1006. doi: 10.1161/CIRCRESAHA.116.305697

Hardy, T. M., and Tollefsbol, T. O. (2011). Epigenetic diet: impact on the epigenome and cancer. *Epigenomics* 3, 503–518. doi: 10.2217/epi.11.71

Harlan, S. M., Guo, D. F., Morgan, D. A., Fernandes-Santos, C., and Rahmouni, K. (2013). Hypothalamic mTORC1 signaling controls sympathetic nerve activity and arterial pressure and mediates leptin effects. *Cell Metab.* 17, 599–606. doi: 10.1016/j.cmet.2013.02.017

Harlan, S. M., and Rahmouni, K. (2013). Neuroanatomical determinants of the sympathetic nerve responses evoked by leptin. *Clin. Auton. Res.* 23, 1–7. doi: 10.1007/s10286-012-0168-4

Hemachandra, A. H., Klebanoff, M. A., Duggan, A. K., Hardy, J. B., and Furth, S. L. (2006). The association between intrauterine growth restriction in the full-term infant and high blood pressure at age 7 years: results from the Collaborative Perinatal Project. *Int. J. Epidemiol.* 35, 871–877. doi: 10.1093/ije/dyl080

Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature* 444, 860–867. doi: 10.1038/nature05485

Ito, T., Funamoto, K., Sato, N., Nakamura, A., Tanabe, K., Hoshiai, T., et al. (2012). Maternal undernutrition induces the expression of hypoxia-related genes in the fetal brain. *Tohoku J. Exp. Med.* 226, 37–44. doi: 10.1620/tjem.226.37

Ito, T., Tanabe, K., Nakamura, A., Funamoto, K., Aoyagi, A., Sato, K., et al. (2011). Aberrant expression of hypoxia-inducible factor 1alpha in the fetal heart is associated with maternal undernutrition. *Tohoku J. Exp. Med.* 224, 163–171. doi: 10.1620/tjem.224.163

Jablonka, E., and Lamb, M. J. (2002). The changing concept of epigenetics. *Ann. N.Y. Acad. Sci.* 981, 82–96. doi: 10.1111/j.1749-6632.2002.tb04913.x

Johansson, S., Norman, M., Legnevall, L., Dalmaz, Y., Lagercrantz, H., and Vanpée, M. (2007). Increased catecholamines and heart rate in children with low birth weight: perinatal contributions to sympathoadrenal overactivity. *J. Intern. Med.* 261, 480–487. doi: 10.1111/j.1365-2796.2007.01776.x

Karlic, H., and Varga, F. (2011). Impact of vitamin D metabolism on clinical epigenetics. *Clin. Epigenet.* 2, 55–61. doi: 10.1007/s13148-011-0021-y

Kurajoh, M., Koyama, H., Kadoya, M., Naka, M., Miyoshi, A., Kanzaki, A., et al. (2015). Plasma leptin level is associated with cardiac autonomic dysfunction in patients with type 2 diabetes: HSCAA study. *Cardiovasc. Diabetol.* 14, 117. doi: 10.1186/s12933-015-0280-6

Labayen, I., Moreno, L. A., Blay, M. G., Blay, V. A., Mesana, M. I., González-Gross, M., et al. (2006). Early programming of body composition and fat distribution in adolescents. *J. Nutr.* 136, 147–152.

Landsberg, L., Aronne, L. J., Beilin, L. J., Burke, V., Igel, L. I., Lloyd-Jones, D., et al. (2013). Obesity-related hypertension: pathogenesis, cardiovascular risk, and treatment–a position paper of the obesity society and The American Society of Hypertension. *Obesity (Silver Spring)* 21, 8–24. doi: 10.1002/oby.20181

Leandro, C. G., da Silva Ribeiro, W., Dos Santos, J. A., Bento-Santos, A., Lima-Coelho, C. H., Falcão-Tebas, F., et al. (2012). Moderate physical training attenuates muscle-specific effects on fibre type composition in adult rats submitted to a perinatal maternal low-protein diet. *Eur. J. Nutr.* 51, 807–815. doi: 10.1007/s00394-011-0259-3

Lomba, A., Martínez, J. A., García-Díaz, D. F., Paternain, L., Marti, A., Campión, J., et al. (2010). Weight gain induced by an isocaloric pair-fed high fat diet: a nutriepigenetic study on FASN and NDUFB6 gene promoters. *Mol. Genet. Metab.* 101, 273–278. doi: 10.1016/j.ymgme.2010.07.017

Lucas, A. (1998). Programming by early nutrition: an experimental approach. *J. Nutr.* 128(2 Suppl.), 401S–406S.

Lyons, C. L., Kennedy, E. B., and Roche, H. M. (2016). Metabolic inflammation-differential modulation by dietary constituents. *Nutrients* 8:E247. doi: 10.3390/nu8050247

Machleidt, F., Simon, P., Krapalis, A. F., Hallschmid, M., Lehnert, H., and Sayk, F. (2013). Experimental hyperleptinemia acutely increases vasoconstrictory sympathetic nerve activity in healthy humans. *J. Clin. Endocrinol. Metab.* 98, E491–E496. doi: 10.1210/jc.2012-3009

Maloyan, A., Muralimanoharan, S., Huffman, S., Cox, L. A., Nathanielsz, P. W., Myatt, L., et al. (2013). Identification and comparative analyses of myocardial miRNAs involved in the fetal response to maternal obesity. *Physiol. Genomics* 45, 889–900. doi: 10.1152/physiolgenomics.00050.2013

Manna, P., and Jain, S. K. (2015). Obesity, oxidative stress, adipose tissue dysfunction, and the associated health risks: causes and therapeutic strategies. *Metab. Syndr. Relat. Disord.* 13, 423–444. doi: 10.1089/met.2015.0095

Mansego, M. L., Milagro, F. I., Campión, J., and Martínez, J. A. (2013). Techniques of DNA methylation analysis with nutritional applications. *J. Nutrigenet. Nutrigenomics* 6, 83–96. doi: 10.1159/000350749

Mazzio, E. A., and Soliman, K. F. (2014). Epigenetics and nutritional environmental signals. *Integr. Comp. Biol.* 54, 21–30. doi: 10.1093/icb/icu049

Milagro, F. I., Campión, J., García-Díaz, D. F., Goyenechea, E., Paternain, L., and Martinez, J. A. (2009). High fat diet-induced obesity modifies the methylation pattern of leptin promoter in rats. *J. Physiol. Biochem.* 65, 1–9. doi: 10.1007/BF03165964

Milagro, F. I., Mansego, M. L., De Miguel, C., and Martínez, J. A. (2013). Dietary factors, epigenetic modifications and obesity outcomes: progresses and perspectives. *Mol. Aspects Med.* 34, 782–812. doi: 10.1016/j.mam.2012.06.010

Mitchell, C., Schneper, L. M., and Notterman, D. A. (2016). DNA methylation, early life environment, and health outcomes. *Pediatr. Res.* 79, 212–219. doi: 10.1038/pr.2015.193

Nanduri, J., and Prabhakar, N. R. (2015). Epigenetic regulation of carotid body oxygen sensing: clinical implications. *Adv. Exp. Med. Biol.* 860, 1–8. doi: 10.1007/978-3-319-18440-1_1

Nascimento, L., Freitas, C. M., Silva-Filho, R., Leite, A. C., Silva, A. B., da Silva, A. I., et al. (2014). The effect of maternal low-protein diet on the heart of adult offspring: role of mitochondria and oxidative stress. *Appl. Physiol. Nutr. Metab.* 39, 880–887. doi: 10.1139/apnm-2013-0452

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., et al. (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 384, 766–781. doi: 10.1016/S0140-6736(14)60460-8

Ng, S. F., Lin, R. C., Laybutt, D. R., Barres, R., Owens, J. A., and Morris, M. J. (2010). Chronic high-fat diet in fathers programs beta-cell dysfunction in female rat offspring. *Nature* 467, 963–966. doi: 10.1038/nature09491

Nuyt, A. M. (2008). Mechanisms underlying developmental programming of elevated blood pressure and vascular dysfunction: evidence from human studies and experimental animal models. *Clin. Sci.* 114, 1–17. doi: 10.1042/CS20070113

Nuyt, A. M., and Alexander, B. T. (2009). Developmental programming and hypertension. *Curr. Opin. Nephrol. Hypertens.* 18, 144–152. doi: 10.1097/MNH.0b013e328326092c

Osmond, C., and Barker, D. J. (2000). Fetal, infant, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women. *Environ. Health Perspect.* 108(Suppl. 3), 545–553. doi: 10.1289/ehp.00108s3545

Ozanne, S. E., and Hales, C. N. (2004). Lifespan: catch-up growth and obesity in male mice. *Nature* 427, 411–412. doi: 10.1038/427411b

Parra, D. C., Iannotti, L., Gomez, L. F., Pachón, H., Haire-Joshu, D., Sarmiento, O. L., et al. (2015). The nutrition transition in Colombia over a decade: a novel household classification system of anthropometric measures. *Arch. Public Health* 73, 12. doi: 10.1186/s13690-014-0057-5

Paulino-Silva, K. M., and Costa-Silva, J. H. (2016). Hypertension in rat offspring subjected to perinatal protein malnutrition is not related to the baroreflex dysfunction. *Clin. Exp. Pharmacol. Physiol.* 43, 1046–1053. doi: 10.1111/1440-1681.12628

Prabhakar, N. R. (2013). Sensing hypoxia: physiology, genetics and epigenetics. *J. Physiol.* 591(Pt 9), 2245–2257. doi: 10.1113/jphysiol.2012.247759

Qasem, R. J., Li, J., Tang, H. M., Browne, V., Mendez-Garcia, C., Yablonski, E., et al. (2015). Decreased liver triglyceride content in adult rats exposed to protein restriction during gestation and lactation: role of hepatic triglyceride utilization. *Clin. Exp. Pharmacol. Physiol.* 42, 380–388. doi: 10.1111/1440-1681.12359

Ravelli, G. P., Stein, Z. A., and Susser, M. W. (1976). Obesity in young men after famine exposure *in utero* and early infancy. *N. Engl. J. Med.* 295, 349–353. doi: 10.1056/NEJM197608122950701

Ribeiro, A. M., Lima Mde, C., de Lira, P. I., and da Silva, G. A. (2015). [Low birth weight and obesity: causal or casual association?]. *Rev. Paul. Pediatr.* 33, 341–349. doi: 10.1016/j.rpped.2014.09.007

Roberts, V. H., Frias, A. E., and Grove, K. L. (2015). Impact of maternal obesity on fetal programming of cardiovascular disease. *Physiology (Bethesda)* 30, 224–231. doi: 10.1152/physiol.00021.2014

Samuelsson, A. M., Matthews, P. A., Argenton, M., Christie, M. R., McConnell, J. M., Jansen, E. H., et al. (2008). Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: a novel murine model of developmental programming. *Hypertension* 51, 383–392. doi: 10.1161/HYPERTENSIONAHA.107.101477

Sawaya, A. L., Martins, P. A., Grillo, L. P., and Florêncio, T. T. (2004). Long-term effects of early malnutrition on body weight regulation. *Nutr. Rev.* 62(7 Pt 2), S127–S133. doi: 10.1111/j.1753-4887.2004.tb00082.x

Sawaya, A. L., and Roberts, S. (2003). Stunting and future risk of obesity: principal physiological mechanisms. *Cad. Saude Publica* 19(Suppl. 1), S21–S28. doi: 10.1590/S0102-311X2003000700003

Siddique, K., Guzman, G. L., Gattineni, J., and Baum, M. (2014). Effect of postnatal maternal protein intake on prenatal programming of hypertension. *Reprod. Sci.* 21, 1499–1507. doi: 10.1177/1933719114530186

Stone, N., Pangilinan, F., Molloy, A. M., Shane, B., Scott, J. M., Ueland, P. M., et al. (2011). Bioinformatic and genetic association analysis of microRNA target sites in one-carbon metabolism genes. *PLoS ONE* 6:e21851. doi: 10.1371/journal.pone.0021851

Szarc vel Szic, K., Declerck, K., Vidakovic, M., and Vanden Berghe, W. (2015). From inflammaging to healthy aging by dietary lifestyle choices: is epigenetics the key to personalized nutrition? *Clin. Epigenetics* 7, 33. doi: 10.1186/s13148-015-0068-2

Tan, H. C., Roberts, J., Catov, J., Krishnamurthy, R., Shypailo, R., and Bacha, F. (2015). Mother's pre-pregnancy BMI is an important determinant of adverse cardiometabolic risk in childhood. *Pediatr. Diabetes* 16, 419–426. doi: 10.1111/pedi.12273

Tarry-Adkins, J. L., Fernandez-Twinn, D. S., Hargreaves, I. P., Neergheen, V., Aiken, C. E., Martin-Gronert, M. S., et al. (2016). Coenzyme Q10 prevents

hepatic fibrosis, inflammation, and oxidative stress in a male rat model of poor maternal nutrition and accelerated postnatal growth. *Am. J. Clin. Nutr.* 103, 579–588. doi: 10.3945/ajcn.115.119834

Taylor, P. D., McConnell, J., Khan, I. Y., Holemans, K., Lawrence, K. M., Asare-Anane, H., et al. (2005). Impaired glucose homeostasis and mitochondrial abnormalities in offspring of rats fed a fat-rich diet in pregnancy. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288, 23. doi: 10.1152/ajpregu.00355.2004

Toyoshima, Y., Tokita, R., Ohne, Y., Hakuno, F., Noguchi, T., Minami, S., et al. (2010). Dietary protein deprivation upregulates insulin signaling and inhibits gluconeogenesis in rat liver. *J. Mol. Endocrinol.* 45, 329–340. doi: 10.1677/JME-10-0102

Victora, C. G., Adair, L., Fall, C., Hallal, P. C., Martorell, R., Richter, L., et al. (2008). Maternal and child undernutrition: consequences for adult health and human capital. *Lancet* 371, 340–357. doi: 10.1016/S0140-6736(07)61692-4

Wang, J., Cao, M., Zhuo, Y., Che, L., Fang, Z., Xu, S., et al. (2016). Catch-up growth following food restriction exacerbates adulthood glucose intolerance in pigs exposed to intrauterine undernutrition. *Nutrition* 32, 1275–1284. doi: 10.1016/j.nut.2016.03.010

Wang, J., Ma, H., Tong, C., Zhang, H., Lawlis, G. B., Li, Y., et al. (2010). Overnutrition and maternal obesity in sheep pregnancy alter the JNK-IRS-1 signaling cascades and cardiac function in the fetal heart. *FASEB J.* 24, 2066–2076. doi: 10.1096/fj.09-142315

Waterland, R. A., and Michels, K. B. (2007). Epigenetic epidemiology of the developmental origins hypothesis. *Annu. Rev. Nutr.* 27, 363–388. doi: 10.1146/annurev.nutr.27.061406.093705

Wei, L. N. (2013). Non-canonical activity of retinoic acid in epigenetic control of embryonic stem cell. *Transcription* 4, 158–161. doi: 10.4161/trns.25395

Wells, J. C. (2007). The programming effects of early growth. *Early Hum. Dev.* 83, 743–748. doi: 10.1016/j.earlhumdev.2007.09.002

Wells, J. C. (2011). The thrifty phenotype: an adaptation in growth or metabolism? *Am. J. Hum. Biol.* 23, 65–75. doi: 10.1002/ajhb.21100

Wells, J. C. (2012). Obesity as malnutrition: the role of capitalism in the obesity global epidemic. *Am. J. Hum. Biol.* 24, 261–276. doi: 10.1002/ajhb.22253

Wensveen, F. M., Jelencic, V., Valentic, S., Šestan, M., Wensveen, T. T., Theurich, S., et al. (2015). NK cells link obesity-induced adipose stress to inflammation and insulin resistance. *Nat. Immunol.* 16, 376–385. doi: 10.1038/ni.3120

West-Eberhard, M. J. (2005a). Phenotypic accommodation: adaptive innovation due to developmental plasticity. *J. Exp. Zool. B Mol. Dev. Evol.* 304, 610–618. doi: 10.1002/jez.b.21071

West-Eberhard, M. J. (2005b). Developmental plasticity and the origin of species differences. *Proc. Natl. Acad. Sci. U.S.A.* 102(Suppl 1), 6543–6549. doi: 10.1073/pnas.0501844102

Zhang, Y., Yuan, M., Bradley, K. M., Dong, F., Anversa, P., and Ren, J. (2012). Insulin-like growth factor 1 alleviates high-fat diet-induced myocardial contractile dysfunction: role of insulin signaling and mitochondrial function. *Hypertension* 59, 680–693. doi: 10.1161/HYPERTENSIONAHA.111.181867

Zohdi, V., Lim, K., Pearson, J. T., and Black, M. J. (2015). Developmental programming of cardiovascular disease following intrauterine growth restriction: findings utilising a rat model of maternal protein restriction. *Nutrients* 7, 119–152. doi: 10.3390/nu7010119

Zohdi, V., Sutherland, M. R., Lim, K., Gubhaju, L., Zimanyi, M. A., and Black, M. J. (2012). Low birth weight due to intrauterine growth restriction and/or preterm birth: effects on nephron number and long-term renal health. *Int. J. Nephrol.* 2012:136942. doi: 10.1155/2012/136942

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ANEXO C – Artigo de coautoria

Safflower (Catharmus tinctorius L.) oil supplementation in overnourished rats during early neonatal development: effects on heart and liver function in the adult

Laís Ribeiro Costa, Patrícia Cavalcanti Macêdo, Janatar Stella Vasconcelos de Melo, Cristiane Moura Freitas, **Aiany Simoes Alves**, Humberto de Moura Barbosa, Eduardo Lira, Mariana Pinheiro Fernandes, Manuella Batista-de-Oliveira-Hornsby, Claudia Lagranha

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ARTICLE

Safflower (*Catharmus tinctorius* L.) oil supplementation in overnourished rats during early neonatal development: effects on heart and liver function in the adult

Laís Ribeiro Costa, Patrícia Cavalcanti Macêdo, Janatar Stella Vasconcelos de Melo, Cristiane Moura Freitas, Aiany Simoes Alves, Humberto de Moura Barbosa, Eduardo Lira, Mariana Pinheiro Fernandes, Manuella Batista-de-Oliveira-Hornsby, and Claudia Lagranha

Abstract: *Carthamus tinctorius* L. (common name: safflower) is an herb whose extracted oil (safflower oil) has been employed in both alternative and conventional medicine in the treatment of disease. Overnutrition during early postnatal life can increase the lifetime risk of obesity and metabolic syndrome. Here we investigate the effect of safflower oil supplementation given during a critical early developmental stage on the eventual occurrence of metabolic disease in overnourished rats. Groups of overnourished or adequately nourished rats were randomly assigned into 2 additional groups for supplementation with either safflower oil (SF) or vehicle for 7 to 30 days. Murinometric data and weights were examined. Serum was collected for measurement of glucose, cholesterol, high-density lipoprotein cholesterol, and triglycerides. Heart and liver oxidative status were also measured. Overnutrition for 7–30 days induced a significant increase in body weight and in values for abdominal circumference, thoracic circumference, body length, and body mass index. SF supplementation did not attenuate the effect of overnutrition on any of these parameters. In addition, overnutrition increased levels of glucose, triglycerides, and very low-density lipid compared with normal controls, but SF supplementation had no effect on these parameters. Measures of oxidative status in heart or liver were not influenced by overnutrition. However, oxidative measures were altered by SF supplementation in both of these organs. The present study reveals that nutritional manipulation during early development induces detrimental effects on metabolism in the adult that are not ameliorated by supplemental SF.

Key words: overnourishment, *Carthamus tinctorius* L. oil, oxidative status, heart, liver.

Résumé : *Carthamus tinctorius* L. (nom commun : carthame) est une plante dont l'huile (huile de carthame) a été utilisée en médecine douce et conventionnelle pour le traitement de maladies. La suralimentation durant la petite enfance peut accroitre le risque à vie de l'obésité et du syndrome métabolique. Dans la présente étude, on examine l'effet de la supplémentation en huile de carthame durant une période critique du développement initial sur l'occurrence éventuelle d'une maladie métabolique chez des rats suralimentés. On divise aléatoirement des rats suralimentés et correctement alimentés en deux groupes, l'un recevant de l'huile de carthame (« SF ») et l'autre, un véhicule, et ce, durant 7 à 30 jours. On examine les masses et les variables murinométriques. On prélève du sérum pour en analyser la teneur en glucose, cholestérol, cholestérol LHD et en triglycérides. On évalue le statut oxydatif du foie et du cœur. La suralimentation durant 7 à 30 jours suscite une augmentation significative de la masse corporelle, du tour de l'abdomen et de la poitrine, de la longueur du corps et de l'indice de masse corporelle. La supplémentation en SF n'atténue pas l'effet de la suralimentation, peu importe la variable. De plus, la suralimentation suscite une augmentation des taux de glucose, de triglycérides et des lipoprotéines de très basse densité comparativement au groupe de contrôle normal et la supplémentation en SF n'a pas d'effet sur toutes ces variables. La suralimentation n'a pas d'effet sur les mesures du statut oxydatif du cœur et du foie. Toutefois, la supplémentation en SF modifie les mesures oxydatives de ces deux organes. D'après la présente étude, la manipulation nutritionnelle durant le développement initial a des effets nuisibles sur le métabolisme de l'adulte et la supplémentation en SF n'apporte pas des améliorations. [Traduit par la Rédaction]

Mots-clés : suralimentation, huile de carthamus tinctorius, statut oxydatif, cœur, foie.

Introduction

The medicinal properties of *Carthamus tinctorius* L. (safflower; SF) were discovered in China over 2500 years ago (Zhao et al. 2009). According to previously published reports, the biological properties attributed to *C. tinctorius* are due to the several compounds isolated from water extracts of the plant, and include flavonoids, alkaloids, carboxylic acids, steroids, and polysaccharides (Sato et al. 1985; Kim et al. 1992; Kazuma et al. 2000; Lee et al. 2002; Roh et al. 2004; Wang et al. 2014).

In traditional Chinese medicine, *C. tinctorius* has been used to treat inflammation and a number of cardiovascular diseases, including stroke, arteriosclerosis, and cardiomyopathy, among oth-

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ers (He 1991; Fan et al. 2009; Zhao et al. 2009; Tien et al. 2010; Li et al. 2013; Gao et al. 2013; Bao et al. 2015). Recent clinical and experimental evidence showing cardioprotective effects for *C. tinctorius* (Koyama et al. 2009; Upadya et al. 2015) lends credence to the traditional use of *C. tinctorius* in the treatment of cardiovascular disease.

A study of normotensive and spontaneously hypertensive rats treated with hydroxysafflower yellow A (HSYA), the main chemical component of SF yellow pigment, showed that HSYA reduces blood pressure and heart rate (Nie et al. 2012). It had been observed previously that HSYA might provide neuroprotection against cerebral ischemia/reperfusion injury through its antioxidant action (Wei et al. 2005). In addition to its effects as an antioxidant, *C. tinctorius* has been shown in humans to decrease low-density lipoprotein cholesterol (LDL-C) levels (Upadya et al. 2015). Furthermore, an earlier study in mice (Koyama et al. 2009) indicated that the reduction of oxidized LDL formation by *C. tinctorius* extract was due primarily to the strong antioxidant activity of the drug.

Corroborating these findings, Bao et al. showed that in mice maintained on a high-fat diet, low doses of the *C. tinctorius* extract (0.1 mg/day) lowered significantly both total cholesterol (TC) and LDL-C levels, and significantly decreased the atherogenic index (AI), a measure of atherosclerosis risk (Bao et al. 2015). In addition, moderate (0.5 mg/day) and high (1.0 mg/day) doses of *C. tinctorius* extract reduced the TC, LDL-C, and triglyceride (TG) levels of hyperlipidemic mice, whereas high-density lipoprotein cholesterol (HDL-C) levels were significantly increased by this treatment. The authors concluded that *C. tinctorius* extract can significantly reduce the levels of serum TC, TG, LDL-C, and AI of adult mice on a high-fat diet, significantly improving lipid metabolism, lowering blood lipids, and preventing atherosclerosis (Bao et al. 2015). However, a study conducted in rats showed that 90 days of HSYA treatment at a dose of 180 mg/kg induces a slight nephrotoxicity, suggesting that high-dose *C. tinctorius* treatment is not without risk (Liu et al. 2004).

Early postnatal life is considered to be a critical window of development in which the individual remains particularly sensitive to environmental and nutritional influences (Smart et al. 1974; Bei et al. 2015). In addition to the deleterious effects of nutritional imbalance on growth rate and morphogenesis during this time, overnutrition during early postnatal life can also place the individual at risk for developing obesity and metabolic syndrome in adulthood (Ji et al. 2014; Bei et al. 2015).

A number of studies have shown that maternal exposure to specific herbs during pregnancy and/or the suckling period can result in adverse effects on the survival and health of the neonate (Eisenberg et al. 1998; Ernst 2002*b*, 2002*a*). On the other hand, other herbal extracts introduced through the placenta or through suckling could prove beneficial to the neonate (Srivastava et al. 2010). The present study had 2 aims: (*i*) to investigate whether supplementation with *C. tinctorius* oil during the suckling period can affect oxidant status and body form in the adult, and (*ii*) to investigate whether the supplementation of overnourished rats with *C. tinctorius* oil during this critical developmental period can ameliorate the deleterious effects of early over-eating on adult metabolic status.

Materials and methods

Animals and housing conditions

The experiments were carried out in accordance with the guidelines of the Institutional Ethics Committee for Animal Research of the Federal University of Pernambuco (approval protocol no. 23 076 035498/2014-57), which comply with the *Principles of Laboratory Animal Care* (National Institutes of Health (NIH 1985)).

Animals were raised from birth until the day of the experiment in a room with a temperature of 23 ± 1 °C and a 12-h light/12-h dark cycle (lights on from 0700 to 1900 h), with free access to water and food, comprising a commercial laboratory chow diet (Purina do Brazil Ltd., Paulinia, São Paulo, Brazil) with 23% protein. After weaning, all pups were housed in groups of 3–4 per polypropylene cage (51 × 35.5 × 18.5 cm).

Overnutrition and *C. tinctorius* supplementation

Female Wistar rats aged 120–150 days and weighing 250 ± 50 g were mated in the proportion of 2 females to 1 male. Females were monitored on a daily basis for the presence of vaginal sperm plugs and once the plug was detected (considered gestational day 0), the female was removed and housed in an individual cage with free access to food and water. After gestation, the pregnant rats delivered 7–12 pups per litter. The pups from 4 to 6 litters were first joined in a common pool. On the second day after birth, litters were standardized to have either 3 or 9 pups to alter the nutritional state of each litter to either overnourished (O; 3 mice per litter; $n = 17$) or nourished (N; 9 mice per litter; $n = 18$), respectively. For the period of 7 through 30 days of age, rats from both experimental groups (O or N) were randomly assigned between rats that received supplementation with *C. tinctorius* (SF) or vehicle (V). The supplementation with SF oil (Nature, Paraíba, Brazil) represented 3% of the daily dietary intake of omega-6. In the placebo group, rats received a vehicle solution consisting of distilled water and Cremophor 0.009% (Sigma–Aldrich; (St. Louis, Mo., USA), the diluent for SF oil. The SF or V groups received 1 daily dose of SF oil or vehicle, respectively, throughout the experimental period, administered via gavage.

Postnatal body weight, murinometric data, heart, and liver weight

The body weights (BWs) of all male Wistar rats at postnatal days 7, 14, 21, 30, and 45 were evaluated to test whether they were influenced by either SF supplementation or overnutrition. Rats were weighed in a semi-analytical digital electronic scale (Marte, Minas Gerais State, Brazil) and their weights were compared with the respective controls.

Heart weight, liver weight, BW, body length (BL; muzzle-to-anus), abdominal circumference (AC; immediately anterior to the forefoot), and thoracic circumference (C; immediately behind the foreleg) were determined in all rats, as described previously (Novelli et al. 2007; da Silva Pedroza et al. 2015). The BW, BL, AC, and C were used to determine the following anthropometric indices: body mass index (BMI) = BW (g)/BL^2 (cm^2); Lee index = cube root of BW (g)/BL (cm); and AC/C ratio.

Blood analysis

Tail blood was collected from fasted (12–14 h) rats to measure glucose levels using a glucometer (G-Tech Free System NoCode, Accumed-Glicomed, Brazil). Animals were then anesthetized with a mixture of 1 g/kg urethane plus 40 mg/kg chloralose (both from Sigma–Aldrich), and blood samples were obtained by cardiac puncture and collected immediately in separate tubes. Approximately 4 mL of blood was placed in a 10-mL tube and gently inverted for 30 s. After 20 min, the sample was centrifuged at 8000 r/min for 10 min. The serum was frozen at –15 °C until assayed for lipid content. Lipid panel analysis was used as an initial broad medical screening tool for abnormalities in lipids. The levels of total cholesterol (TC), HDL-C, and TG were measured using available commercial kits (Labtest, Lagoa Santa, MG, Brazil). The levels of very-low–density lipoprotein cholesterol (VLDL-C) were calculated using the Friedwald formula (VLDL = TG/5). AI, the parameter most used to evaluate cardiovascular risk, was determined by the following equation (Dobiásová and Frohlich 2001; Jurgonski et al. 2012):

$$AI = [\log(TG/HDL\text{-}C)]$$

Drugs and reagents

All drugs and reagents were purchased from Sigma–Aldrich (Sinc Pernambuco, Brazil).

Heart and Liver preparations for biochemical analysis

After animals were anesthetized and blood samples collected, heart and liver were immediately collected and frozen for further analysis. Homogenates of heart and liver tissues were prepared in 50 mmol/L Tris buffer containing 1 mmol/L ethylenediaminetetraacetic acid (EDTA) (pH 7.4), 1 mmol/L sodium orthovanadate, 200 μg/mL phenylmethylsulfonyl fluoride and centrifuged at 4000 r/min for 10 min at 4 °C. The supernatant was collected and used in the following experiments as described below. Concentration of protein in supernatant was estimated using bovine serum albumin as standard (Bradford 1976).

Oxidative stress biomarkers

Evaluation of malondialdehyde (MDA) production

A total of 0.3 mg/mL of either heart or liver homogenate was used to measure MDA production following reaction with thiobarbituric acid (TBA) at 100 °C according to the method of Draper (Draper et al. 1993). In the TBA test reaction, MDA or MDA-like substances react to produce a pink pigment with a maximum absorption at 535 nm. The reaction was developed by the sequential addition to the sample of 30% TBA and Tris-HCl (3 mmol/L) followed by thorough mixing and centrifugation at 2500 g for 10 min. Supernatant was transferred to another tube and 0.8% TBA (*v*/*v*) was added before mixing and boiling for 30 min. After cooling, the absorbance of the organic phase was read at 535 nm in a spectrophotometer (Nascimento et al. 2014).

Evaluation of carbonyl content

Protein oxidation was assessed using the procedure described by Reznick and Packer (1994). Tricyclic antidepressant (30% *w*/*v*) was added to the samples (0.3 mg/mL heart and liver homogenates) on ice and the mix was centrifuged for 14 min at 4000 r/min. The pellet was suspended in 10 mmol/L 2,4-dinitrophenylhydrazine and immediately incubated in a dark room for 1 h, shaking after each 15 min. The samples were then washed and centrifuged 3 times in ethyl/acetate buffer and the final pellet was suspended in 6 mol/L guanidine hydrochloride, incubated for 30 min at 37 °C, and absorbance read at 370 nm.

Total and protein-bound sulfhydryl group content

Total and protein-bound sulfhydryl group content were determined as described by Aksenov and Markesbery (2001). The reduction of 5,5′-dithiobis(2-nitrobenzoic acid) by thiol groups was measured in homogenates of 0.5 mg/mL heart and 0.45 mg/mL liver, resulting in the generation of a yellow-stained compound, TNB, whose absorption is measured spectrophotometrically at 412 nm. The sulfhydryl content is inversely correlated to oxidative damage to proteins. Results were calculated as mmol/mg protein.

Superoxide dismutase (SOD) assay

The determination of total SOD enzyme activity was performed according to the method of Misra and Fridovich (1972). Supernatants (0.2 mg/mL heart and 0.1 mg/mL liver) were collected from homogenized tissues following centrifugation, and were incubated with 880 μL sodium carbonate (0.05%, pH 10.2, 0.1 mmol/L EDTA) at 37 °C before development of the reaction by the addition of 30 mmol/L epinephrine (in 0.05% acetic acid). SOD activity was determined from the kinetics of the inhibition of adrenaline autoxidation at 480 nm expressed as U/mg protein.

Catalase (CAT) assay

CAT activity was measured according to the method described by Aebi (1984). The principle of the assay is based on the determination of the rate constant (k) of H_2O_2 decomposition, which under our conditions of temperature and pH was defined as 4.6×10^7. The assay content was composed of 50 mmol/L phosphate buffer (pH 7.0), 300 mmol/L H_2O_2, and samples of 0.3 mg/mL heart and 0.08 mg/mL liver homogenate. The rate constant of the enzyme was determined by measuring the change in absorbance (at 240 nm) per minute over a 4-min period at 20 °C, and the CAT activity was expressed as U/mg protein.

Glutathione S-transferase (GST) activity

GST is an antioxidant enzyme involved in the detoxification of a wide range of toxic agents, including peroxide and alkylating agents present in the tissues. The activity of GST was measured according to the method described by Habig et al. (1974). The principle of the assay is based on the determination through absorbance spectroscopy of the conjugation of 1-chloro, 2,4-dinitrobenzene (CDNB) with reduced glutathione (GSH). Absorbance was measured at 340 nm at 30 °C. One unit of enzyme conjugates 10.0 nmol of CDNB with reduced GSH per minute.

Statistical analysis

All data were plotted and the statistical analysis performed using GraphPad Prism 6.0 software (GraphPad Software Inc., La Jolla, Calif., USA). Two-way ANOVA with nutritional state (nourished, overnourished) and supplementation (safflower, vehicle) were conducted on each variable tested (body, heart, and liver weights; murinometric data; blood glycemia; lipid profile; AI; heart oxidative stress; and liver oxidative stress). Post hoc tests were recommended based on the results of the tests of normality (Kolmogorov–Smirnov test). Data with only 2 values for 1 interesting nominal variable, such as overnutrition (O/V vs. N/V) or SF oil (N/SF vs. N/V), were analyzed under subgroups using an unpaired t test. Differences were considered statistically significant when $p < 0.05$.

Results

Postnatal body weight, murinometric data, and tissue ratios

Two-way ANOVA with nutritional state (nourished vs. overnourished) and supplementation (SF vs. vehicle) were conducted on each variable (postnatal BW, BMI, Lee index, AC/C ratio, and tissue ratios). We found a significant effect of the nutritional state of the rats between 7 days up to 45 days on the values of these variables (Fig. 1). Overnutrition per se (O/V vs. N/V) induced a significantly higher postnatal BW (Fig. 1), and also increased values of AC, C, BL, and BMI (Table 1) compared with normally nourished rats. On the other hand, supplementation with SF oil influenced neither the postnatal BW (SF vs. V, unpaired t test, $p > 0.05$) (Fig. 1), nor the murinometric data (AC, C, BL, BMI, Lee index, and AC/C ratio; Table 1). Regarding the tissue ratio, only in liver did we observe a significant difference in this ratio between O/V and N/V (unpaired t test, $p < 0.05$; Table 1).

Blood glycaemia, lipid profile, and AI

There was a significant increase of the fasting glycemia levels in the overnourished rats (O/SF and O/V) compared with their respective nourished controls (N/SF and N/V, $p < 0.05$, 2-way ANOVA). Two-way ANOVA showed that both factors (nutrition and supplementation) induced significant differences for the values of blood glycemia. In addition, SF oil (N/SF vs. N/V, unpaired t test, $p < 0.05$) induced a significant increase in fasting glycemia levels, TG, and VLDL. Overnutrition (O/V vs. N/V, unpaired t test, $p < 0.05$) also increased glycemia levels, TG, and VLDL. However, neither the nutritional state nor the presence or absence of SF oil significantly changed TC, HDL, or AI (2-way ANOVA, $p > 0.05$; Table 2).

Oxidative status and SF supplementation

We observed that SF supplementation during a critical period in heart development induces a significant increase in lipid per-

Fig. 1. Postnatal body weights in nourished and overnourished rats supplemented with safflower (SF) during lactation period. *, $p < 0.05$.

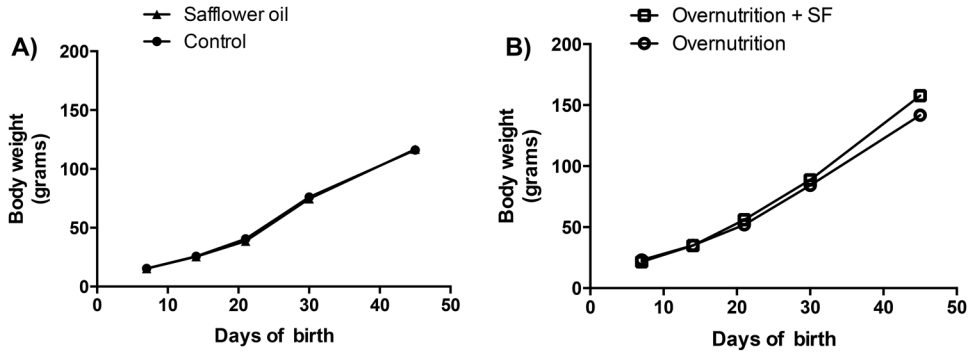


Table 1. Murinometric evaluation on the effect of SF supplementation during lactation in nourished and overnourished rats.

	BW	AC	C	BL	BMI	LI	AC/C	HW/BW	LW/BW
N/V	120.24±4.33a	12.11±0.23a	9.94±0.24a	16.56±0.35a	0.42±0.01a	0.30±0.006	1.21±0.02	0.67±0.04	1.49±0.05a
N/SF	116.78±6.28	11.68±0.22	9.61±0.20	16.89±0.45	0.39±0.02	0.29±0.007	1.22±0.006	0.58±0.03	1.55±0.09
O/V	138.40±7.45a	13.33±0.34a	10.83±0.31a	17.64±0.33a	0.51±0.03a	0.30±0.007	1.21±0.04	0.68±0.02	1.82±0.11a
O/SF	157.67±9.94	13.5±0.28	10.75±0.35	17.94±0.29	0.48±0.03	0.29±0.006	1.28±0.02	0.60±0.02	1.48±0.05

Note: Values are means ± SE. $n = 8$ for each group and analysis. Values from different groups that are marked with the same letters were significantly different. AC, abdominal circumference; BW, body weight; BL, body length; BMI, body mass index; C, thoracic circumference; LI, Lee index; N, nourished rats; O, overnourished rats; SF, safflower oil supplementation; V, vehicle.

Table 2. Lipid profile of nourished and overnourished rats supplemented with SF during lactation.

	FG (mg/dL)	TC (mg/dL)	TG (mg/dL)	VLDL (mg/dL)	HDL (mg/dL)	AI (AU)
N/V	93.67±3.59bc	96.51±4.76	34.37±1.50ab	6.87±0.30ab	34.70±2.80	1.01±0.02
N/SF	85.00±0.68ac	99.27±7.89	62.89±7.30a	12.58±1.46a	42.13±3.16	1.12±0.04
O/V	121.86±7.24b	81.55±4.07	50.42±7.46b	10.08±1.49b	40.62±1.08	1.07±0.05
O/SF	119.83±7.19a	99.07±5.93	63.94±3.88	12.79±0.78	42.65±2.33	1.11±0.04

Note: Values are means ± SE. $n = 8$ for each group and analysis. Values from different groups that are marked with the same letters were significantly different. AI, atherogenic index; FG, fasting glycaemia; HDL, high-density lipoprotein; N, nourished rats; O, overnourished rats; SF, safflower oil supplementation; TC, total cholesterol; TG, triglyceride; V, vehicle; VLDL, very-low–density lipoprotein.

oxidation (N/V: 67.6 ± 8.0; N/SF: 207.7 ± 47.4 μmol/mg protein; $p < 0.05$), but no difference in oxidative status was observed between the overnourished and normally nourished groups (N/V: 67.6 ± 8.0; O/V: 57.6 ± 15.8 μmol/mg protein). Furthermore, no difference was observed in carbonyl levels (N/V: 3.5 ± 0.34 vs. N/SF: 3.0 ± 0.31 μmol/mg protein; N/V: 3.5 ± 0.34 vs. O/V: 3.2 ± 0.31 μmol/mg protein) or in total thiol content (N/V: 19.3 ± 1.3 vs. N/SF: 21.1 ± 0.5 mmol/mg protein; N/V: 19.3 ± 1.3 vs. O/V: 21.7 ± 1.2 mmol/mg protein). Measurement of enzymatic antioxidant defense revealed a significant increase induced by SF supplementation (SOD activity N/V: 4.7 ± 0.4 vs. N/SF: 7.0 ± 0.9 U/mg protein, $p < 0.05$; CAT activity N/V: 2.9 ± 0.2 vs. N/SF: 4.2 ± 0.5 U/mg protein, $p < 0.05$ and GST activity N/V: 0.9 ± 0.1 vs. N/SF: 1.5 ± 0.2 U/mg protein, $p < 0.01$). However, when we compared nourished with overnourished groups we did not observe significant differences in any enzymatic activity (SOD activity N/V: 4.7 ± 0.4 vs. O/V: 5.2 ± 0.5 U/mg protein; CAT activity N/V: 2.9 ± 0.2 vs. O/V: 3.4 ± 0.3 U/mg protein; and GST activity N/V: 0.9 ± 0.1 vs. O/V: 1.0 ± 0.1 U/mg protein, Figs. 2D–2F). Moreover, in overnourished rats, GST activity was increased in the supplemented group (GST activity O/V: 1.05 ± 0.07 vs. O/SF: 1.49 ± 0.04 U/mg protein, $p < 0.05$, Fig. 2F).

In regard to the liver, we observed that SF supplementation increased lipid peroxidation in nourished group (N/V: 17.3 ± 4.9; N/SF: 40.8 ± 5.2 μmol/mg protein; $p < 0.01$) but found no effect of supplementation in the overnourished group (N/V: 17.3 ± 4.9 vs. O/V: 17.0 ± 4.8 μmol/mg protein). Moreover, no difference was observed in carbonyl levels (N/V: 3.6 ± 0.5 vs. N/SF: 4.1 ± 0.3 μmol/mg protein; N/V: 3.6 ± 0.5 vs. O/V: 5.1 ± 1.1 μmol/mg protein) or in total thiol content (N/V: 11.3 ± 0.63 vs. N/SF: 9.2 ± 0.86 mmol/mg protein; N/V: 11.3 ± 0.63 vs. O/V: 10.2 ± 1.4 mmol/mg protein; Fig. 3). In contrast to the heart, enzymatic antioxidant defense in the liver was unchanged with SF supplementation in nourished group (SOD activity N/V: 5.8 ± 0.6 vs. N/SF: 5.06 ± 0.8 U/mg protein; CAT activity N/V: 27.2 ± 3.8 vs. N/SF: 49.0 ± 12.2 U/mg protein; GST activity N/V: 55.7 ± 2.3 vs. N/SF: 59.6 ± 2.1 U/mg protein). However, when we compared nourished with overnourished groups we observed significantly greater SOD activity in the overnourished group (SOD: N/V: 5.9 ± 0.6 vs. O/V: 9.5 ± 1.3 U/mg protein, $p < 0.05$; CAT: N/V: 27.2 ± 3.8 vs. O/V: 28.2 ± 6.3 U/mg protein; and GST: N/V: 55.7 ± 2.3 vs. O/V: 57.1 ± 9.2 U/mg protein; Fig. 3).

Discussion

To the best of our knowledge, this is the first investigation of the effects of both overnutrition and SF oil supplementation during the critical developmental window of lactation on murinometric parameters, blood profile, AI, and heart and liver oxidative status. The present data show that SF oil consumption in nourished rats during the suckling period modulates blood parameters (FG, TG, VLDL) and increases antioxidant defense in heart, but causes no changes in liver. In addition, our data demonstrate that overnutrition during this critical period of development negatively affects both murinometric and blood parameters, with no changes in the oxidative status of either heart or liver.

Our study has potential clinical importance, since it has been noted that users of herbal medicine products including *C. tinctorius*

Fig. 2. Oxidative stress biomarkers in heart from nourished and overnourished rats supplemented with safflower (SF) during lactation. Data are presented as means ± SE. (A) Malondialdehyde (MDA) concentration; (B) carbonyls content; (C) total sulfhydryl content; (D) superoxide dismutase (SOD) activity; (E) catalase (CAT) activity; (F) Glutathione-*S*-transferase (GST) activity. mM, mmol; prot, protein; μM, μmol. *, Differences using 2-way ANOVA, $p < 0.05$; †, differences using Student's *t* test, $p < 0.05$.

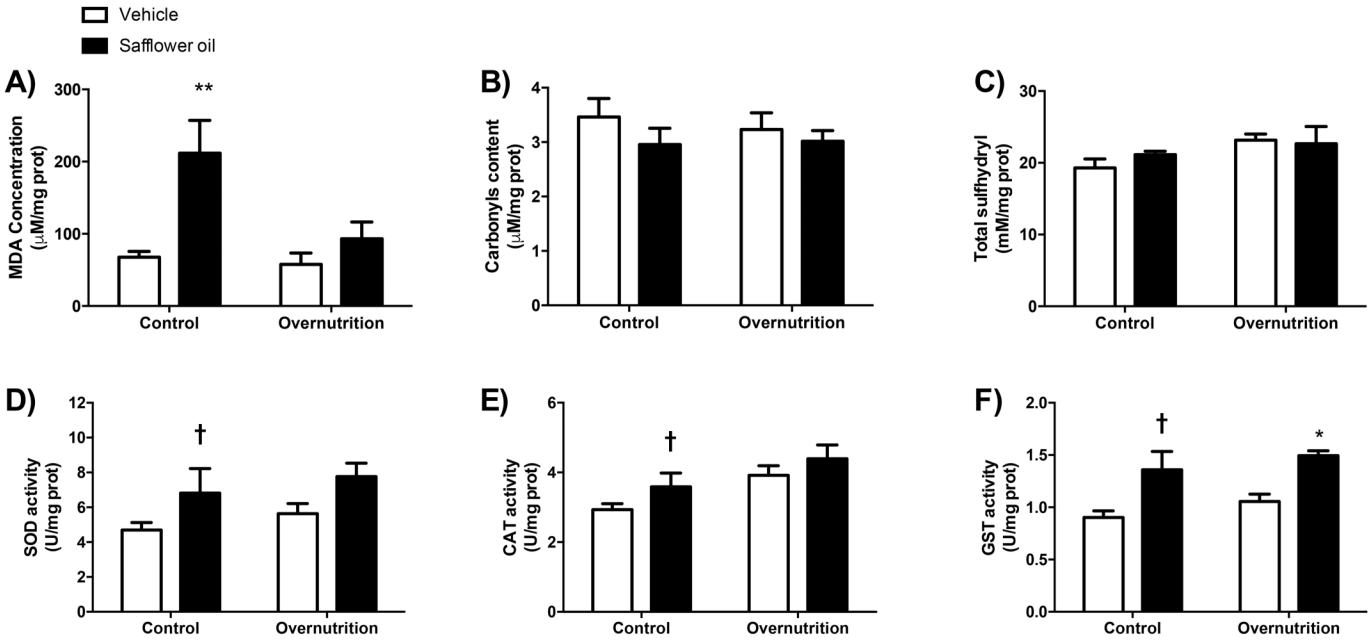
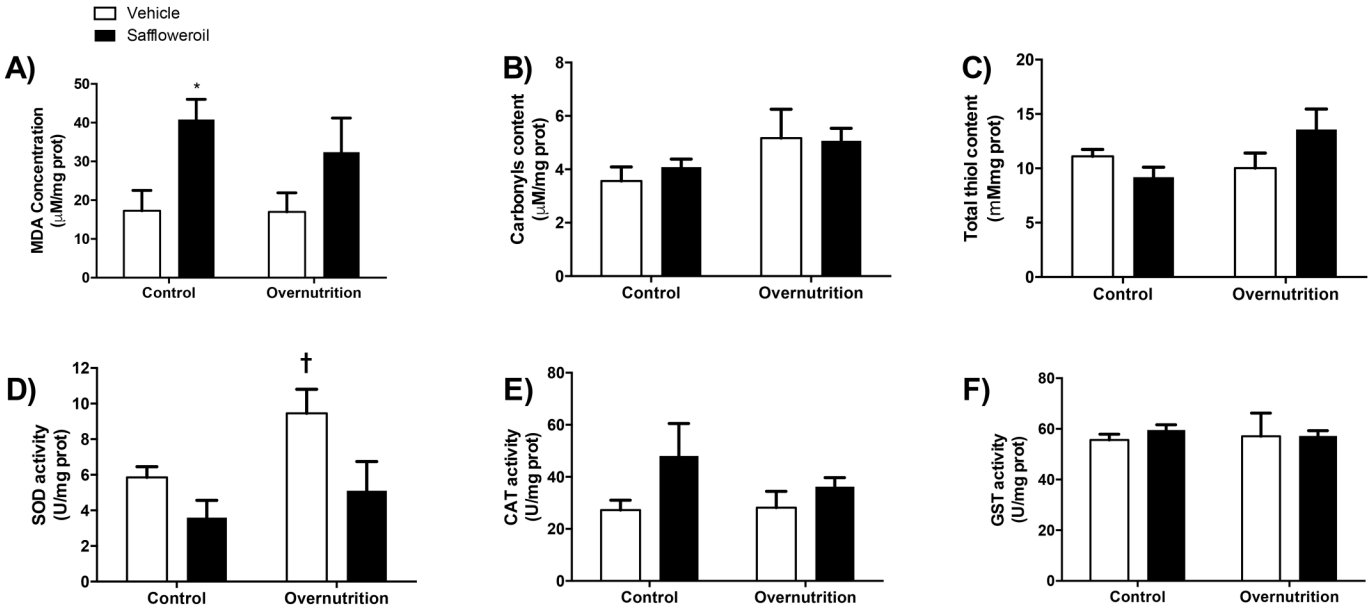


Fig. 3. Oxidative stress biomarkers in liver from nourished and overnourished rats supplemented with safflower (SF) during lactation. Data are presented as means ± SE. (A) Malondialdehyde (MDA) concentration; (B) carbonyls content; (C) total sulfhydryl content; (D) superoxide dismutase (SOD) activity; (E) catalase (CAT) activity; (F) glutathione-*S*-transferase (GST) activity. *, Differences using 2-way ANOVA, $p < 0.05$; †, differences using Student's *t* test, $p < 0.05$. mM, mmol; prot, protein; μM, μmol.



are predominantly women (Eisenberg et al. 1998), and it has furthermore been suggested that pregnant women in particular frequently use herbal medicine products believing that they are "natural and therefore free of risks" (Ernst 2002*b*, 2002*a*). However, previous studies reported an association between maternal exposure to *C. tinctorius* extract and congenital malformations in their offspring, demonstrating that *C. tinctorius* can cross the human placenta to affect the fetus. *Catharmus tinctorius* may also be present in breast milk where it can result in perinatal effects on newborns (Nobakht et al. 2000; Louei Monfared and Salati 2012). Thus, an evaluation of how *C. tinctorius* supplementation during lactation can affect body composition, blood lipids, and oxidative status in overnourished rats adds valuable insight into the literature assessing the risks and benefits of *C. tinctorius* supplementation in early development.

Experimental data shows that a high-SF diet can alter adiposity and result in effective amelioration of diet-induced obesity (Zhang et al. 2010). Additionally, previous investigations demonstrate beneficial effects of the consumption of yellow SF by hyperlipidemic mice on lipid profile (Bao et al. 2015) and also show cardio-

protective effects of the supplement (Upadya et al. 2015). However, in our hands the consumption of SF did not improve lipid profile in a significant manner, nor did it result in a decrease BW. Contradictions between the present findings and earlier reports could be due to the timing of SF supplementation, since in our model the SF oil was the given during the lactation period but in other studies it was administered to adult rats. Our findings should alert investigators studying the effects of SF and other oils (e.g., LC-PUFA) to the relevance of temporal and developmental considerations in the effects of these oils on physiologic indicators of health and disease. Investigators should also take into account the question of how "natural" this herbal oil supplementation is given that peanut products, sunflower, and SF oils fed to mother rats, guinea pig, rhesus monkeys, and baboons have been shown to induce predictable changes in tissue polyunsaturated fatty acid composition that are abnormal in free-living and mammals (Brenna 2011).

Additionally, our data show that SF supplementation positively modulates enzymatic defense, but only in the heart, not the liver. Furthermore, even the increase in protective enzymatic activity in the heart was not enough in our model to ameliorate oxidative damage as measured by increased lipid peroxidation. According to previous papers, the active compounds present in *C. tinctorius* have the ability to improve antioxidant activity and to reduce the manifestations of cardio-cerebrovascular disease (Zhu et al. 2003; Wang et al. 2007; Yang et al. 2016). Additionally, it was shown that HYSA of *C. tinctorius* attenuated hepatic disease in rats by decreasing oxidative stress (Wang et al. 2013; Gao et al. 2015). It was a surprise to us that the positive health effects of SF observed by others researchers were not seen in our study. We suspect that one reason for the lack of effect of SF in our study was the timing of the supplementation, since our experimental design involved a long period between supplementation and evaluation (i.e., between the lactation period and adulthood), allowing a possible "wash-out" of SF effects on some parameters, whereas earlier studies performed both supplementation and evaluation in the adult animal.

Supporting our present findings with liver tissue, a recent study of rats fed a high-fat diet enriched with SF demonstrated an increase in oxidative stress biomarkers in plasma, and in liver mitochondria associated with an increase in peroxidability index and steatosis (Crescenzo et al. 2015). The author of the study suggests that the major factor adding to the increased oxidative stress in blood and liver tissue was the significant increase in cellular content of polyunsaturated fatty acid, since polyunsaturated fatty acids are prone to be oxidized by the reactive oxygen species. In addition, the author suggested that the increased hepatic steatosis observed in rats fed long term with a high fat diet enriched with SF could be due to the increased lipid storage ($n6/n3$ ratio) and concluded that diets containing elevated amounts of polyunsaturated fats could represent a predisposing factor for the development of liver steatosis/liver disease. Furthermore, Choi et al. (2013) demonstrated that α-terpineol, a monoterpene component of *C. tinctorius,* predisposes the individual to hepatic steatosis by inducing activation of the AMP-activated protein kinase-sterol regulatory element-binding protein-1 pathway (Choi et al. 2013).

In summary, this study provides new evidence that nutritional manipulation during a critical developmental period (i.e., nursing stage) in the rat may result in harmful effects in the adult associated with redox impairment. Taken together, these data add to the growing body of evidence that predisposition to certain diseases (cardiovascular disease and metabolic syndrome) is actually initiated at a very early age due in part to an increase in oxidative stress.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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References

Aebi, H. 1984. Catalase in vitro. Methods Enzymol. **105**: 121–126. doi:10.1016/S0076-6879(84)05016-3. PMID:6727660.

Aksenov, M.Y., and Markesbery, W.R. 2001. Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. Neurosci. Lett. **302**: 141–145. doi:10.1016/S0304-3940(01)01636-6. PMID:11290407.

Bao, L.D., Wang, Y., Ren, X.H., Ma, R.L., Lv, H.J., and Agula, B. 2015. Hypolipidemic effect of safflower yellow and primary mechanism analysis. Genet. Mol. Res. **14**: 6270–6278. doi:10.4238/2015.June.9.14. PMID:26125829.

Bei, F., Jia, J., Jia, Y.Q., Sun, J.H., Liang, F., Yu, Z.Y., and Cai, W. 2015. Long-term effect of early postnatal overnutrition on insulin resistance and serum fatty acid profiles in male rats. Lipids Health Dis. **14**: 96. doi:10.1186/s12944-015-0094-2. PMID:26302954.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**: 248–254. doi:10.1016/0003-2697(76)90527-3. PMID:942051.

Brenna, J.T. 2011. Animal studies of the functional consequences of suboptimal polyunsaturated fatty acid status during pregnancy, lactation and early postnatal life. Matern. Child Nutr. **7**(S2): 59–79. doi:10.1111/j.1740-8709.2011.00301.x. PMID:21366867.

Choi, Y.J., Sim, W.C., Choi, H.K., Lee, S.H., and Lee, B.H. 2013. alpha-Terpineol induces fatty liver in mice mediated by the AMP-activated kinase and sterol response element binding protein pathway. Food Chem. Toxicol. **55**: 129–136. doi:10.1016/j.fct.2012.12.025. PMID:23274539.

Crescenzo, R., Bianco, F., Mazzoli, A., Giacco, A., Cancelliere, R., di Fabio, G., et al. 2015. Fat Quality Influences the Obesogenic Effect of High Fat Diets. Nutrients, **7**: 9475–9491. doi:10.3390/nu7115480. PMID:26580650.

da Silva Pedroza, A.A., Lopes, A., Mendes da Silva, R.F., Braz, G.R., Nascimento, L.P., Ferreira, D.S., et al. 2015. Can fish oil supplementation and physical training improve oxidative metabolism in aged rat hearts? Life Sci. **137**: 133–141. doi:10.1016/j.lfs.2015.07.021. PMID:26231695.

Dobiásová, M., and Frohlich, J. 2001. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FER(HDL)). Clin. Biochem. **34**: 583–588. doi:10.1016/S0009-9120(01)00263-6. PMID:11738396.

Draper, H.H., Squires, E.J., Mahmoodi, H., Wu, J., Agarwal, S., and Hadley, M. 1993. A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. Free Radic. Biol. Med. **15**: 353–363. doi:10.1016/0891-5849(93)90035-S. PMID:8225017.

Eisenberg, D.M., Davis, R.B., Ettner, S.L., Appel, S., Wilkey, S., Van Rompay, M., and Kessler, R.C. 1998. Trends in alternative medicine use in the United States, 1990–1997: results of a follow-up national survey. JAMA **280**: 1569–1575. PMID:9820257.

Ernst, E. 2002*a*. Herbal medicinal products during pregnancy: are they safe? BJOG **109**: 227–235. PMID:11950176.

Ernst, E. 2002*b*. Herbal medicinal products during pregnancy? Phytomedicine, **9**: 352–354. doi:10.1078/0944-7113-00149. PMID:12120817.

Fan, L., Zhao, H.Y., Xu, M., Zhou, L., Guo, H., Han, J., et al. 2009. Qualitative evaluation and quantitative determination of 10 major active components in *Carthamus tinctorius* L. by high-performance liquid chromatography coupled with diode array detector. J. Chromatogr. A, **1216**: 2063–2070. PMID:18394634.

Gao, L.N., Cui, Y.L., Wang, Q.S., and Wang, S.X. 2013. Amelioration of Danhong injection on the lipopolysaccharide-stimulated systemic acute inflammatory reaction via multi-target strategy. J. Ethnopharmacol. **149**: 772–782. doi:10.1016/j.jep.2013.07.039. PMID:23954279.

Gao, L.N., Yan, K., Cui, Y.L., Fan, G.W., and Wang, Y.F. 2015. Protective effect of *Slavia miltiorrhiza* and *Carthamus tinctorius* extract against lipopolysaccharide-induced liver injury. World J. Gastroenterol. **21**(30): 9079–9092. PMID:26290634.

Habig, W.H., Pabst, M.J., Fleischner, G., Gatmaitan, Z., Arias, I.M., and Jakoby, W.B. 1974. The identity of glutathione *S*-transferase B with ligandin, a major binding protein of liver. Proc. Natl. Acad. Sci. U. S. A. **71**: 3879–3882. doi:10.1073/pnas.71.10.3879. PMID:4139704.

He, G.F. 1991. Resources of medicinal plants in China. Mem. Inst. Oswaldo Cruz, **86**(S2): 9–12. doi:10.1590/S0074-02761991000600005. PMID:1842021.

Ji, C., Dai, Y., Jiang, W., Liu, J., Hou, M., Wang, J., et al. 2014. Postnatal overfeeding promotes early onset and exaggeration of high-fat diet-induced nonalcoholic fatty liver disease through disordered hepatic lipid metabolism in rats. J. Nutr. Biochem. **25**: 1108–1116. doi:10.1016/j.jnutbio.2014.06.010. PMID:25154569.

Jurgonski, A., Juskiewicz, J., Zdunczyk, Z., and Król, B. 2012. Caffeoylquinic acid-rich extract from chicory seeds improves glycemia, atherogenic index, and antioxidant status in rats. Nutrition, **28**: 300–306. doi:10.1016/j.nut.2011.06.010. PMID:22014632.

Kazuma, K., Takahashi, T., Sato, K., Takeuchi, H., Matsumoto, T., and Okuno, T.

2000. Quinochalcones and flavonoids from fresh florets in different cultivars of Carthamus tinctorius L. Biosci. Biotechnol. Biochem. **64**: 1588–1599. doi:10.1271/bbb.64.1588. PMID:10993143.

Kim, M.N., Scao-Bogaert, F.L., and Paris, M. 1992. Flavonoids from Carthamus tinctorius Flowers. Planta Med. **58**: 285–286. doi:10.1055/s-2006-961460. PMID:17226471.

Koyama, N., Suzuki, K., Furukawa, Y., Arisaka, H., Seki, T., Kuribayashi, K., et al. 2009. Effects of safflower seed extract supplementation on oxidation and cardiovascular risk markers in healthy human volunteers. Br. J. Nutr. **101**: 568–575. PMID:18590590.

Lee, J.Y., Chang, E.J., Kim, H.J., Park, J.H., and Choi, S.W. 2002. Antioxidative flavonoids from leaves of *Carthamus tinctorius*. Arch. Pharm. Res. **25**: 313–319. doi:10.1007/BF02976632. PMID:12135103.

Li, L., Yang, Y., Hou, X., Gu, D., Hang, B., Abdulla, R., et al. 2013. Bioassay-guided separation and purification of water-soluble antioxidants from *Carthamus tinctorius* L. by combination of chromatographic techniques. Sep. Purif. Technol. **104**: 200–207. doi:10.1016/j.seppur.2012.11.027.

Liu, Z., Li, C., Li, M., Li, D., and Liu, K. 2004. The subchronic toxicity of hydroxysafflor yellow A of 90 days repeatedly intraperitoneal injections in rats. Toxicology, **203**: 139–143. doi:10.1016/j.tox.2004.06.007. PMID:15363589.

Louei Monfared, A., and Salati, A.P. 2012. The effects of Carthamus tinctorius L. on placental histomorphology and survival of the neonates in mice. Avicenna J. Phytomed. **2**: 146–152. PMID:25050244.

Misra, H.P., and Fridovich, I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. **247**: 3170–3175. PMID:4623845.

Nascimento, L., Freitas, C.M., Silva-Filho, R., Leite, A.C., Silva, A.B., da Silva, A.I., et al. 2014. The effect of maternal low-protein diet on the heart of adult offspring: role of mitochondria and oxidative stress. Appl. Physiol. Nutr. Metab. **39**: 880–887. doi:10.1139/apnm-2013-0452. PMID:24905448.

Nie, P.H., Zhang, L., Zhang, W.H., Rong, W.F., and Zhi, J.M. 2012. The effects of hydroxysafflor yellow A on blood pressure and cardiac function. J. Ethnopharmacol. **139**: 746–750. doi:10.1016/j.jep.2011.11.054. PMID:22197825.

NIH. 1985. Principles of Laboratory Animal Care, NIH publication 85-23. National Institutes of Health, Bethesda, MD. USA.

Nobakht, M., Fattahi, M., Hoormand, M., Milanian, I., Rahbar, N., and Mahmoudian, M. 2000. A study on the teratogenic and cytotoxic effects of safflower extract. J. Ethnopharmacol. **73**: 453–459. doi:10.1016/S0378-8741(00)00324-X. PMID:11090999.

Novelli, E.L., Diniz, Y.S., Galhardi, C.M., Ebaid, G.M., Rodrigues, H.G., Mani, F., et al. 2007. Anthropometrical parameters and markers of obesity in rats. Lab. Anim. **41**: 111–119. doi:10.1258/002367707779399518. PMID:17234057.

Reznick, A.Z., and Packer, L. 1994. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol. **233**: 357–363. PMID:8015470.

Roh, J.S., Han, J.Y., Kim, J.H., and Hwang, J.K. 2004. Inhibitory effects of active compounds isolated from safflower (*Carthamus tinctorius* L.) seeds for melanogenesis. Biol. Pharm. Bull. **27**: 1976–1978. doi:10.1248/bpb.27.1976. PMID:15577216.

Sato, H., Kawagishi, H., Nishimura, T., Yoneyama, S., Yoshimoto, Y., Sakamura, S., et al. 1985. Serotobenine, a novel phenolic amide from safflower seeds (*Carthamus tinctorius* L.). Agric. Biol. Chem. **49**: 2969–2974. doi:10.1080/00021369.1985.10867182.

Smart, J.L., Adlard, B.P., and Dobbing, J. 1974. Further studies of body growth and brain development in "small-for-dates" rats. Biol. Neonate. **25**: 135–150. doi:10.1159/000240686. PMID:4451688.

Srivastava, J.K., Shankar, E., and Gupta, S. 2010. Chamomile: A herbal medicine of the past with bright future. Mol. Med. Rep. **3**(6): 895–901. PMID:21132119.

Tien, Y.C., Lin, J.Y., Lai, C.H., Kuo, C.H., Lin, W.Y., Tsai, C.H., et al. 2010. *Carthamus tinctorius* L. prevents LPS-induced TNFalpha signaling activation and cell apoptosis through JNK1/2-NFkappaB pathway inhibition in H9c2 cardiomyoblast cells. J. Ethnopharmacol. **130**: 505–513. PMID:20538053.

Upadya, H., Devaraju, C.J., and Joshi, S.R. 2015. Anti-inflammatory properties of blended edible oil with synergistic antioxidants. Indian J. Endocrinol. Metab. **19**: 511–519. doi:10.4103/2230-8210.159063. PMID:26180768.

Wang, C., Zhang, D., Li, G., Liu, J., Tian, J., Fu, F., and Liu, K. 2007. Neuroprotective effects of safflor yellow B on brain ischemic injury. Exp. Brain Res. **177**: 533–539. doi:10.1007/s00221-006-0705-2. PMID:17006684.

Wang, C.Y., Liu, Q., Huang, Q.X., Liu, J.T., He, Y.H., Lu, J.J., and Bai, X.Y. 2013. Activation of PPARgamma is required for hydroxysafflor yellow A of *Carthamus tinctorius* to attenuate hepatic fibrosis induced by oxidative stress. Phytomedicine, **20**: 592–599. doi:10.1016/j.phymed.2013.02.001. PMID:23523101.

Wang, Y., Chen, P., Tang, C., Wang, Y., Li, Y., and Zhang, H. 2014. Antinociceptive and anti-inflammatory activities of extract and two isolated flavonoids of *Carthamus tinctorius* L. J. Ethnopharmacol. **151**: 944–950. doi:10.1016/j.jep.2013.12.003. PMID:24333963.

Wei, X., Liu, H., Sun, X., Fu, F., Zhang, X., Wang, J., et al. 2005. Hydroxysafflor yellow A protects rat brains against ischemia-reperfusion injury by antioxidant action. Neurosci. Lett. **386**: 58–62. doi:10.1016/j.neulet.2005.05.069. PMID:16002219.

Yang, M., Orgah, J., Zhu, J., Fan, G., Han, J., Wang, X., et al. 2016. Danhong injection attenuates cardiac injury induced by ischemic and reperfused neuronal cells through regulating arginine vasopressin expression and secretion. Brain Res. **1643**: 516–523. PMID:27107944.

Zhang, Z., Li, Q., Liu, F., Sun, Y., and Zhang, J. 2010. Prevention of diet-induced obesity by safflower oil: insights at the levels of PPARalpha, orexin, and ghrelin gene expression of adipocytes in mice. Acta Biochim. Biophys. Sin. (Shanghai) **42**: 202–208. PMID:20213045.

Zhao, G., Zheng, X.W., Gai, Y., Chu, W.J., Qin, G.W., and Guo, L.H. 2009. Safflower extracts functionally regulate monoamine transporters. J. Ethnopharmacol. **124**: 116–124. doi:10.1016/j.jep.2009.04.002. PMID:19527825.

Zhu, H., Wang, Z., Ma, C., Tian, J., Fu, F., Li, C., et al. 2003. Neuroprotective effects of hydroxysafflor yellow A: in vivo and in vitro studies. Planta Med. **69**: 429–433. doi:10.1055/s-2003-39714. PMID:12802724.