UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE – UFCSPA
CURSO DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Iberê Machado Kostrycki

Resposta Anti-inflamatória do Exercício Físico Agudo não Ocorre em Camundongos Obesos Expostos ao Material Particulado Fino.

Porto Alegre
2016

Iberê Machado Kostrycki

Resposta Anti-inflamatória do Exercício Físico Agudo não Ocorre em Camundongos Obesos Expostos ao Material Particulado Fino.

Dissertação de Mestrado submetida ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Ciências da Saúde de Porto Alegre, como requisito para a obtenção do grau de Mestre em Ciências da Saúde.

Orientador: Dra. Cláudia Ramos Rhoden
Co-orientador: Dr. Thiago Gomes Heck

Porto Alegre
2016

Catalogação na Publicação

Kostrycki, Iberê Machado
Resposta anti-inflamatória do exercício físico agudo não ocorre em camundongos obesos expostos ao material particulado fino / Iberê Machado Kostrycki. -- 2016.
88 p. : il., graf., tab. ; 30 cm.

Dissertação (mestrado) -- Universidade Federal de Ciências da Saúde de Porto Alegre, Programa de Pós-Graduação em Ciências da Saúde, 2016.

Orientador(a): Profa Dra. Cláudia Ramos Rhoden ; coorientador(a): Prof Dr. Thiago Gomes Heck.

1. Obesidade. 2. Material Particulado. 3. Exercício. 4. Proteínas de Choque Térmico HSP70. I. Título.

Sistema de Geração de Ficha Catalográfica da UFCSPA com os dados fornecidos pelo(a) autor(a).

AGRADECIMENTOS

Certamente não conquistamos nossos objetivos sozinhos. Portanto, gostaria de agradecer a todos que de alguma forma foram importantes nesta etapa:

À orientadora Professora Cláudia Ramos Rhoden, pela confiança e oportunidade em conceder-me a oportunidade de realização deste sonho.

Aos colegas e amigos do GPeF, Analú, Bethânia, Carolain, Eliara, Eloísa, Fernanda, Guilherme, Jaíne, João, Jéssica, Lucas, Lílian, Maicon, Marcos, Marieli, Marlon, Pauline, Renan, Yana e Yohanna pelos ensinamentos, apoio e companheirismo do início até o final dos experimentos, sem vocês não seria possível a realização deste trabalho. Muito obrigado!

A minha esposa e companheira Terezinha Torres por caminhar ao meu lado com tanto carinho, paciência e compreensão nesses últimos meses, te amo meu amor!

Quero agradecer a minha irmã Xana Machado Kostrycki pelo apoio incondicional em todos os momentos, muito obrigado minha irmã querida, te amo!

Aos amigos Ale, Dudu, Ítalo, Lucas e Rafael pelas boas risadas e ótima companhia aí pelas “bandas de Porto Alegre”. Dudu obrigado por abrir as portas da tua casa, pelos intensos debates e discussões, e, por ouvir tantos desabafos meu amigo!

A professora Mirna Stela Ludwig pelo carinho, paciência e principalmente pelos ensinamentos em nosso grupo. Também quero agradecer o acolhimento nesse período em Ijuí. Muito obrigado Profe!

Ao colega e amigo Antônio Miragem, pela confiança, atenção e compreensão “sempre” que preciso. Obrigado professor!

Por fim, agradeço imensamente ao professor Thiago Gomes Heck, co-orientador, por todos esses anos de constante aprendizado, amizade e paciência. Desejo agradecer também pelo exemplo que és pra mim como pessoa, cientista e professor. Muito obrigado!

RESUMO

Desafios ao organismo como a obesidade, poluição atmosférica e a realização de exercício físico (EF), podem promover alterações na concentração intracelular de Proteínas de Choque Térmico de 70 kDa (iHSP70, sabidamente anti-inflamatórias) e na concentração plasmática da sua forma induzível (eHSP72, com ações pró-inflamatórias). Além disso, a razão entre [eHSP72/iHSP70], Índice H, pode representar um importante biomarcador de processos subclínicos que ocorrem no organismo diante destes desafios. Assim, neste trabalho, buscamos avaliar o efeito do EF agudo em camundongos obesos expostos ao material particulado fino quanto à razão intra e extracelular de proteínas de choque térmico e variáveis de estresse oxidativo do coração e do pulmão. Foram utilizados 60 camundongos B6129SF2 (B6) machos, de 30 dias de idade, separados em dois grupos por 16 semanas: Ração Padrão (CTR, n=29) e Dieta Hiperlipídica (HFD, n=31) *ad libitum*. Após as 16 semanas de dieta os animais foram adaptados a natação (3 dias, 10min, água 30$\pm$1°C) e separados nos seguintes grupos: repouso que receberam via intranasal salina (CTR, n=6 e HFD, n=8) ou $MP_{2,5}$ (PM, n=7 e PM+HFD, n=7); exercício agudo de intensidade moderada com salina (MIE, n=10 e HFD+MIE n=9) ou $MP_{2,5}$ (PM+MIE, n=6 e HFD+PM+MIE, n=7). Natação por 20 minutos com carga de 4% do peso corporal adicionada a cauda e instilação intranasal de $MP_{2,5}$ (50µg/10µL). Foi avaliado consumo de ração, perfil biométrico e glicêmico, marcadores de estresse oxidativo, concentração de eHSP72 plasmática, de iHSP70 nos tecidos pulmonar e cardíaco, o índice H. Não encontramos diferenças na expressão de iHSP70 nos tecidos avaliados. No entanto, o exercício agudo de intensidade moderada gerou uma menor concentração de eHSP72 no plasma de camundongos. Além disso, levou a diminuição do índice H em relação ao coração e ao pulmão do grupo CTR+MIE e em relação ao pulmão do grupo PM+MIE. Estes dados indicam que a realização de exercício agudo de moderada intensidade gera um quadro anti-inflamatório em camundongos sedentários não obesos, mesmo que expostos ao $MP_{2,5}$, o que não ocorre na obesidade.

Palavras - chave: Exercício. Obesidade. Material Particulado fino. HSP70. eHSP72.

ABSTRACT

Challenges to the body homeostasis as obesity, air pollution and physical exercise can promote alterations on intracellular levels of anti-inflammatory 70 kDa Heat Shock Protein (iHSP70) and on plasma concentrations of its stress inducible 72 kDa isoform (eHSP72, with pro-inflammatory role). The versatility of these proteins to induced and mark different responses related to inflammation may be an important biomarker immune-inflammatory the effect of physical exercise associated with exposure to the three conditions studied here. Furthermore, the [eHSP72/iHSP70] ratio, H index, may represent and important biomarker of the subclinical and health condition. In this work, we evaluated the effect of acute exercise in obese mice exposed to fine particulate matter as intra and extracellular ratio of heat shock proteins and oxidative stress heart and lung variables. We used 60 mice (B6129SF2) with 30 days old, divided in two groups for 16 weeks: standard diet (CTR, n =29) or high fat diet (HFD, n = 31). After 16 weeks the animals were adapted to the exercise of swimming (2 days, 10 minutes, water 30 ± 1ºC) and separated again: 10µL saline or $PM_{2.5}$ intranasal instillation (50µg/10µL). Thus, all groups were immediately subjected to a swimming workout at moderate intensity (MIE) for 20 minutes or maintained at rest, performing the following groups: CTR n=6; CTR+MIE n=10; PM n=7; PM+MIE n=6; HFD n=8; HFD+MIE n=9; HFD+PM n=7; HFD+PM+MIE n=7. Were evaluated: Food consumption, biometric and glycemic profile, oxidative stress markers, plasma eHSP72 concentration, iHSP70 expression in cardiopulmonary tissues and H index. We found no differences in iHSP70 expression. However, acute moderate intensity exercise generates a lower plasma concentration of eHSP72. Also, exercise reduce H index related to heart and lung tissues in CTR+MIE group and related to lung tissue in PM+MIE group, suggesting an anti-inflammatory response only in non-obese animal. We conclude that acute moderate intensity exercise produced an anti-inflammatory effect in sedentary mice, even if exposed to $PM_{2.5}$ and this effect did not occur in obesity.

Keywords: Physical Exercise. Obesity. Fine Particulate Matter. HSP70. eHSP72.

LISTA DE ABREVIATURAS

ANOVA: Análise de Variância

AUC: Área sob a curva

CAT: Catalase

DM2: Diabetes mellitus tipo 2

EDTA: *ethylenediaminetetraacetic acid*

EF: Exercício Físico

eHSP72: Proteína de Choque Térmico extracelular de 72 kDa

EO: Estresse Oxidativo

ERO: Espécies Reativas de Oxigênio

GTT: Teste de Tolerância à Glicose

HFD: Dieta Hiperlipídica

HSP70: Proteína de Choque Térmico de 70 kDa

iHSP70: Proteína de Choque Térmico intracelular de 70 kDa

IL-1β: Interleucina 1 beta

IL-1ra: Receptor de interleucina 1

IL-2: Interleucina 2

IL-6: Interleucina 6

IL-10: Interleucina 10

i.p.: Intraperitoneal

i.p. – GTT: Teste de Tolerância à Glicose via intraperitoneal

IMC: Índice de Massa Corporal

kDa: quilodalton

KPi: *Potassium Phosphate Buffer*

LPO: Lipoperoxidação

MDA: Malondialdeído

MIE: Exercício físico de intensidade moderada

MP: Material particulado fino

$MP_{2,5}$: Material Particulado fino <2,5 μM

NF-κB: Fator de transcrição nuclear κB

OMS: Organização Mundial da Saúde

PMSF: *Phenylmethanesulfonyl Fluoride*

SDS: *Sodium Dodecyl Sulfate*

SOD: Superóxido Dismutase

sTNF-R: Receptor Solúvel do Fator de Necrose Tumoral

TAB: Tecido adiposo branco epididimal

TBARS: Teste de Substâncias Reativas ao Ácido Tiobarbitúrico

TCA: Ácido tricloroacético

TNF-α: fator de necrose tumoral alfa

TRIS: *2-amino-2-hydroxymethyl-propane-1,3-diol*

SUMÁRIO

1. INTRODUÇÃO

A obesidade é uma doença complexa, multifatorial, que está fortemente associada com diversas comorbidades (SMITH; SMITH, 2016), caracterizada pelo balanço energético positivo e considerada atualmente um importante problema de saúde pública em diferentes culturas. A obesidade e o sobrepeso aumentaram sua prevalência de forma global sendo 28% em adultos e 47% em crianças no período de 1980 a 2013. Estimativas atuais mostram que existem aproximadamente 2,1 bilhões de pessoas com sobrepeso ou com obesidade no mundo (NG et al., 2014).

Hábitos cada vez mais sedentários das populações têm corroborado para o aumento da prevalência da obesidade e doenças relacionadas. No Brasil, em recente levantamento realizado em todas as capitais, com indivíduos acima de 18 anos pela, Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico (VIGITEL), apontou que a frequência de sedentários no conjunto da população é de 48,7%. Este estudo considerou sedentários, aqueles indivíduos que não alcançam um nível suficiente de atividade física moderada semanal de 150min (BRASIL, 2015).

As alterações metabólicas decorrentes do modo de vida moderno são estabelecidas como os principais fatores de risco para desenvolvimento de diabetes (WHO, 2003). Estudos têm demonstrado que indicadores antropométricos de excesso de tecido adiposo na região abdominal estão correlacionados à ocorrência de algumas doenças como diabetes *mellitus* tipo 2 (DM2) e outras como hipertensão arterial, dislipidemias e também risco coronariano elevado (CAVALCANTI et al., 2009).

Muita atenção tem sido dada ao tecido adiposo depois que o quadro de inflamação crônica de baixo grau, característica da obesidade, surgiu como um elo entre obesidade e doenças metabólicas como o DM2 (HOTAMISLIGIL, 2006). O acúmulo de tecido adiposo tem sido associado com biomarcadores de estresse oxidativo (KEANEY et al., 2003). Indivíduos diabéticos, devido à condição de hiperglicemia, podem induzir aumento de Espécies Reativas de Oxigênio (ERO), além de reduzirem sua capacidade de defesa antioxidante favorecendo um quadro de estresse oxidativo (MARTÍN-GALLÁN et al., 2003).

Assim, estudos que abordem estratégias de prevenção e/ou tratamento da obesidade e síndromes metabólicas relacionadas, ou estudos que propiciam uma melhor compreensão dos

mecanismos fisiopatológicos dessas doenças, são de suma importância para a compreensão e desenvolvimento de estratégias de prevenção e tratamento em saúde. Logo, modelos experimentais são essenciais para estudo dos mecanismos patológicos de doenças humanas como a obesidade e doenças relacionadas (KOYA; KANASAKI, 2011).

Nesse sentido, o modelo que mais se assemelha ao desenvolvimento da obesidade humana e tem sido considerado adequado é o modelo de obesidade exógena. Modelo no qual o objetivo é gerar um balanço energético positivo e observar alterações metabólicas em animais, semelhantes aos efeitos da obesidade em humanos. Assim, são frequentemente adotadas metodologias de exposição prolongada a dietas ricas em gorduras (hiperlipídicas) (ESTADELLA et al., 2004).

Além da obesidade, outro problema de ordem de saúde global é a má qualidade do ar ao qual estamos expostos, principalmente em países industrializados (SLOVIC et al., 2015). Correntemente, pesquisas epidemiológicas e toxicológicas vêm fornecendo evidências dos efeitos da poluição atmosférica na saúde humana, ainda que com níveis de exposição menores do que os padrões recomendados pela Organização Mundial da Saúde (OMS), confirmando um problema de saúde pública (COTE et al., 2008).

A obesidade e a exposição à poluentes atmosféricos, dentre os quais se destaca o material particulado de 2,5μm ($MP_{2,5}$), podem apresentar mecanismos comuns de agravo à saúde. Ambos, estão intimamente relacionados ao aumento desregulado de citocinas inflamatórias, logo, podem levar ao consequente aumento de Espécies Reativas de Oxigênio (ERO), estresse oxidativo e inflamação sistêmica (FURUKAWA et al., 2004; MEIER et al., 2014).

Além disso, obesidade e o sobrepeso corporal podem tornar o indivíduo mais suscetível aos efeitos da poluição atmosférica, como visto pelo aumento da prevalência de doenças cardiovasculares e acidente vascular encefálico (QIN et al., 2015). Partículas que apresentam diâmetro aerodinâmico menor que 2,5 μm, formadas a partir dos processos de combustão, incluindo fontes como veículos, usinas, incêndios, queimadas agrícolas e processos industriais têm a capacidade de invadir o trato respiratório e sistema vascular (PEARSON et al., 2010), sendo um grande risco a saúde da população.

A exposição prolongada a níveis elevados de poluentes atmosféricos está significativamente associada ao elevado risco de desenvolvimento de DM2 (WANG et al., 2014). Estudos com animais demonstram que a inalação de $MP_{2,5}$ pode gerar desequilíbrio autonômico e estresse oxidativo, fatores associados ao desenvolvimento de inflamação pulmonar em ratos (RHODEN et al., 2004). Ainda, a inalação de $MP_{2,5}$ pode levar à alteração do tônus vasomotor, inflamação vascular, adiposidade, aterosclerose e resistência insulínica (XU et al., 2011).

Em contrapartida, no combate as doenças crônicas e suas comorbidades, têm-se a recomendação de prática regular de exercícios moderados a vigorosos pela OMS e por diversas sociedades acadêmico-científicas (ELJSVOGELS; THOMPSON, 2015). Quando realizado de forma aguda, por indivíduos sedentários e sem planejamento, o exercício físico também representa desafios ao organismo, podendo desencadear alterações no sistema redox e quadro inflamatório (THRALL et al., 2007). A explicação deve-se ao fato que em situação de repouso, as defesas antioxidantes presentes nos tecidos são suficientes para controlar grande parte da atividade oxidativa, no entanto, durante a realização de exercício físico (principalmente extenuante), ocorre aumento do consumo de oxigênio nas células de diferentes tecidos, o qual é diretamente proporcional à formação de ERO (BANERJEE et al., 2003). Em indivíduos sedentários, os tecidos não são capazes de debelar tal demanda oxidativa (FISHER-WELLMAN et al., 2009), podendo ocorrer processo inflamatório associado (VAN HELVOORT et al., 2005).

Neste trabalho, buscamos avaliar o efeito do exercício físico agudo em condições de risco à saúde: na obesidade e na exposição à poluição atmosférica. Ainda, temos o entendimento de que essas condições (obesidade, poluição e exercício físico) podem ser avaliadas por variáveis comuns: pelas alterações metabólicas, pelo estresse oxidativo e pelo quadro inflamatório, este último, analisado através da Proteína de Choque Térmico de 70 kDa (HSP70).

Estímulos estressores como a obesidade (CHUNG et al., 2008), poluição atmosférica (KIDO et al., 2011) e exercício físico (MILNE; NOBLE, 2002) podem promover alterações na expressão de HSP70. Tem sido proposto que devido à versatilidade dessa proteína, em induzir diferentes respostas relacionadas à inflamação, de acordo com sua localização, a HSP70 pode representar um importante marcador para o estado imunoinflamatório durante o exercício físico (KRAUSE et al., 2015) e em muitos tipos de doenças (RODRIGUES-

KRAUSE et al., 2012). Resumidamente, enquanto no ambiente intracelular (iHSP70) essa proteína ativa diversas vias de sinalização anti-inflamatórias, principalmente através da inativação do fator de transcrição nuclear NF-κB (JONES et al., 2011), fora da célula (eHSP70) induz o efeito oposto (ASEA, 2008).

O quadro de obesidade, devido à expansão do tecido adiposo, aumenta os níveis circulantes de citocinas pró-inflamatórias como o fator de necrose tumoral alfa (TNF-α) e a IL-1β (CHUNG et al., 2008), as quais em conjunto com o quadro de hiperglicemia, aumentam a produção de ERO resultando em estresse oxidativo, disfunção mitocondrial e ativação de NF-κB (NEWSHOLME; KRAUSE, 2014). Assim, notavelmente o aumento da gordura corporal pode determinar o conteúdo de HSP70 (RODRIGUES-KRAUSE et al., 2012).

A exposição ao MP induz a expressão de HSP70 *in vitro* e *in vivo*. O aumento do conteúdo de HSP70 *in vitro* está relacionado à proteção e sobrevivência celular (GUALTIERI et al., 2008). A exposição crônica *in vivo* ao MP demonstrou aumentar o nível de eHSP70 (plasma), podendo ser um importante mediador imunológico, contribuindo à agravos como disfunção vascular e eventos cardiovasculares (KIDO et al., 2011). Desta forma, tem sido proposto que a HSP70 possa ser utilizada como um biomarcador para avaliações precoces dos efeitos nocivos à saúde causados pela exposição à poluição atmosférica (MUKHOPADHYAY et al., 2003).

É amplamente aceito que a realização de exercício físico agudo e/ou crônico modula a atividade e expressão de HSP70 em vários tecidos humanos e animais (DIMAURO et al., 2016). Além disso, resultados com animais indicam que o exercício físico induz mudanças no nível de HSP70 no coração e pulmão (LOLLO et al., 2013). E como já indicado, a expressão dessa proteína é dependente da intensidade do exercício realizado (MILNE; NOBLE, 2002). Enquanto sessões agudas de exercício sinalizam uma situação de estresse fisiológico para todos os sistemas (HECK et al., 2011), podendo gerar um aumento momentâneo na concentração de eHSP70 (FEBBRAIO et al., 2002), com o treinamento essa resposta inicial tende a diminuir (MORTON et al., 2009), assim, gerando adaptações ao estresse e consequente menor concentração de eHSP70.

Interessantemente, a expressão de HSP70 em diferentes células e sua exportação para a circulação ocorre em situações de desafio ao organismo, como nas três dimensões em estudo (obesidade, exposição à poluição e exercício físico). Assim, o objetivo deste estudo foi avaliar

o efeito do exercício físico agudo em camundongos obesos expostos ao material particulado fino quanto à razão intra e extracelular de proteínas de choque térmico e variáveis de estresse oxidativo do coração e do pulmão.

1.1 Obesidade

A prevalência da obesidade tem atingido proporções epidêmicas nos últimos anos, estima-se que existam aproximadamente 2,1 bilhões de pessoas obesas ou com sobrepeso no mundo, o que representa quase 30% da população mundial. Definida como o acúmulo de gordura anormal ou excessiva, a obesidade é uma doença crônica degenerativa multifatorial, caracterizada pelo balanço energético positivo, e considerada atualmente um importante problema de saúde pública de ordem global, tanto para países desenvolvidos como para os em desenvolvimento (WHO, 2015).

O padrão ouro adotado, como critério para o diagnóstico do sobrepeso e obesidade, são técnicas de imagem como ressonância magnética, tomografia computadorizada e absorciometria com raios-X de dupla energia (dexa). Porém devido ao alto custo e a falta de equipamentos necessários, seu uso na prática clínica torna-se indisponível (ABESO, 2010). O critério indicado pela Organização Mundial da Saúde (OMS), devido a sua aplicabilidade e baixo custo, é o Índice de Massa Corporal (IMC), calculado a partir da massa corporal do indivíduo em quilogramas, dividido pelo quadrado de sua altura em metros (kg/m^2). Desta forma, são classificados com sobrepeso corporal indivíduos de IMC $\geq$ 25 kg/m^2 e com obesidade IMC $\geq$ 30 kg/m^2 (WHO, 2015). O número de indivíduos nessas condições já ultrapassa o número de pessoas desnutridas, o que demonstra aceitação global por alimentos palatáveis ricos em gorduras. No Brasil 52,5% dos homens e 58,4% das mulheres com mais de 20 apresentam sobrepeso e/ou obesidade (NG et al., 2014).

As alterações metabólicas decorrentes deste modo de vida são estabelecidas como os principais fatores de risco para uma série de doenças crônicas não transmissíveis (DCNT) (WHO, 2003). Estudos têm demonstrado que indicadores antropométricos de excesso de tecido adiposo na região abdominal estão correlacionados à ocorrência de DM2 e de algumas doenças como hipertensão arterial, dislipidemias e também risco coronariano elevado (CAVALCANTI; CARVALHO; BARROS, 2009).

Atualmente 382 milhões de pessoas são afetadas pelo DM2 e todos os anos 4,9 milhões de pessoas morrem em decorrência da doença. Existem previsões, de acordo com as tendências atuais, que 592 milhões de pessoas terão a doença em 2035 (INTERNATIONAL DIABETES FEDERATION, 2013). Essa pandemia de síndromes metabólicas e transtornos relacionados com a obesidade sugerem um aumento proporcional na prevalência de DM2, e

este, é um dos principais fatores de morbidade e mortalidade em todo mundo (HOSSAIN et al., 2007).

A etiologia para o aumento das taxas de prevalência da obesidade tem sido associada a um estilo de vida inadequado, caracterizado principalmente pelo excesso da ingestão de alimentos altamente energéticos, ricos em gordura. Além disso, o aumento da inatividade física, devido à natureza cada vez mais sedentária de muitas formas de trabalho, mudança em modos de transporte e crescente urbanização contribuem para essa alta prevalência (WHO, 2015).

Desde que a obesidade passou a ser reconhecida como uma doença inflamatória, diversos estudos tem encontrado relação entre a doença e alterações metabólicas e endócrinas do tecido adiposo. Em indivíduos obesos, o tecido adiposo aumenta a síntese de adipocinas (citocinas) de efeito pró-inflamatório, como a interleucina-6 (IL-6), o fator de necrose tumoral alfa (TNF-α) e os fatores do complemento B, C3 e D (adipsina). Todas elas têm funções imunológicas, produzidas no adipócito, em resposta a estímulos infecciosos ou inflamatórios (COSTA; DUARTE, 2006). Embora algumas destas moléculas tenham, sobretudo, ação autócrina ou parácrina, algumas contribuem para a inflamação sistêmica de baixo grau, presente no DM2 (BERG; SCHERER, 2005)

Assim, a inflamação crônica de baixo grau, que é mediada principalmente por células do sistema imune inato e adaptativo surgiu como um elo importante entre obesidade e alterações metabólicas (HOTAMISLIGIL, 2006). O tecido adiposo deixa de ser considerado apenas um reservatório de energia e passa a ser reconhecido como órgão endócrino, com múltiplas funções e papel central na gênese da resistência à insulina (RI) (MOHAMED-ALI; PINKNEY; COPPACK, 1998), intolerância à glicose, hipertensão arterial e dislipidemias (DEFRONZO, 1997; TIMAR; SESTIER; LEVY, 2000).

A RI e a conseqüência do declínio na secreção de insulina pelo pâncreas, são o princípio da patogênese do DM2 (KAHN, 1994; GRODSKY, 1999; REAVEN, 2011). Na fase inicial do DM2, a tolerância normal à glicose é preservada devido à compensação pela hipersecreção de insulina (hiperinsulinemia), porém com aumento constante da RI a hiperinsulinemia compensatória torna-se insuficiente, ocorre exaustão das células β pancreática, resultando em hiperglicemia sustentada e DM2 (DIABETES, 2010).

Existem crescentes evidências que mostram que o estresse oxidativo (EO) desempenha um papel chave em processos patológicos observados no DM2. Estudos indicam que o EO é responsável em gerar disfunções nas células β pancreáticas e resistência à insulina, as duas marcas do DM2 (GIACCO; BROWNLEE, 2010; HENRIKSEN et al., 2011).

EO é o desequilíbrio bioquímico apresentado quando a produção de radicais livres ou de ERO excede a capacidade antioxidante natural do organismo, resultando em dano oxidativo (HALLIWELL; GUTTERIDGE, 1999). Logo, o EO torna o organismo suscetível e contribui para o desenvolvimento de inúmeras patologias, especialmente aquelas de natureza crônica ou degenerativa (WEXLER, 2007), como podemos salientar, na condição de indivíduos obesos (VAN GAAL; MERTENS; DE BLOCK, 2006) e/ou diabéticos (GIACCO; BROWNLEE, 2010).

Em decorrência da hiperglicemia, gerada no quadro de DM2, indivíduos acometidos com a doença são predispostos a desenvolver um perfil pró-oxidante e pró-inflamatório, uma vez que apresentam aumento da produção de ERO e diminuição da capacidade de defesa antioxidante (VANDERJAGT et al., 2001), bem como aumento de citocinas inflamatórias (SHARMA et al., 2012).

A obesidade e doenças associadas, atualmente representam um dos maiores desafios para a ciência básica e pesquisas clínicas. É evidente que o desenvolvimento de modelos animais apropriados é crucial para estudos da patogênese e terapia destas complexas desordens metabólicas (CALIXTO, 2012).

Assim como os seres humanos, os roedores tendem a ganhar peso corporal com ingestão de dieta hiperlipídica, e os parâmetros utilizados para confirmação de um estado obeso, assim como em humanos pode ser o IMC, determinado a partir da relação entre peso corporal em gramas, dividido pelo comprimento nasoanal do animal em centímetros quadrados (g/cm^2) e a adiposidade abdominal (NOVELLI et al., 2007).

Diversos estudos têm utilizado dietas hiperlipídicas para reproduzir modelos de obesidade em roedores. Desde a década de 40, quando foi descrita a primeira intervenção nutricional, estudos subseqüentes revelaram que este modelo experimental promove hiperglicemia e resistência à insulina (IKEMOTO et al., 1996; WOODS et al., 2003),

demonstrando, que dietas hiperlipídicas podem ser utilizadas para criar um modelo válido de obesidade em roedores (BUETTNER et al., 2007).

Neste modelo, acrescenta-se ou associam-se à ração padrão, substâncias altamente calóricas (CESARETTI; KOHLMANN JUNIOR, 2006). Por gerar um balanço energético positivo e observar alterações no metabolismo dos animais que se assemelhem aos efeitos da obesidade em humanos, este tem sido considerado um modelo experimental adequado (ESTADELLA et al., 2004). As consequências metabólicas do consumo contínuo de dietas hiperlipídicas também estão associadas ao prejuízo no metabolismo glicêmico, estimulando uma produção anormal de glicose, causando hiperinsulinemia e resistência à insulina, EO e inflamação (REAVEN, 1988).

1.2 Poluição Atmosférica

A poluição do ar é um problema de saúde ambiental que tem afetado todos os países desenvolvidos ou em desenvolvimento em todo o mundo. Definida como qualquer forma de matéria ou energia com intensidade, concentração, tempo ou características que possam tornar o ar impróprio e nocivo à saúde, ao bem estar público e à qualidade de vida de uma comunidade (BRASIL, 2012), a poluição atmosférica foi responsável por cerca de 7 milhões de mortes em todo o mundo só no ano de 2012 (WHO, 2014).

O termo "poluente" diz respeito a substâncias químicas, partículas ou gases tóxicos, introduzidas no meio ambiente por diversas fontes e que causam efeitos adversos aos seres vivos e/ou no ecossistema (BRASIL, 2012). A OMS lista uma série de componentes tóxicos para a saúde relacionados à poluição do ar. Estes podem ser emitidos para a atmosfera como dióxido de enxofre, monóxido de carbono e óxido de nitrogênio, e também quando formados a partir de reações químicas, a exemplo do ozônio e o material particulado (MP) (WHO, 2006).

A relação entre poluição atmosférica e problemas de saúde tem sido constantemente estudada nos últimos 15 anos, e a literatura mundial tem demonstrado que variações tóxicas dos poluentes no ambiente afetam a saúde humana de diferentes maneiras e níveis de gravidade, reduzindo a expectativa de vida e aumentando a taxa de mortalidade principalmente em grandes centros urbanos (SLOVIC et al., 2015).

Devido ao maior efeito sobre a saúde humana, o MP tem recebido maior ênfase em pesquisas científicas epidemiológicas e experimentais. MP é uma mistura de partículas líquidas e sólidas em suspensão no ar. Sua composição e tamanho dependem das fontes de emissão e condições meteorológicas presentes no ambiente. O MP é classificado de acordo com seu tamanho aerodinâmico, mas também pode ser classificado em partículas inaláveis ou grossas cujo diâmetro é menor que 10 μm (MP_{10} - 2,5 a 10 μm), partículas finas de até 2,5 μm ($MP_{2,5}$) e ultrafinas cujo o tamanho é inferior a 0,1 μm ($MP_{0,1}$) (BRAGA et al., 2001).

O tamanho aerodinâmico da partícula possui relação inversamente proporcional ao potencial de deposição no trato respiratório e os efeitos associados à saúde. Logo, quanto menor a partícula, maiores são os efeitos produzidos. Neste contexto, destaca-se a poluição atmosférica por $MP_{2,5}$, formado a partir dos processos de combustão, incluindo fontes como

veículos, usinas, incêndios, queimadas agrícolas e processos industriais que tem a capacidade de invadir o trato respiratório e sistema vascular (PEARSON et al., 2010) podendo gerar efeitos tóxicos em órgãos específicos (BURCH, 2002).

Portanto, estabelecer padrões de controle da qualidade do ar, pode contribuir para prevenção de problemas ambientais e da saúde da população (RODRIGUES et al., 2015). A OMS estabeleceu em 2005 padrões de poluição recomendáveis, visando o menor efeito sobre a saúde: para a concentração de $MP_{2,5}$, foi estabelecida uma média diária de 25μg/m^3 e anual de 10μg/m^3, mas os países poderiam adotar distintos padrões, de acordo com suas especificidades locais (WHO, 2005).

No Brasil, os padrões de qualidade do ar foram estabelecidos pela resolução do Conselho Nacional do Meio Ambiente (CONAMA) nº 03/90 e foi estabelecida apenas a média anual de MP_{10} de 150μg/m^3 (BRASIL, 1990). No entanto, em 2013 o Conselho Estadual do Meio Ambiente do Estado de São Paulo (CONSEMA), definiu novos critérios para MP_{10} e estabeleceu o padrão para $MP_{2,5}$, até então inexistente no Brasil. Assim, foram estabelecidos os limites diários para $MP_{2,5}$ em 60μg/m^3 e anuais em 20μg/m^3 (CONSEMA, 2013).

Com base em inúmeros estudos epidemiológicos e grande observação clínica, o $MP_{2,5}$ tem sido considerado como o principal responsável pelos efeitos cardiovasculares adversos da poluição atmosférica na saúde humana (BROOK et al., 2010). Estudos mostram a relação da exposição ao $MP_{2,5}$ na disfunção endotelial e formação da placa aterosclerótica (KILINÇ et al., 2011), com o aumento da pressão arterial (CHAN et al., 2015), com o infarto agudo do miocárdio (CESARONI et al., 2014), e em outras doenças como câncer de pulmão (POPE et al., 2002) e doenças respiratórias (XING et al., 2016).

Pesquisa realizada pela *Global Burden of Disease* que avalia a mortalidade e incapacidade por doenças graves, lesões e fatores de risco, estabeleceu em 2010 a poluição atmosférica por $MP_{2,5}$ como o sexto maior fator de risco para mortalidade prematura global. A carga da exposição ao poluente é maior que a combinação de doenças como malária e HIV-AIDS (IHME, 2012; APTE et al., 2015).

O desequilíbrio bioquímico, gerado após a exposição a agentes pró-oxidantes, como o $MP_{2,5}$, é o fator patogênico comum entre as doenças relacionadas a poluição atmosférica

(GONZÁLEZ-FLECHA, 2004). Os mecanismos moleculares de doenças provocadas pela exposição ao $MP_{2,5}$ incluem mecanismos diretos, nos quais o $MP_{2,5}$ entra em contato diretamente com a corrente sanguínea e com outros órgãos, gerando inflamação pulmonar local, aumento de ERO e consequente EO (NEMMAR et al., 2002), e mecanismos indiretos, onde o $MP_{2,5}$ pode desencadear uma cascata inflamatória relacionada a sua deposição no pulmão, gerando assim, aumento de citocinas inflamatórias como Proteína C Reativa (PCR), IL-6, IL-8 e IL-1β, caracterizando um quadro de inflamação sistêmica (MEIER et al., 2014).

Assim, o quadro de obesidade (tópico 1.1) e de exposição à poluição atmosférica, destacado acima pelo $MP_{2,5}$, podem apresentar mecanismos em comum. A obesidade, está intimamente relacionada ao aumento desregulado de citocinas inflamatórias, assim como, o resultado da exposição ao $MP_{2,5}$. Logo, ambos podem levar ao consequente aumento de ERO, EO e inflamação sistêmica (FURUKAWA et al., 2004; MEIER et al., 2014).

Além disso, indivíduos obesos ou com sobrepeso corporal, podem ser mais suscetíveis aos efeitos da poluição atmosférica, observado por estudos epidemiológicos que indicam o aumento da prevalência de doenças cardiovasculares e acidente vascular encefálico em população exposta ao $MP_{2,5}$ (QIN et al., 2015). Além disso, a exposição prolongada a níveis elevados de poluentes atmosféricos está significativamente associada ao elevado risco de desenvolvimento de DM2 (WANG et al., 2014).

Diversos estudos têm utilizado modelos experimentais com animais, na busca de uma melhor compreensão dos mecanismos fisiopatológicos relacionados aos efeitos nocivos da poluição atmosférica à saúde. Para tal, existem diferentes modelos *in vivo* que mimetizam os efeitos da poluição atmosférica. Vale destacar métodos em câmaras, em que o ar é propulsionado do ambiente externo para a área interna da câmara onde os animais ficam expostos (SUN et al., 2009), concentrador de partículas, onde são utilizadas durante um período de tempo as concentrações reais de partículas existentes no meio ambiente (RHODEN et al., 2004) e métodos de instilação intratraqueal (YAN et al., 2011) e intranasal (ZANCHI et al., 2008).

O método de instilação intranasal, conforme utilizado em nosso estudo, o animal é contido pelo manipulador, e então é realizada a administração via intranasal da suspensão em líquido, da concentração conhecida do poluente em teste, diretamente na narina do animal, que por reflexo de apnéia inala a suspensão (OSIER; OBERDÖRSTER, 1997). Este tem sido

um método eficiente em relacionar os efeitos da poluição atmosférica no desenvolvimento de doenças crônicas respiratórias e cardiometabólicas (BROOK et al., 2010).

Estudos com animais demonstram que a inalação de $MP_{2,5}$ pode gerar desequilíbrio autonômico e EO, fatores associados ao desenvolvimento de inflamação pulmonar em ratos (RHODEN et al., 2004). XU et al. (2011) mostraram que a inalação de $MP_{2,5}$ pode levar à alteração do tônus vasomotor, inflamação vascular, adiposidade, aterosclerose e RI. Considerando que indivíduos com obesidade também apresentam desequilíbrio autonômico e EO a combinação apresenta-se como um risco à saúde.

1.3 Exercício Físico

Compreende-se por exercício físico (EF) todos os movimentos corporais planejados, organizados e repetidos com o objetivo de manter ou melhorar um ou mais componentes da aptidão física (CASPERSEN et al. 1985). O EF caracteriza-se por uma situação que retira o organismo de sua homeostase, pois implica no aumento instantâneo da demanda energética da musculatura exercitada e, consequentemente, do organismo como um todo. As respostas geradas no organismo dependem do tipo de EF realizado, variando quanto ao seu volume, freqüência e intensidade (BRUM; NEGRÃO, 2004).

Atualmente, evidências científicas têm suportado que o EF deve ser entendido como uma das formas de tratamento para DCNT. Estudos demonstram que uma mudança no estilo de vida, visando o aumento do gasto calórico, através da prescrição de EF regular, aliado ao controle alimentar, visando diminuir a ingestão calórica, fazem parte de uma primeira linha de tratamento da obesidade e síndromes relacionadas como DM2 (LEON; MADDOX, 2015).

Foi demonstrado por KNOWLER et al. (2002), em ensaio clínico com indivíduos obesos em alto risco de desenvolvimento de DM2, que a adoção de um estilo de vida adequado, visando à diminuição do peso corporal inicial em 7%, através de práticas regulares de exercícios físicos moderados (150min/semana), seguido de uma dieta com baixo teor gordura, obteve maior eficácia na incidência de DM2 do que o tratamento farmacológico com Metformina, medicamento hipoglicemiante.

Nesse sentido, têm-se recomendações de prática regular de exercícios moderados à vigorosos pela OMS e por sociedades acadêmico-científicas, no combate de doenças crônicas e suas comorbidades (ELJSVOGELS; THOMPSON, 2015). É atual a discussão sobre qual a melhor modalidade e intensidade de exercício para que sejam promovidos benefícios, metabólicos (LLOPIS et al., 2015) para indivíduos saudáveis e para indivíduos com obesidade e/ou DM2 (LITTLE et al., 2014).

Embora sejam relatados muitos efeitos benéficos do treinamento físico regular no controle glicêmico (UMPIERRE et al., 2011), evidências recentes demonstraram que uma sessão (30min.) de exercício aeróbico de intensidade moderada reduz substancialmente a hiperglicemia no dia seguinte em indivíduos com DM2 (VAN DIJK et al., 2012).

No entanto, quando realizado de forma aguda, por indivíduos obesos, em ambientes de exposição à poluição atmosférica, a prática de EF pode representar desafios extremos ao organismo, deixando de trazer benefícios e oferecendo risco ao indivíduo (THRALL et al., 2007).

A principal razão para este debate é que, mesmo durante o exercício agudo de baixa intensidade, há aumento significativo da ventilação pulmonar, assim, a taxa de deposição de MP durante o EF pode ser cerca de cinco vezes maior do que na situação de repouso (DAIGLE et al., 2003). Cabe destacar que tais eventos tóxicos são potencialmente maiores em sessões agudas de EF realizada por indivíduos sedentários quando comparadas a indivíduos treinados (WEN et al., 2009).

A explicação deve-se ao fato que em situação de repouso, as defesas antioxidantes presentes nos tecidos são suficientes para controlar grande parte da atividade oxidativa, no entanto, durante a realização de exercício físico, ocorre aumento do consumo de oxigênio nas células de diferentes tecidos, o qual é diretamente proporcional à intensidade do exercício e consequente formação de ERO (BANERJEE et al., 2003). Em indivíduos sedentários, os tecidos não são capazes de debelar tal demanda oxidativa (FISHER-WELLMAN et al., 2009), podendo ocorrer processo inflamatório associado (VAN HELVOORT et al., 2005).

Neste sentido, tanto humanos como animais utilizam sistemas de defesa antioxidante para neutralizar o dano oxidativo e proteger as células da ação de ERO (ZANCHI et al., 2008). Esse mecanismo de defesa envolve estratégias enzimáticas como aumento na atividade de enzimas antioxidantes como a superóxido dismutase (SOD), catalase (CAT), ou ainda adaptações moleculares como aumento na expressão de proteínas de choque térmico (HSP) (MILNE; NOBLE, 2002).

Por outro lado, estudos têm relatado que o exercício agudo é capaz de gerar uma resposta imunológica anti-inflamatória, mediada por citocinas com ação anti-inflamatória (PETERSEN; PEDERSEN, 2005). As citocinas são pequenos polipeptídeos, que foram originalmente descobertos por terem funções imunoreguladoras, assim, podendo atuar com funções tanto pró quanto anti-inflamatória (AKIRA; KISHIMOTO, 1992).

A principal mediadora na resposta anti-inflamatória durante o EF é a IL-6. A atividade reguladora do processo inflamatório da IL-6 vem sendo considerada como o principal agente

regulador da resposta de fase aguda no EF. Essa citocina é produzida em concentrações mais elevadas pelo tecido muscular esquelético estriado, leucócitos e células endoteliais, via sinalização das citocinas pró-inflamatórias e das ERO, sendo sua secreção relacionada à intensidade, duração e quantidade de massa muscular envolvida no EF. Os níveis dessa citocina podem aumentar de modo exponencial, até 100 vezes, em resposta ao exercício e declina no período pós-exercício (PETERSEN; PEDERSEN, 2005). As ações anti-inflamatórias da IL-6 são diversificadas, incluindo efeitos inibitórios sobre a produção e secreção, principalmente de TNF-α, estímulo da síntese das citocinas anti-inflamatórias como receptor antagonista de IL-1 (IL-1ra) e IL-10, além do estímulo a liberação de receptores solúveis para TNF-α (sTNF-R) (PETERSEN; PEDERSEN, 2005).

Considerando que desafios ao organismo geram uma situação de estresse e que dependendo da intensidade pode causar efeitos danosos ao mesmo, e por outro lado, o fato de alterações moderadas poderem moldar capacidades fisiológicas através do desenvolvimento de ajustes internos (HOCHACHKA, 2002). Destacam-se os efeitos da realização de exercício físico, que desafia o organismo durante o esforço, mas promove adaptações que podem ser benéficas diante de outras adversidades do ambiente interno (obesidade) e externo (poluição atmosférica).

Assim, é plausível inferir que este efeito do EF, simultaneamente sobre o sistema imunológico (equilíbrio pró/anti-inflamatório) e sobre o balanço das atividades pró e antioxidantes nos diferentes órgãos, possa ter um biomarcador em comum, que indique com precisão o processo saúde/doença, assim como servir de referência sobre os processos biológicos subclínicos que ocorrem no organismo quanto a prescrição de exercício físico: a HSP70 (HECK, 2011).

1.4 Proteínas de Choque Térmico

As proteínas de choque térmico, do inglês *Heat Shock Protein (HSP)*, são consideradas parte de uma família de proteínas conhecidas como proteínas de estresse. Inicialmente, foram identificadas como proteínas induzidas pelo estresse térmico, quando observou-se o surgimento de um novo padrão de espessamento cromossomal em células de glândulas salivares de *Drosophila Buskii*, fato que representava a transcrição para síntese de HSP após a exposição celular a temperaturas elevadas (DE MAIO, 2011).

Primeiramente, as HSPs foram descritas pela sua ação de chaperona molecular, devido à capacidade de impedir a agregação proteica, auxiliando o remodelamento e a manutenção de proteínas estruturais, auxiliar no transporte de proteínas através da membrana, a degradação de proteínas instáveis e o remodelando de proteínas desnaturadas (JOHNSON; FLESHNER, 2006). Contudo, foi verificado que se trata não só de um mecanismo de defesa celular complexo e altamente conservado, como também desempenha papel fundamental a preservação da sobrevivência celular sob condições adversas (GUPTA et al., 2007), situação designada como *stress response* (resposta ao estresse) (MUKHOPADHYAY et al., 2003). Embora a descoberta das HSPs tenha ocorrido sob condições de choque térmico, outros estímulos estressores como a obesidade, poluição atmosférica e EF podem promover alterações na expressão destas proteínas (CHUNG et al., 2008; KIDO et al., 2011; MILNE; NOBLE, 2002).

As HSPs são usualmente divididas em duas grandes classes, de acordo com seu peso molecular: as pequenas, com peso molecular menor que 40 kDa, e as com alto peso molecular, entre 60 e 100 kDa. Apresentam baixa expressão sob condições basais (HSPs constitutivas) e são aumentadas em resposta a fatores estressores (HSPs induzíveis). A classe mais conservada e estudada ao longo dos anos é a de peso molecular de aproximadamente 70 kDa (HSP70) (VELICHKO et al., 2013).

Além da ação clássica de chaperona molecular, a HSP70 apresenta um efeito intracelular que merece um maior destaque: a inibição da ativação do fator de transcrição nuclear NF-κB (JONES et al., 2011). NF-κB é um fator de transcrição originalmente descoberto em linfócitos-B que são essenciais para armar as respostas inflamatórias a uma variedade de sinais, função imunológica, ativação de células endoteliais e controle do crescimento celular (BARNES; KARIN, 1997). Resumidamente, pode-se considerar que a

indução da expressão HSP70, aumentando o conteúdo intracelular (iHSP70) desta proteína, pode representar um potencial fator citoprotetor e anti-inflamatório, por evitar a desnaturação de outras proteínas intracelulares, por influenciar na sinalização que desencadeia a apoptose e, fundamentalmente, por inibir a excessiva ativação do NF-κB que pode trazer prejuízos celulares (HECK et al., 2011).

Em contraste a seu efeito intracelular anti-inflamatório, a HSP70 quando encontrada na circulação, ou seja, no ambiente extracelular (eHSP70), seus níveis circulantes estão correlacionados com prejuízos no balanço energético, alteração no *status* pró e anti-inflamatório e com o desequilíbrio dos sistemas pró e antioxidante do organismo (WALSH et al., 2001; MILNE; NOBLE, 2002; CAMPISI et al., 2003; WHITHAM; FORTES, 2008).

Por outro lado, a ausência e/ou inibição da expressão desta protein resulta em vulnerabilidade da célula ao estresse (RUBIO et al., 2002) podendo gerar apoptose celular (ADACHI et al., 2009). Um defeito na resposta ao estresse, como geralmente observado em quadros crônicos de inflamação, tem sido associado a muitas doenças relacionadas com a obesidade (DI NASO et al., 2015), como resistência à insulina, DM2 e esteatose hepática não alcoólica (NEWSHOLME; HOMEM DE BITTENCOURT, 2014). Resumidamente, a capacidade de uma célula em detectar corretamente e iniciar uma resposta é fundamental para sua sobrevivência.

Tem sido proposto que, devido à versatilidade da HSP70, em induzir diferentes respostas relacionadas à inflamação de acordo com sua localização, esta proteína pode representar um importante marcador para o estado imunoinflamatório durante o exercício físico (KRAUSE et al., 2015) e em muitos tipos de doenças (RODRIGUES-KRAUSE et al., 2012).

Portanto, níveis ideais desta proteína na circulação parecem ser fundamentais na manutenção da homeostase do organismo (HECK et al., 2011). Além disso, o equilíbrio na concentração eHSP70 e iHSP70, como já sugerido em outros estudos (YANG et al., 2008; MAGALHÃES et al., 2010; RODRIGUES-KRAUSE et al., 2012), mensurados pelo cálculo matemático da razão eHSP72/iHSP70 (HECK, 2011), pode representar um importante biomarcador do processo saúde doença, assim como servir de referência sobre os processos biológicos subclínicos que ocorrem no organismo como a obesidade (KRAUSE et al., 2015).

Assim, neste trabalho, buscaremos avaliar o efeito do EF agudo em condições de risco à saúde: na obesidade e na exposição ao $MP_{2,5}$. Ainda, temos o entendimento de que essas

condições (obesidade, poluição e exercício físico) podem ser avaliadas por fatores em comum: pelas alterações metabólicas, pelo EO e pelo quadro inflamatório, este último, quantificado através da razão eHSP70/iHSP70.

Interessantemente, a expressão de HSP70 em diferentes células e sua exportação para a circulação ocorre em situações de desafio ao organismo nas três dimensões em estudo: obesidade (tópico 1.1), exposição à poluição (tópico 1.2) e exercício físico (tópico 1.3).

OBJETIVO GERAL

Avaliar o efeito do exercício físico em camundongos obesos expostos ao material particulado fino quanto à razão intra e extracelular de proteínas de choque térmico e variáveis de estresse oxidativo do coração e do pulmão.

Objetivos Específicos

Avaliar o efeito do exercício físico em camundongos obesos expostos ao material particulado fino na/no(s):

- Perfil biométrico (massa corporal, IMC, Adiposidade Abdominal);

- Perfil Glicêmico;

- Concentração plasmática de eHSP70;

- Níveis de lipoperoxidação e atividade das enzimas antioxidantes CAT e SOD do coração e pulmão;

- Expressão de iHSP70 no coração e no pulmão;

- Razão eHSP72/iHSP70 (plasma/coração e plasma/pulmão).

REFERÊNCIAS

ABESO, ASSOCIAÇÃO BRASILEIRA ESTUDO DA OBESIDADE E SÍNDROME METABÓLICA. Atualização das diretrizes para o tratamento Farmacológico da obesidade e do sobrepeso. **Abeso**, v. 76, p. 4–18, 2010.

ADACHI, H. et al. Heat shock proteins in neurodegenerative diseases: pathogenic roles and therapeutic implications. **International journal of hyperthermia : the official journal of European Society for Hyperthermic Oncology, North American Hyperthermia Group**, v. 25, n. 8, p. 647–54, dez. 2009.

AKIRA, S.; KISHIMOTO, T. IL-6 and NF-IL6 in acute-phase response and viral infection. **Immunological reviews**, v. 127, p. 25–50, jun. 1992.

APTE, J. S. et al. Addressing Global Mortality from Ambient PM2.5. **Environmental science & technology**, v. 49, n. 13, p. 8057–66, 7 jul. 2015.

ASEA, A. Heat shock proteins and toll-like receptors. **Handbook of experimental pharmacology**, n. 183, p. 111–27, jan. 2008.

BANERJEE, A. K. et al. Oxidant, antioxidant and physical exercise. **Molecular and cellular biochemistry**, v. 253, n. 1-2, p. 307–12, nov. 2003.

BARNES, P. J.; KARIN, M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. **The New England journal of medicine**, v. 336, n. 15, p. 1066–71, 10 abr. 1997.

BERG, A. H.; SCHERER, P. E. Adipose tissue, inflammation, and cardiovascular disease. **Circulation research**, v. 96, n. 9, p. 939–49, 13 maio 2005.

BRAGA, A. et al. Poluição atmosférica e saúde humana. **Revista USP**, 2001.

BRASIL. **Qualidade do ar**. Disponível em: <http://www.mma.gov.br/cidades-sustentaveis/qualidade-do-ar>. Acesso em: 6 mar. 2016.

BRASIL. **VIGITEL 2014: Vigilância de fatores de Risco para doenças crônicas por inquérito telefônico**. [s.l: s.n.].

BRASIL, C. N. DO M. A.-C. **Resolução CONAMA n. 3 de 28 de junho de 1990. Diário Oficial da União, 22/08/1990.**, 1990. Disponível em: <http://www.mma.gov.br/port/conama/legiabre.cfm?codlegi=100>

BROOK, R. D. et al. Particulate matter air pollution and cardiovascular disease: An update to

the scientific statement from the American Heart Association. **Circulation**, v. 121, n. 21, p. 2331–78, 1 jun. 2010.

BRUM, P. C.; NEGRÃO, C. E. Adaptações agudas e crônicas do exercício físico no sistema cardiovascular. **Rev Paul Educ Fís**, v. 18, n. ago, n. esp., p. 21–31, 2004.

BUETTNER, R.; SCHÖLMERICH, J.; BOLLHEIMER, L. C. High-fat diets: modeling the metabolic disorders of human obesity in rodents. **Obesity (Silver Spring, Md.)**, v. 15, n. 4, p. 798–808, abr. 2007.

BURCH, W. M. Passage of Inhaled Particles Into the Blood Circulation in Humans * Response. **Circulation**, v. 106, n. 20, p. 141e–142, 12 nov. 2002.

CALIXTO, M. C. **Efeito da ativação da AMPK na exacerbação da inflamação pulmonar alérgica em camundongos obesos.** [s.l.] Biblioteca Digital da Unicamp, 21 jul. 2012.

CAMPISI, J.; LEEM, T. H.; FLESHNER, M. Stress-induced extracellular Hsp72 is a functionally significant danger signal to the immune system. **Cell stress & chaperones**, v. 8, n. 3, p. 272–86, jan. 2003.

CASPERSEN, C. J.; POWELL, K. E.; CHRISTENSON, G. M. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. **Public health reports (Washington, D.C. : 1974)**, v. 100, n. 2, p. 126–31, jan. 1985.

CAVALCANTI, C. B. DOS S.; CARVALHO, S. C. B. DO E.; BARROS, M. V. G. DE. Anthropometric indicators of abdominal obesity: review of the papers indexed on SciELO electronic library. **Rev. bras. cineantropom. desempenho hum**, p. 217–225, 2009.

CESARETTI, M. L. R.; KOHLMANN JUNIOR, O. Modelos experimentais de resistência à insulina e obesidade: lições aprendidas. **Arquivos Brasileiros de Endocrinologia & Metabologia**, v. 50, n. 2, p. 190–197, abr. 2006.

CESARONI, G. et al. Long term exposure to ambient air pollution and incidence of acute coronary events: prospective cohort study and meta-analysis in 11 European cohorts from the ESCAPE Project. **BMJ (Clinical research ed.)**, v. 348, p. f7412, jan. 2014.

CHAN, S. H. et al. Long-Term Air Pollution Exposure and Blood Pressure in the Sister Study. **Environmental health perspectives**, v. 123, n. 10, p. 951–8, out. 2015.

CHUNG, J. et al. HSP72 protects against obesity-induced insulin resistance. **Proceedings of the National Academy of Sciences of the United States of America**, v. 105, n. 5, p. 1739–44, 5 fev. 2008.

CONSEMA. **Revisão dos padrões de qualidade do ar e aprimoramento da gestão integrada da qualidade do ar no Estado de São Paulo.** Disponível em: <<http://www.cetesb.sp.gov.br/tecnologia/camaras/gt_ar/>. Acesso em: 31 mar. 2016.

COSTA, J.; DUARTE, J. Tecido adiposo e adipocinas. **Acta Med Port**, 2006.

COTE, I.; SAMET, J.; VANDENBERG, J. J. U.S. air quality management: local, regional and global approaches. **Journal of toxicology and environmental health. Part A**, v. 71, n. 1, p. 63–73, jan. 2008.

DAIGLE, C. C. et al. Ultrafine particle deposition in humans during rest and exercise. **Inhalation toxicology**, v. 15, n. 6, p. 539–52, maio 2003.

DE MAIO, A. Extracellular heat shock proteins, cellular export vesicles, and the Stress Observation System: a form of communication during injury, infection, and cell damage. It is never known how far a controversial finding will go! Dedicated to Ferruccio Ritossa. **Cell stress & chaperones**, v. 16, n. 3, p. 235–49, maio 2011.

DEFRONZO, R. A. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidaemia and atherosclerosis. **The Netherlands journal of medicine**, v. 50, n. 5, p. 191–7, maio 1997.

DI NASO, F. C. et al. Obesity depresses the anti-inflammatory HSP70 pathway, contributing to NAFLD progression. **Obesity (Silver Spring, Md.)**, v. 23, n. 1, p. 120–9, jan. 2015.

DIABETES, A. A. Diagnosis and classification of diabetes mellitus. **Diabetes care**, v. 33 Suppl 1, p. S62–9, jan. 2010.

DIMAURO, I.; MERCATELLI, N.; CAPOROSSI, D. Exercise-induced ROS in Heat Shock Proteins response. **Free Radical Biology and Medicine**, 26 mar. 2016.

ELJSVOGELS, T. M. H.; THOMPSON, P. D. Exercise Is Medicine At Any Dose ? **JAMA : the journal of the American Medical Association**, v. 314, n. 18, p. 1915–1916, 2015.

ESTADELLA, D. et al. Effect of Palatable Hyperlipidic Diet on Lipid Metabolism of Sedentary and Exercised Rats. **Nutrition**, v. 20, n. 2, p. 218–224, 2004.

FEBBRAIO, M. A. et al. Exercise induces hepatosplanchnic release of heat shock protein 72 in humans. **The Journal of physiology**, v. 544, n. Pt 3, p. 957–62, 1 nov. 2002.

FISHER-WELLMAN, K.; BELL, H. K.; BLOOMER, R. J. Oxidative stress and antioxidant defense mechanisms linked to exercise during cardiopulmonary and metabolic disorders. **Oxidative medicine and cellular longevity**, v. 2, n. 1, p. 43–51, jan. 2009.

FURUKAWA, S. et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. **The Journal of clinical investigation**, v. 114, n. 12, p. 1752–61, dez. 2004.

GIACCO, F.; BROWNLEE, M. Oxidative stress and diabetic complications. **Circulation Research**, v. 107, n. 9, p. 1058–1070, 2010.

GONZÁLEZ-FLECHA, B. Oxidant mechanisms in response to ambient air particles. **Molecular aspects of medicine**, v. 25, n. 1-2, p. 169–82, jan. 2004.

GRODSKY, G. M. The importance of rapid insulin secretion: revisited. **Diabetes technology & therapeutics**, v. 1, n. 3, p. 259–60, jan. 1999.

GUALTIERI, M. et al. Organic compounds in tire particle induce reactive oxygen species and heat-shock proteins in the human alveolar cell line A549. **Environment international**, v. 34, n. 4, p. 437–42, maio 2008.

GUPTA, S. C. et al. Induction of hsp70, alterations in oxidative stress markers and apoptosis against dichlorvos exposure in transgenic Drosophila melanogaster: modulation by reactive oxygen species. **Biochimica et biophysica acta**, v. 1770, n. 9, p. 1382–94, set. 2007.

HALLIWELL, B.; GUTTERIDGE, J. M. C. Free radicals in biology and medicine, second edition. **Free Radical Biology and Medicine**, v. 10, n. 6, p. 449–450, jan. 1999.

HECK, T. G. **Razão entre o conteúdo extracelular e intracelular de HSP70 como um sinal de alerta imunológico e marcador de intensidade de exercício**. [s.l.] Universidade Federal do Rio Grande do Sul - UFRGS, 2011.

HECK, T. G.; SCHÖLER, C. M.; DE BITTENCOURT, P. I. H. HSP70 expression: does it a novel fatigue signalling factor from immune system to the brain? **Cell biochemistry and function**, v. 29, p. 215–226, 2011.

HENRIKSEN, E. J.; DIAMOND-STANIC, M. K.; MARCHIONNE, E. M. Oxidative stress and the etiology of insulin resistance and type 2 diabetes. **Free radical biology & medicine**, v. 51, n. 5, p. 993–9, 1 set. 2011.

HOCHACHKA, P. . Biochemical Adaptation: Mechanism and Process in Physiological Evolution. **Oxfort University Press**, p. 480, 1 jan. 2002.

HOSSAIN, P.; KAWAR, B.; EL NAHAS, M. Obesity and diabetes in the developing world--a growing challenge. **The New England journal of medicine**, v. 356, n. 3, p. 213–5, 18 jan. 2007.

HOTAMISLIGIL, G. S. Inflammation and metabolic disorders. **Nature**, v. 444, n. 7121, p.

860–7, 14 dez. 2006.

IHME, I. FOR H. M. AND E. **Global Burden of Disease Study 2010 (GBD 2010) Population Estimates 1970-2010**. Disponível em: <http://ghdx.healthdata.org/record/global-burden-disease-study-2010-gbd-2010-population-estimates-1970-2010>. Acesso em: 30 mar. 2016.

IKEMOTO, S. et al. High-fat diet-induced hyperglycemia and obesity in mice: differential effects of dietary oils. **Metabolism: clinical and experimental**, v. 45, n. 12, p. 1539–46, dez. 1996.

INTERNATIONAL DIABETES FEDERATION. **International Diabetes Federation Annual Report 2013**. Disponível em: <http://www.idf.org/publications/annual-report>. Acesso em: 6 mar. 2016.

JOHNSON, J. D.; FLESHNER, M. Releasing signals, secretory pathways, and immune function of endogenous extracellular heat shock protein 72. **Journal of leukocyte biology**, v. 79, n. 3, p. 425–34, mar. 2006.

JONES, Q. et al. Heat shock proteins protect against ischemia and inflammation through multiple mechanisms. **Inflammation & allergy drug targets**, v. 10, n. 4, p. 247–59, ago. 2011.

KAHN, C. R. Banting Lecture. Insulin action, diabetogenes, and the cause of type II diabetes. **Diabetes**, v. 43, n. 8, p. 1066–84, ago. 1994.

KEANEY, J. F. et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. **Arteriosclerosis, thrombosis, and vascular biology**, v. 23, n. 3, p. 434–9, 1 mar. 2003.

KIDO, T. et al. Diesel exhaust inhalation induces heat shock protein 70 expression in vivo. **Inhalation toxicology**, v. 23, n. 10, p. 593–601, 25 ago. 2011.

KILINÇ, E. et al. Factor XII activation is essential to sustain the procoagulant effects of particulate matter. **Journal of thrombosis and haemostasis : JTH**, v. 9, n. 7, p. 1359–67, jul. 2011.

KNOWLER, W. C. et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. **The New England journal of medicine**, v. 346, n. 6, p. 393–403, 7 fev. 2002.

KOYA, D.; KANASAKI, K. Biology of obesity: Lessons from animal models of obesity.

Journal of Biomedicine and Biotechnology, v. 2011, 2011.

KRAUSE, M. et al. The chaperone balance hypothesis: the importance of the extracellular to intracellular HSP70 ratio to inflammation-driven type 2 diabetes, the effect of exercise, and the implications for clinical management. **Mediators of inflammation**, v. 2015, p. 249205, jan. 2015.

LEON, B. M.; MADDOX, T. M. Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research. **World journal of diabetes**, v. 6, n. 13, p. 1246–58, 10 out. 2015.

LITTLE, J. P. et al. Effects of high-intensity interval exercise versus continuous moderate-intensity exercise on postprandial glycemic control assessed by continuous glucose monitoring in obese adults. **Applied physiology, nutrition, and metabolism = Physiologie appliquée, nutrition et métabolisme**, v. 39, n. 7, p. 835–41, 2014.

LLOPIS, P. Q.; REIG GARCÍA-GALBIS, M. Control glucémico a través del ejercicio físico en pacientes con diabetes mellitus tipo 2; revisión sistemática. **Nutrición Hospitalaria**, v. 31, n. 4, p. 1465–1472, 2015.

LOLLO, P. C. B. et al. Differential response of heat shock proteins to uphill and downhill exercise in heart, skeletal muscle, lung and kidney tissues. **Journal of sports science & medicine**, v. 12, n. 3, p. 461–6, jan. 2013.

MAGALHÃES, F. DE C. et al. Heat and exercise acclimation increases intracellular levels of Hsp72 and inhibits exercise-induced increase in intracellular and plasma Hsp72 in humans. **Cell stress & chaperones**, v. 15, n. 6, p. 885–95, nov. 2010.

MARTÍN-GALLÁN, P. et al. Biomarkers of diabetes-associated oxidative stress and antioxidant status in young diabetic patients with or without subclinical complications. **Free radical biology & medicine**, v. 34, n. 12, p. 1563–74, 15 jun. 2003.

MEIER, R. et al. Associations of short-term particle and noise exposures with markers of cardiovascular and respiratory health among highway maintenance workers. **Environmental health perspectives**, v. 122, n. 7, p. 726–32, jul. 2014.

MILNE, K. J.; NOBLE, E. G. **Exercise-induced elevation of HSP70 is intensity dependent**. [s.l: s.n.]. v. 93

MOHAMED-ALI, V.; PINKNEY, J. H.; COPPACK, S. W. Adipose tissue as an endocrine and paracrine organ. **International journal of obesity and related metabolic disorders :**

journal of the International Association for the Study of Obesity, v. 22, n. 12, p. 1145–58, dez. 1998.

MORTON, J. P. et al. The exercise-induced stress response of skeletal muscle, with specific emphasis on humans. **Sports medicine (Auckland, N.Z.)**, v. 39, n. 8, p. 643–62, jan. 2009.

MUKHOPADHYAY, I. et al. Heat shock response: hsp70 in environmental monitoring. **Journal of biochemical and molecular toxicology**, v. 17, n. 5, p. 249–54, jan. 2003.

NEMMAR, A. et al. Passage of inhaled particles into the blood circulation in humans. **Circulation**, v. 105, n. 4, p. 411–4, 29 jan. 2002.

NEWSHOLME, P.; DE BITTENCOURT, P. I. H. The fat cell senescence hypothesis: a mechanism responsible for abrogating the resolution of inflammation in chronic disease. **Current opinion in clinical nutrition and metabolic care**, v. 17, n. 4, p. 295–305, jul. 2014.

NEWSHOLME, P.; KRAUSE, M. Diet, obesity, and reactive oxygen species–implications for diabetes and aging. In: **Systems Biology of Free Radicals and Antioxidant 01:3361–3374**. [s.l: s.n.].

NG, M. et al. 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**, v. 384, n. 9945, p. 766–781, 28 maio 2014.

NOVELLI, E. L. B. et al. Anthropometrical parameters and markers of obesity in rats. **Laboratory animals**, v. 41, n. 1, p. 111–9, jan. 2007.

OSIER, M.; OBERDÖRSTER, G. Intratracheal inhalation vs intratracheal instillation: differences in particle effects. **Fundamental and applied toxicology : official journal of the Society of Toxicology**, v. 40, n. 2, p. 220–7, dez. 1997.

PEARSON, J. F. et al. Association between fine particulate matter and diabetes prevalence in the U.S. **Diabetes care**, v. 33, n. 10, p. 2196–201, out. 2010.

PETERSEN, A. M. W.; PEDERSEN, B. K. The anti-inflammatory effect of exercise. **Journal of applied physiology (Bethesda, Md. : 1985)**, v. 98, n. 4, p. 1154–62, abr. 2005.

POPE, C. A. et al. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. **JAMA**, v. 287, n. 9, p. 1132–41, 6 mar. 2002.

QIN, X.-D. et al. Gender-specific differences of interaction between obesity and air pollution on stroke and cardiovascular diseases in Chinese adults from a high pollution range area: A large population based cross sectional study. **The Science of the total environment**, v. 529,

p. 243–8, 1 out. 2015.

REAVEN, G. M. Role of insulin resistance in human disease. **Diabetes**, v. 37, n. 12, p. 1595–607, dez. 1988.

REAVEN, G. M. Insulin resistance: the link between obesity and cardiovascular disease. **The Medical clinics of North America**, v. 95, n. 5, p. 875–92, set. 2011.

RHODEN, C. R. et al. N-acetylcysteine prevents lung inflammation after short-term inhalation exposure to concentrated ambient particles. **Toxicological sciences : an official journal of the Society of Toxicology**, v. 79, n. 2, p. 296–303, jun. 2004.

RODRIGUES, C. G. et al. Projeção da mortalidade e internações hospitalares na rede pública de saúde atribuíveis à poluição atmosférica no Estado de São Paulo entre 2012 e 2030. **Revista Brasileira de Estudos de População**, v. 32, n. 3, p. 489–509, dez. 2015.

RODRIGUES-KRAUSE, J. et al. Divergence of intracellular and extracellular HSP72 in type 2 diabetes: Does fat matter? **Cell Stress and Chaperones**, v. 17, n. 3, p. 293–302, 2012.

RUBIO, E. et al. Programmed cell death in the neurulating embryo is prevented by the chaperone heat shock cognate 70. **The European journal of neuroscience**, v. 15, n. 10, p. 1646–54, maio 2002.

SHARMA, A.; BERNATCHEZ, P. N.; DE HAAN, J. B. Targeting endothelial dysfunction in vascular complications associated with diabetes. **International journal of vascular medicine**, v. 2012, p. 750126, jan. 2012.

SLOVIC, A. D. et al. How Can Urban Policies Improve Air Quality and Help Mitigate Global Climate Change: a Systematic Mapping Review. **Journal of Urban Health**, n. x, 2015.

SMITH, K. B.; SMITH, M. S. Obesity Statistics. **Primary care**, v. 43, n. 1, p. 121–35, mar. 2016.

SUN, Q. et al. Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. **Circulation**, v. 119, n. 4, p. 538–46, 3 fev. 2009.

THRALL, G. et al. A systematic review of the effects of acute psychological stress and physical activity on haemorheology, coagulation, fibrinolysis and platelet reactivity: Implications for the pathogenesis of acute coronary syndromes. **Thrombosis research**, v. 120, n. 6, p. 819–47, jan. 2007.

TIMAR, O.; SESTIER, F.; LEVY, E. Metabolic syndrome X: a review. **The Canadian journal of cardiology**, v. 16, n. 6, p. 779–89, 1 jun. 2000.

UMPIERRE, D. et al. Physical activity advice only or structured exercise training and association with HbA1c levels in type 2 diabetes: a systematic review and meta-analysis. **JAMA**, v. 305, n. 17, p. 1790–9, 4 maio 2011.

VAN DIJK, J.-W. et al. Exercise therapy in type 2 diabetes: is daily exercise required to optimize glycemic control? **Diabetes care**, v. 35, n. 5, p. 948–54, maio 2012.

VAN GAAL, L. F.; MERTENS, I. L.; DE BLOCK, C. E. Mechanisms linking obesity with cardiovascular disease. **Nature**, v. 444, n. 7121, p. 875–880, 14 dez. 2006.

VAN HELVOORT, H. A. C. et al. Systemic inflammatory response to exhaustive exercise in patients with chronic obstructive pulmonary disease. **Respiratory medicine**, v. 99, n. 12, p. 1555–67, dez. 2005.

VANDERJAGT, D. J. et al. Oxidative stress indices in IDDM subjects with and without long-term diabetic complications. **Clinical biochemistry**, v. 34, n. 4, p. 265–70, jun. 2001.

VELICHKO, A. K. et al. Mechanisms of heat shock response in mammals. **Cellular and molecular life sciences : CMLS**, v. 70, n. 22, p. 4229–41, nov. 2013.

WALSH, R. C. et al. Exercise increases serum Hsp72 in humans. **Cell stress & chaperones**, v. 6, n. 4, p. 386–93, out. 2001.

WANG, B. et al. Effect of long-term exposure to air pollution on type 2 diabetes mellitus risk: a systemic review and meta-analysis of cohort studies. **European journal of endocrinology / European Federation of Endocrine Societies**, v. 171, n. 5, p. R173–82, 8 nov. 2014.

WEN, X.-J. et al. Association of self-reported leisure-time physical inactivity with particulate matter 2.5 air pollution. **Journal of environmental health**, v. 72, n. 1, p. 40–4; quiz 45, jan. 2009.

WEXLER, B. Antioxidants: Natural Defense Against Oxidative Stress. p. 32, 2007.

WHITHAM, M.; FORTES, M. B. Heat shock protein 72: release and biological significance during exercise. **Frontiers in bioscience : a journal and virtual library**, v. 13, p. 1328–39, jan. 2008.

WHO. **Ambient (outdoor) air quality and health**. 2005. Disponível em: <http://www.who.int/mediacentre/factsheets/fs313/en/>. Acesso em: 29 fev. 2016.

WHO, W. H. O. Diet, nutrition and the prevention of chronic diseases. **WHO Technical Report Series. Geneve: WHO**, 2003.

WHO, W. H. O. **Air Quality Guidelines**. Disponível em: <<http://goo.gl/XQRNLZ>>.

Acesso em: 31 mar. 2016.

WHO, W. H. O. **Air quality guidelines**. Disponível em: <<http://goo.gl/XQRNLZ>>. Acesso em: 30 mar. 2016.

WHO, W. H. O. **Obesity and overweight.** Disponível em: <http://www.who.int/mediacentre/factsheets/fs311/en/>. Acesso em: 3 jun. 2016.

WOODS, S. C. et al. A controlled high-fat diet induces an obese syndrome in rats. **The Journal of nutrition**, v. 133, n. 4, p. 1081–7, abr. 2003.

XING, Y. et al. The impact of PM2 . 5 on the human respiratory system. v. 8, n. I, p. 69–74, 2016.

XU, Z. et al. Ambient particulate air pollution induces oxidative stress and alterations of mitochondria and gene expression in brown and white adipose tissues. **Particle and fibre toxicology**, v. 8, p. 20, jan. 2011.

YAN, Y.-H. et al. Enhanced insulin resistance in diet-induced obese rats exposed to fine particles by instillation. **Inhalation toxicology**, v. 23, n. 9, p. 507–19, ago. 2011.

YANG, X. et al. Association between heat-shock protein 70 gene polymorphisms and DNA damage in peripheral blood lymphocytes among coke-oven workers. **Mutation research**, v. 649, n. 1-2, p. 221–9, 8 jan. 2008.

ZANCHI, A. C. et al. Chronic nasal instillation of residual-oil fly ash (ROFA) induces brain lipid peroxidation and behavioral changes in rats. **Inhalation toxicology**, v. 20, n. 9, p. 795–800, jul. 2008.

Artigo

(Periódico Internacional: European Journal of Applied Physiology)

Anti-inflammatory response of acute exercise does not occur in obese mice exposed to fine particulate matter

Iberê Machado Kostrycki[1,2]; Guilherme Wildner[1]; Fernanda Giesel Baldissera[1,2]; Yohanna Hannah Donato[1]; Analú Bender dos Santos[1]; Cláudia Ramos Rhoden[2]; Thiago Gomes Heck[1,3]

[1]Research Group in Physiology, Department of Life Sciences, Regional University of Northwestern Rio Grande do Sul State (UNIJUI), Ijuí, RS, Brazil.

[2]Postgraduate Program in Health Sciences (PPGCS-UFCSPA), Porto Alegre, RS, Brazil.

[3]Postgraduate Program in Integral Attention to Health (PPGAIS-UNIJUI/UNICRUZ), Ijuí, RS, Brazil.

Corresponding author

Thiago Gomes Heck, PhD

Research Group in Physiology, Department of Life Sciences, Regional University of Northwestern Rio Grande do Sul State (UNIJUI), Rua do Comércio, 3000 – Bairro Universitário, Ijuí, RS, Brazil, CEP: 98700-000.

Fone: 55 55 33320522

gomesheck@yahoo.com

Abstract

Challenges to the body as obesity, air pollution and exercise can cause alterations in the intracellular content of 70 kDa Heat Shock Proteins (iHSP70, anti-inflammatory) and in the plasmatic content of its inducible form (eHSP72, pro-inflammatory). Besides that, [eHSP72/iHSP70] ratio can represent an important biomarker of subclinical processes of the body under challenges. Thus, the present work the effect of acute exercise in obese mice exposed to fine particulate matter ($PM_{2.5}$) as intra and extracellular ratio of heat shock proteins and oxidative stress heart and lung variables. Were used 60 B6129SF2 (B6) male mices, 30 days old, divided in two groups for 16 weeks: standard feed (CTR, n=29) and high fat diet (HFD, N=31) *ad libitum*. After 16 weeks, animals were adapted to the swimming (3 days, 10 min, water 30$\pm$1°C) and allocated in the followed groups: repose that received saline via intranasal (CTR, n=6 and HFD, n=8) or $PM_{2.5}$ (PM, n=7 and PM+HFD, n=7); acute exercise of moderate intensity plus saline (MIE, n=10 and HFD+MIE, n=9) or $PM_{2.5}$ (PM+MIE, n=6 and HFD+PM+MIE, n=7). Swimming for 20 min with 4% of load upon animal weight added to the tail and intranasal instillation of $PM_{2.5}$ (50 µg/10 µL). Feed intake, biometric and glycemic profile, oxidative stress biomarkers, plasmatic concentration of eHSP72, cardiopulmonary content of iHSP70 and H index were available. Differences in the iHSP70 of tissues were not found. Therefore, acute exercise of moderate intensity caused low eHSP72 content in the plasma. Besides that, promoted decreased of H index upon heart and lung tissues of the CTR+MIE animals and upon lung on the PM+MIE animals. This founding indicates moderate acute exercise cause anti-inflammatory profile in not obese sedentary mices, despite $PM_{2.5}$ exposure, which does not occur in obesity.

Keywords: Exercise. Obesity. Particulate Matter. HSP70.

Abbreviations

AUC - Area Under the Curve

BMI - Body Mass Index

CAT - Catalase

eHSP72 - extracellular 72 kDa Heat Shock Proteins

GTT- Glucose Tolerance Test

HFD - High Fat Diet

HSP70 - 70 kDa Heat Shock Proteins of

iHSP70 - intracellular 70 kDa heat shock proteins

MIE - Moderate Intensity Exercise

$PM_{2.5}$ - Fine Particulate Matter

ROS - reactive oxigen species

SOD - Superoxide dismutase

T2DM - Type 2 Diabetes

TCA: Ácido tricloroacético

TNF-α: fator de necrose tumoral alfa

TRIS: *2-amino-2-hydroxymethyl-propane-1,3-diol*

Introduction

Obesity is a multifactorial disease strongly associated with diverse comorbidities (Smith and Smith 2016) considerate an important public health problem in different cultures with increasing prevalence (Ng et al. 2014). Currently nearly 30% of the adult global population does not reach enough levels of moderate exercise and the sedentary habits collaborate to the increase of obesity prevalence (Hallal et al. 2012). The metabolic alterations resulting from low active lifestyle as increasing abdominal adiposity are established as major risk factors to the type 2 diabetes (T2DM) development (WHO, 2003; Cavalcanti et al. 2009). Adipose tissue may be consider as central organ in low grade chronic inflammation profile in T2DM (Hotamisligil 2006). In addition, adiposity has been associated with oxidative stress (Keaney et al. 2003; Martín-Gallán et al. 2003) presenting in experimental models with high-fat diet (HFD) treatment that mimicking the development of human obesity (Estadella et al. 2004; Koya and Kanasaki 2011).

Other global health problem is the air pollution exposure by fine particulate matter ($PM_{2.5}$) (Slovic et al. 2015). $PM_{2.5}$ is formed from combustion processes, including vehicles, power plants, fires, burning agricultural and industrial work, and it is able to invade the respiratory tract and vascular system (Pearson et al. 2010), promoting risk to the population. Epidemiological and toxicological studies have evidenced the adverse effects of $PM_{2.5}$ on human health, even under exposure to low levels than the recommended by the World Health Organization (WHO) (Cote et al. 2008). Findings from animal studies demonstrate $PM_{2.5}$ inhalation is able to cause autonomic imbalance and thus pulmonary and cardiac oxidative stress, which it has been associated with the development of pulmonary inflammation in rats (Rhoden et al. 2004).

Obesity and exposure to $PM_{2.5}$ may have common mechanisms, as redox imbalance and inflammatory process (Furukawa et al. 2004; Meier et al. 2014). Obesity can turns the individual more susceptible to the effects of $PM_{2.5}$, as seen by the increasing prevalence of cardiovascular disease and stroke (Qin et al. 2015). In addition, prolonged exposure to high $PM_{2.5}$ levels is associated with increased risk of developing T2DM (Wang et al. 2014). $PM_{2.5}$ inhalation can also lead to alterations of vasomotor tone, vascular inflammation, adiposity, atherosclerosis and insulin resistance (Xu et al. 2011). Together, HFD intake and $PM_{2.5}$ exposure may increase body weight, adiposity, fasting glycemic levels and corroborate to glucose intolerance in mice (Goettems-Fiorin et al. 2016).

In contrast, to prevent and treat many chronic diseases and its comorbidities regular practice of moderade intensity exercise (MIE) have been recommended (Eljsvogels and Thompson 2015). At rest, tissue antioxidant defenses are sufficient to control most of the oxidative activity, however, during the course of exercise, there is an increase of oxygen consumption in cells of different tissues, which is directly proportional to oxidative stress (Banerjee et al. 2003). Besides the well-known benefits of exercise training, acutely performed physical activity may also trigger positive alterations, demonstrated in single moderate swimming session in rats (Silveira et al. 2007).

Stressor stimuli such as obesity (Chung et al. 2008), air pollution (Kido et al. 2011) and exercise (Milne and Noble 2002) may cause alterations in cellular response to stress, characterized by changes in expression of the 70 kDa heat shock protein (HSP70). It has been proposed versatility of this protein, once intracellular environment (iHSP70) activates anti-inflammatory signaling pathways (Jones et al. 2011), while outside cell, especially the inducible form of 72 kDa (eHSP72) under stress conditions induces the opposite effect (Asea 2008).

The measure of eHSP72 levels in plasma may represent an important biomarker for imunoinflammatory state during exercise (Krause et al. 2015), T2DM, obesity (Rodrigues-Krause et al. 2012) and $PM_{2.5}$ exposure (Goettems-Fiorin et al. 2016). Chronic exposure *in vivo* to $PM_{2.5}$ showed to increase the level of eHSP72 (plasma) and may be an important immune mediator, contributing to diseases such as vascular dysfunction and cardiovascular events (Kido et al. 2011). Thus, it has been proposed HSP70 can be used as a biomarker for early assessments of the harmful health effects caused by exposure to air pollution (Mukhopadhyay et al. 2003). It is known acute and/or chronic exercise modulate the expression of iHSP70 in several human and animal tissues (Dimauro et al. 2016). In addition, findings with animal models suggest exercise changes iHSP70 level in heart and lung (Milne and Noble 2002; Lollo et al. 2013). Acute exercise sessions signal a physiological stress situation for all system (Heck et al. 2011), which cause a momentary increase in the expression of HSP72 (Febbraio et al. 2002). However, training tends to decrease this response (Morton et al. 2009) and then, generates adaptations to stress and consequent lower expression of HSP72, setting up an anti-inflammatory response. Also, recently investigated, the eHSP72/iHSP70 ratio works as a biomarker of the subclinical process mediated by combination of some factor listed above (HFD and $PM_{2.5}$) (Goettems-Fiorin et al. 2016). In

this work, we investigated the effects of a single exercise session exposed to $PM_{2.5}$ in high-fat diet treated mice on cardiac and pulmonary oxidative stress and iHSP70 analysis and eHSP72/iHSP70 ratio.

Material and methods

Animals

Sixty male B6129SF2 (B6) mice (30 day-old; 60 animals) from Jackson Laboratory (Bar Harbor, ME, USA) were reproduced in the Regional University of Northwestern State's Rio Grande do Sul, Life Sciences Department, Animal Care Facility, and maintained under controlled temperature (23 ± 1 °C) in a 12/12 h light/dark cycle (lights on at 7 a.m.), housed in plastic cages (40 x 33 x 17 cm; 3 to 5 animals per cage). Throughout the experiments, the animals had free access to water and fed *ad libitum*. The investigation followed all ethical rules established by Arouca's Act (Federal Law 11794/2008) and the Guide for Care and Use of Experimental Animals published by the National Institutes of Health (NIH publication no. 85-23, revised in 1996). All the procedures were approved by the Regional University of Northwestern State's Rio Grande do Sul Committee of Animal Welfare (CEUA-UNIJUÍ, protocol # 011/2013).

Experimental design

At 30 days old the mice were randomly separated in two groups, receiving the standard diet (CTR, n=29) or high-fat diet (HFD, n=31) for 16 weeks. Animals from CTR group received pelleted diet consisted of crude protein, mineral material, fibrous matter and minerals (Nuvilab CR-1, commercially obtained from Nuvital Nutrientes SA.; total metabolizable energy: 16.6 MJ/kg, being 11.4 % as fats, 62.8% as carbohydrates and 25.8% as proteins). To the HFD groups, was provided a lard-based diet (37.4% w/w; total metabolizable energy: 22.8 MJ/kg, being 58.3% as fats, 24.5% as carbohydrates, and 17.2% as proteins) (Bock et al. 2015; Goettems-Fiorin et al. 2016). Biometric profile (weight, naso-anal length and BMI) and intraperitoneal Glucose Tolerance Test via intraperitoneal (i.p. – GTT) were realized in the animals at 30 days-old and at 4^{th} , 8^{th}, 12^{th} and 16^{th} week in both groups. Also were measured food and water consumption weekly.

At the end of 16^{th} week the animals were familiarized to swim exercise. Each mice swam 10 min in a glass tank filled with 20 cm of water at 30 ± 1 °C. After two days of

familiarization, animals remained 24 h without any manipulation and were randomly separated again in two groups: animals that received $PM_{2.5}$ by intranasal instillation (50μg/10μL) or saline (vehicle). After $PM_{2.5}$ or saline instillation, the animals were submitted to acute swimming session under moderate intensity or at rest. The details of each group are described in the table 1.

Moderate Intensity Exercise

Swimming exercise was performed one time (acute exercise). Mice performed moderate exercise intensity (MIE) by adding load coupled to the base of the tail with adhesive tape (4% of the animal body weight), in 20 cm deep water at 30 ± 1ºC for 20 min. Animals from control group remained by the same 20 min in shallow water (2 ± 1 cm deep) at the same temperature. After exercise session, was collected blood from the distal part of the tail (~25 μL) to measure lactate by specific strips and lactimeter (Accutrend® Lactate, Roche).

Atmospheric pollution – Fine Particulate Matter ($PM_{2.5}$)

The pollutant used in the experiment was $PM_{2.5,}$ collected in polycarbonate filter through a gravimetric collector, on the terrace of the Faculty of Medicine, University of São Paulo (USP) in São Paulo, Brazil, as previously described (Maatz et al. 2009). The exposure site was located close to a monitoring station of the State of São Paulo Sanitation Agency. It is estimated that at least 100,000 vehicles circulate daily on the main and lateral street (~83% cars, ~10% diesel vehicles, ~6% motorcycles). There are no industries or significant biomass sources in the surrounding area. Trace element determinations of PM content were carried out by neutron activation analysis and their concentration were expressed by ng per m^3 of air, as follows: As, 12.91±0.53; Br, 8.88±0.39; Cl, 8.88±0.39; Co, 1.14±0.04; Fe, 1.15±0.03; La, 2.33±0.29; Mn, 27.5±2.2; Sb, 8.73±0.08; Sc, 0.141±0009; Th 0.351±0.050. Likewise, PM sulfur concentration, determined by X-ray fluorescence analysis, was 1.424±0.08 μm/m^3. Briefly, after exposure (24 h), the filter was removed and retained particles were obtained by sonication, with ultrasound bath in seven sessions (50 min each) and resuspended in saline solution at a dose of 50 μg/10 μL. The process of nasotropic instillation was performed once before exercise session with an automatic pipette, with 10 μL of solution in the nostril of the animal. This procedure induces apnea reflex promoting the inhalation of the pollutant. Control groups received 10 μL of saline solution.

Biometric profile

Biometric profile was evaluated before (30 days-old), at 4th, 8th, 12th and 16th week. Body mass (g) by semi-analytical balance (Marte®) and naso-anal length (cm) by scale were measured. These data were used to calculate the body mass index (BMI): animal body mass (g) divided by squared of body length (cm^2) (Novelli et al. 2007). Adiposity was evaluated upon white adipose tissue (WAT) from epididymis. WAT was collected and weighted by analytical balance.

Glucose Tolerance Test (GTT)

The glucose tolerance test was performed before the experiment in the 4th, 8th, 12th, 16th weeks of intervention in all animals. Food was withdrawn in the night before experiments (12 h before). Glycemia was measured as described above immediately before and at 30 and 120 min after glucose (1 g/kg in saline solution, *i.p.*) administration. The glycemic response during GTT was evaluated by the AUC method (USA-FAO, 1997).

Sample processing

Finished exercise session or rest, the animals were killed by decapitation to collect blood, heart and lung. Plasma was obtained by centrifugation (blood with EDTA; 3000 rpm during 10 min) and stored at - 20°C. Heart and lung were collected, washed in saline, freeze-clamped in liquid nitrogen and stored for further homogenization. Tissue samples were homogenized in two different buffers. In order to measure oxidative stress variables (lipid peroxidation, and SOD and CAT activities) the samples were homogenized in potassium phosphate buffer pH 7.4, and to determine HSP70 concentration by Western blot the samples were homogenized in SDS 0.1% buffer (*w/v*). All tissues were homogenized (still frozen) in respective buffers, containing PMSF (Phenyl-Methyl-Sulfonyl Fluoride, 100 μM).

Protein quantitation

Protein quantitation in the tissues was measured by Bradford (1976) spectrophotometric method upon 595 nm, and was used bovine serum albumin as standard.

Oxidative stress biomarkers

Lipid peroxidation was measured by TBARS method (Buege and Aust 1978), which the homogenates were precipitated with trichloroacetic acid (TCA) 10% (w/v), centrifuged and incubated during 15 min at 100 °C with thiobarbituric acid (TBA). Next, absorbances were verified by spectrophotometer at 535 nm.

Catalase and Superoxide Dismutase Activities

Enzymatic activity of SOD was proceded by inhibition of pyrogallol auto oxidation and measured by spectrophotometric at 420 nm (Marklund and Marklund 1974). Data were expressed as Units of SOD per mg of protein. Enzymatic activity of CAT was determined by by the decomposition of hydrogen peroxide at 25 °C and 240 nm (Aebi 1984). Data were expressed as pmol of CAT per mg of protein.

SDS-PAGE and immunoblot analysis of iHSP70

In order to examine HSP70 content in the heart and lung, the samples were processed for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli 1970). Equivalent amount of protein from each tissue sample (~40 μg) and equivalent volume from each plasma sample (10 μL) were mixed with Laemmli's gel loading buffer [50 mM Tris (10% w/v), SDS (10% v/v), glycerol (10% v/v), 2-mercaptoethanol and 2 mg/ml bromphenol blue] in a ratio of 1:1, boiled for 5 min and electrophoresed in a 10% polyacrylamide minigel for 4 h at 15 mA/gel. Proteins were transferred onto nitrocellulose membranes (GE HealthCare) according to the electrotransfer (Bio-Rad) manufacturer's instructions (1 h, 100 V) and transferred bands were visualized with 0.3% (w/v) Red Ponceau S (Sigma) in 3% (w/v) trichloroacetic acid solution. For immunoblotting procedures the membranes were washed in wash buffer [TBS-Tween 20 solution (0.1% w/v)] and blocked in 1% (w/v) nonfat dry milk in wash buffer. Next, membranes were incubated overnight (at 2 – 8 °C) in mouse anti-HSP70 monoclonal antibody (Sigma H5147) which recognizes both the HSP73 (HSPA8 gene) and the HSP72 (HSPA1A/HSPA1B genes) forms, at 1:100 dilution. Membranes were washed and incubated for 1h (at room temperature) in horseradish peroxidase (HRP) antibody, labelled anti-mouse IgG (whole molecule) secondary antibody (Sigma A9044), at 1:15000 dilution. Membranes were washed and the HSP70 detection was performed by enhanced chemiluminescence method (ECL Plus RPN2132, GE). Protein bands were revealed upon film (Amersham Hyperfilm ECL, GE Health Care), photodocumented and quantified by ImageJ software (http://imagej.nih.gov/). Tissues actin detection were

performed by Coomassie Blue-stained gel [Coomassie Blue R 250 (0.1% w/v), glacial acetic acid (10% v/v) and methanol (40% (v/v)], photodocumented and quantified by ImageJ software for normalization.

Plasmatic eHSP72 concentration

Plasmatic eHSP72 concentration was determined by high sensitive kit (HSP70 ELISA Kit, ENZO Life Sciences, EKS-715) and the data was expressed as ng of eHSP70 per mL of plasma (ng/mL).

H-index calculation – Extra/intracellular ratio of HSP70

It can be considerate that eHSP72 release to the extracellular *milieu* characterize a pro-inflammatory state, while intracellular expression of iHSP70 represents a greater anti-inflammatory role. In this context, was established the eHSP72/iHSP70 ratio, or H-index, considerating [eHSP72/iHSP70] at basal state as 1:1=1 (CTR group) as a normal condition, as proposed by (Heck 2011) and Krause et al. (2015). The H- Index represents immune-inflammatory balance in situations as exercise (Heck, 2011), as well as in experimental model of HFD intake and $PM_{2.5}$ exposure (Goettems-Fiorin et al. 2016). Thus, H-index was calculated from measure plasma/heart and plasma/lung ratios of HSP70 [eHSP72/iHSP70]. Data were expressed as arbitrary units.

Statistical Analysis

Kolmogorov-Smirnov normality test was applied before all analysis. Student *t* test was used to compare diet effects (HFD *vs* standard diet). One-way analysis of variance (ANOVA) followed by post-hoc Tukey's test were performed to compare the groups upon oxidative stress variables. Significance level was set at $P < 0.05$. Data were analyzed using Graph Pad Prism 5.0 software. Values are presented as mean ± standard deviation.

Results

Animals submitted to HFD, when compared to CTR mice, consumed less amount of feed per week (figure 1A) and lower energy content (kcal) in 13 of the 16 weeks during this study (figure 1B). Even so, from the 4^{th} and 8^{th} weeks of experiment they demonstrated higher body mass (figure 2A) and BMI (figure 2B) than animals fed with standard chow

respectively. As expected, high-fat diet intake promoted increase in adiposity (figure 2C) and glycemia (figure 2D), and worsed glucose tolerance test response since the 4th week of treatment (figure 3F). Animals of HFD group presented higher glycemia than CTR mice (CTR = 150 ± 24 mg/dL; HFD = 173 ± 30 mg/dL; P = 0.02). These data allow us to observe the effects of the acute exercise and $PM_{2.5}$ combination in normal and obesogenic/diabetogenic mice.

Moderate intensity was confirmed by blood lactate concentration measurement (HFD = 5.5 ± 1.1; MIE = 4.3 ± 0.5; HFD+MIE = 5.5 ± 0.9 mmol/L; PM+MIE = 5.0 ± 0.9). The animals from standard diet treatment (MIE and PM+MIE) performed completely the exercise session (20 min) without effect of $PM_{2.5}$ exposure and presented higher lipid peroxidation levels in lung (figure 4A). In other hand, high-fat diet treated animals presented lower capacity of exercise (HFD+MIE = 13.4 ± 4.6 min.; HFD+PM+MIE = 12.1 ± 7.0 min; $P<0.05$) and the same lipid peroxidation levels in lung (Figure 4A), both when compared to the CTR group animals.

MIE and $PM_{2.5}$ exposure triggered increase of the SOD activity in lung (both compared to CTR). However, animals submitted to $PM_{2.5}$ exposure and exercise combination presented lower SOD activity than animals only submitted to the of $PM_{2.5}$ exposure (figure 4B). It was not found changes in the CAT activity in lung (figure 4C).

Animals from HFD and HFD+MIE groups demonstrated lower lipid peroxidation levels in heart than CTR and CTR+MIE groups, respectively (figure 5A). There was no changes in the enzymatic activity of SOD (figure 5B), but was observed higher activity of CAT in the animals from HFD and HFD+MIE groups when compared to animals from CTR and CTR+MIE groups in heart, respectively (figure 5C).

Exercise, high-fat diet intake, $PM_{2.5}$ exposure, or the combination of the groups, not caused alteration of the iHSP70 concentration in the heart (figure 6A) and lung (figure 6B). On the other hand, it has observed lower eHSP72 concentration in plasma of the animals submitted to MIE and $PM_{2.5}$ exposure when compared to CTR animals (figure 6C). Exercise caused lower H-index levels in the plasma/heart ratio (figure 7A) and plasma/lung ratio (figure 7B), and animals submitted to both exercise and $PM_{2.5}$ exposure decreased H-index level only in the lung (figure 7B). Decreasing H-index was observed as effect of MIE only in standard diet-treated mice and no H-index alteration in high-fat diet treated mice.

Discussion

Our study shows a single exercise session under moderate intensity is able to reduce eHSP72 concentration in plasma and trigger a protection effect upon cardiopulmonary pro- and anti-inflammatory state in mice, evaluated by H-index ([eHSP72/iHSP70] ratio), even when exposed to $PM_{2.5}$. These effects have not observed in animals under obesogenic/diabetogenic process induced by high-fat diet intake. Some protocol considerations and implications of this effect are discussed below.

Animals fed with high-fat diet presented lower feed intake in grams (g) and kilocalories (kcal), when compared to animals fed with standard diet. However, high-fat diet intake triggered increase of body weight and BMI in the mice, suggesting animals from HFD group developed higher efficiency in converting minor amounts of calories in body mass (g). Studies have differed if the increase in energy consumption is necessary to induces obesity in mice (Ziotopoulou et al. 2000; Collins et al. 2004; Alexander et al. 2006), but as described by Townsend et al. (2008) the increase of energy intake may not be required for increasing adiposity due to high-fat diet intake. The type of fat used in the high-fat diet preparations may contribute to adiposity development, regardless the amount of caloric intake, once saturated fats, as used in this study (lard), are recommended for obesity animal models with several metabolic changes in rodents (Buettner et al. 2006). In this way, animals from HFD groups presented higher adipose tissue weight (~3.5 fold) when compared to CTR group. These findings suggest energy storage on white adipose tissue are regulated as a response to factors directly related to the fat amount on the diet, and not directly related with the amount of energy consumed in kcal (Hariri and Thibault 2010).

Intervention with high-fat diet (over 40%) is an appropriate model for studies upon obesity, glucose intolerance, insulin resistance and T2DM. However, there is no agreement about which mice strain is more susceptible to the metabolic damage caused by high-fat diet (Surwit et al. 1995). Recently, metabolic differences have been suggested between mice strains C57BL/6J and B6.129SF2/J in response to high-fat diet intake. It was suggested that B6 mice used in our study should be more resistant to the effects of high-fat diet due to decreased intestinal absorption of lipids, which would characterize the strain as less susceptible to the effects of high-fat diet (Bock et al. 2015). In the study of Bock et al (2015) lower responsiveness to GTT and alterations in fasting glucose were observed after 4 weeks of high-fat diet intake in adult animals. The authors also observed alterations in the fasting

glucose in this same strain, but didn't find differences in the GTT after 28 weeks. These metabolic responses alterations may have been caused by methodological variation among studies. In contrast to the research cited above, our study exposes the animals after weaning (four weeks old). Corroborating with King et al. (2014), our data shows that the effects of high-fat diet after weaning result pronounced adiposity and metabolic disorders than animals exposed to high-fat diet intake only on adult period. Notwithstanding, the high-fat diet treated mice in our study presented different oxidative and HSPs responses to exercise and $PM_{2.5}$ exposure. Thus, the high-fat diet used in our study is a suitable model to induce obesity in B6129SF2 mice.

Besides the obesity (increased BMI, body mass and adipose tissue) the model in study allows pathophysiological investigation of alterations resulting due obesity in the development of a chronic state of low-grade inflammation and thereafter an anti-inflammatory response alteration diabetes' characteristic (Rummel et al. 2016), resulting in consequences to the respiratory (Cho and Shore 2016) and cardiovascular systems (Homem de Bittencourt et al. 2007). Those alterations may compromise the response of mice to exercise (Rosa et al. 2011).

Constantly used in human or animal studies, blood lactate concentration reflects the effort intensity made in response to an exercise session (Ferreira et al. 2007; Goodwin et al. 2007). In our study, that measure after swimming protocol confirmed the moderate exercise condition in the groups. Swimming exercise model has advantages over the treadmill running, since swimming is a natural ability of the animal. However, in opposite to running protocols, findings related to swimming intensity performed in mice are still limited (Gobatto et al. 2001). Ferreira et al. (2007) showed that the maximum blood lactate concentration stabilization occurs at 3.0 mmol/L in mice at ~15 m/min in treadmill, corresponding to 60% of the maximum speed of a running test. Gobatto et al. (2009) reported that the equivalent to metabolic transition of swimming exercise on mice occurs at a 4.6% loading of body mass and at 5.78 ± 0.29 mmol/L of blood lactate. In our study, animals which performed swimming exercise under 4% load showed blood lactate concentration at 4.3 ± 0.5 (MIE) and 5.5 ± 0.9 mmol/L (HFD + PM + MIE). Although not statistically different, the values closer to 5.5 mmol/L demonstrated by animals fed with high-fat diet may be related to early fatigue of these animals, which failed to perform 20 minutes of swimming (HFD+MIE e HFD+PM+MIE). Furthermore, similar results of blood lactate (cited above) and time of

exercise were found by Gobatto et al. (2009), but with ordinary mice. Authors suggest that sedentary mice do not support loads higher than 5% of its body mass for 25 min. Our animals were submitted to 4% load and the adverse effect of high-fat diet in the exercise performance was evident in our study. The fatigable effect may be related with high levels of eHSP72 in plasma during exercise, and represents a neuroimmunomodulatin signaling that may causes fatigue sensation (Heck et al. 2011). Thus, considering obesity as a factor that triggers low grade chronic inflammation, eHSP72 levels increasing (Rodrigues-Krause et al. 2012) and unbalance in the eHSP72/iHSP70 ratio (Krause et al. 2015), the fatigue showed by HFD groups submitted to the exercise may be related to blunted anti-inflammatory response and development of early fatigue state.

Aerobic exercise is able to increase free radicals production that may result in oxidative stress in many tissues (Davies et al. 1982). However, most studies related to oxidative stress and acute exercise are applied using maximum and submaximal effort protocols (Fisher-Wellman and Bloomer 2009). Prior findings report that exercise increases lipid peroxidation in the lung, suggesting that this condition induces oxidative stress in this tissue. Prigol et al. (2009) evaluated oxidative damage 1h after swimming exercise and observed that the acute exercise (20 min) caused increase of lipid peroxidation in the lung of mice. Exposure condition to urban $PM_{2.5}$ during 20 min of moderate swimming exercise in rats is enough to induce a modest increase in lipid peroxidation levels in the lung and heart (Heck et al., 2014). Only with more time of exercise (60 min, according to Heck et al., 2014) or a higher dose of particles (500 µg) (Heck et al. 2014) it is possible to observe lung and heart lipid peroxidation oxidative damage by acutely air pollution and exercise combination. Our data (50µg of $PM_{2.5}$ intranasally) represents one day in urban environment with levels within preconized limits of air pollution. Thus, our data corroborates with other studies, once increasing oxidative damage levels immediately after swimming session seems to be dependent of the time of exercise and exposure level of air pollution.

Increasing in SOD activity in the lung of animals from MIE and PM groups suggests similar responses of this enzyme in exercise and polluted air conditions. However, SOD activity attenuated lung oxidative damage under pollutant exposure. It suggests that a higher SOD enzymatic activity may be effective against lung oxidative stress (Ghio et al. 2002). Unlike the pulmonary tissue, we did not find increasing of lipid peroxidation in the heart tissue. These results indicate specific responses of tissues to exercise, as previously described

about $PM_{2.5}$ exposure (Damiani and Piva 2012). Furthermore, the intensity of exercise was not enough to trigger oxidative damage in the heart of mice (Gao et al. 2014). It was observed a lower level of oxidative damage in the heart of the animals from HFD and HFD+MIE groups, regardless of rest or exercise conditions. This finding can be explained due higher CAT activity, generating defensive effect against lipid peroxidation in the heart and suggesting an important antioxidant response in this tissue under high-fat diet intake.

Under challenges to the homeostasis, cells from different tissues increase iHSP70 expression (cellular stress response) and also export this protein to circulation (Febbraio et al. 2002; Noble et al. 2008). High plasmatic levels of eHSP72 are correlated to energetic balance impairment, alteration on pro-/anti-inflammatory status and redox state balance (Walsh et al. 2001; Milne and Noble 2002; Campisi et al. 2003; Whitham and Fortes 2008). On the other hand, absence or inhibition of HSP70 expression demonstrated cell vulnerability to the stress (Rubio et al. 2002) and may generate apoptosis (Adachi et al. 2009).

Regardless the conditions evaluated in this study as obesity, air pollution and exercise, we did not find alterations in the expression of iHSP70 in the lung and heart. We expected a lower intracellular expression of this protein in the animals chronically exposed to the high-fat diet intake, since obesity is capable of causing a decrease in the iHSP70 levels is muscle (Chung et al. 2008), liver and adipose tissue (Di Naso et al. 2015). This decrease has relation to glucose intolerance (Krause et al. 2015) and insulin resistance in obese (Henstridge et al. 2010). However, heart and lung did not show iHSP70 content alterations by high-fat diet intake. Defect in response to stress, as commonly observed in chronic cases of inflammation, has been associated to many obesity-related diseases (Di Naso et al. 2015), as insulin resistance, T2DM and non alcoholic hepatic steatosis (Newsholme and Homem de Bittencourt 2014). Briefly, the ability of a cell to properly detect and initiate a response is essential for its survival.

Due to the versatility of HSP70 to induce different responses related to inflammation according to its location, it's proposed that this protein may represent an important marker for immunoinflammatory state during exercise (Krause et al. 2015). Therefore, ideal levels of this protein in the circulation appear to be fundamental in the maintenance of homeostasis (Heck et al. 2011). In addition, [eHSP72/iHSP70] ratio balance measured by mathematical calculation H-index, as suggested by Heck (2011) and reported in other studies (Yang et al. 2008; Magalhães et al. 2010; Rodrigues-Krause et al. 2012), may represent an important

biomarker of health/disease process, as well as serve as references on subclinical biological processes that occur in the body (Heck, 2011). Our study shows that the performance of acute moderate-intensity exercise generates a lower concentration of eHSP72 in the plasma of mice. In addition, it confirms the effect of exercise on reducing H-index in the heart and lung of the animals of CTR+MIE group (0.37 and 0.34 *versus* 1.00) and only lung of the animals from PM+MIE group (0.39 *versus* 1.00), suggesting a beneficial effect of the exercise on the status pro-and anti-inflammatory, on behalf of the second, indicating an anti-inflammatory response in these animals (Keller et al. 2004; Heck 2011; Krause et al. 2015).

The anti-inflammatory response to acute exercise is attributed to increased circulating levels of known anti-inflammatory cytokines as IL-6 (Petersen and Pedersen 2005), and it generates increasing on IL-1Rα, sTNF-R and IL-10 (Ostrowski et al. 1999; Ostrowski et al. 2000; Steensberg et al. 2003). Acutely in humans, exercise is able to measure the inflammatory response by IL-6 release, which is produced and released from the muscle contraction (Starkie et al. 2003). In animals, acute exercise is also able to normalize the excessive expression of pro-inflammatory cytokines (TNF-α) (Keller et al. 2004). Moderate exercise generates a 20 time increasing in plasma concentration of IL-6, demonstrating this intensity exerts important effect on the expression of this anti-inflammatory cytokine (Fischer et al. 2004). This response does not occur at the same manner under obesity, once obese may respond to exercise with an overly pro-inflammatory profile (e.g. increased levels of TNF-α and IL-2) (Rosa et al. 2011). Thus, according to our data, we suggest the performance of acute moderate-intensity exercise produces an anti-inflammatory process in sedentary mice, even exposed to $PM_{2.5}$, which does not occur in obesity. This response has confirmed unprecedented way by cardiopulmonary H-index calculation.

Conclusion

Our study provides evidences based on HSP70 status and oxidative stress parameters about benefits of acute exercise of moderate intensity on cardiopulmonary system, even exposed to $PM_{2.5}$. Further, our data indicates that the benefits of a single session of acute exercise at moderate intensity may not occur in obesity.

Author Contribution

IMK completed all the experiments described in this manuscript. GW and YHD performed biometric and metabolic profile. LCW and ABS performed experiments on oxidative stress parameters. FGB and GW performed western blot analyses. All authors were involved in analyzing the results. TGH, IMK, FGB and GW co-wrote the paper. TGH and CRR designed the study. TGH and CRR provided experimental advice and helped with manuscript revision. All the authors had final approval of the submitted and published versions.

Acknowledgements

The authors would like to thank to colleagues from the Laboratory of Oxidative Stress and Atmospheric Pollution (UFCSPA) and Research group in Physiology for their technical support.

Financial Support

This work was supported by Federal University of Health Sciences of Porto Alegre (UFCSPA) and by grants from Research Support Foundation of the State of Rio Grande do Sul (PqG-2013 - FAPERGS, process: 002106-2551/13-5 and PqG, #ARD/PPP/FAPERGS/CNPq 08/2014 both to TGH). IMK, ABS and FGB were recipients of scholarships from the Coordination for the Improvement of Higher Education Personnel (CAPES) and GW from Research Support Foundation of the State of Rio Grande do Sul (FAPERGS).

Declaration of Interest

The authors declare that they do not have competing financial interests.

References

Adachi H, Katsuno M, Waza M, et al (2009) Heat shock proteins in neurodegenerative diseases: pathogenic roles and therapeutic implications. Int J Hyperthermia 25:647–54. doi: 10.3109/02656730903315823

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–6.

Alexander J, Chang GQ, Dourmashkin JT, Leibowitz SF (2006) Distinct phenotypes of obesity-prone AKR/J, DBA2J and C57BL/6J mice compared to control strains. Int J Obes (Lond) 30:50–9. doi: 10.1038/sj.ijo.0803110

Asea A (2008) Heat shock proteins and toll-like receptors. Handb Exp Pharmacol 111–27. doi: 10.1007/978-3-540-72167-3_6

Banerjee AK, Mandal A, Chanda D, Chakraborti S (2003) Oxidant, antioxidant and physical exercise. Mol Cell Biochem 253:307–12.

Bock PM, Krause M, Schroeder HT, et al (2015) Oral supplementations with l-glutamine or l-alanyl-l-glutamine do not change metabolic alterations induced by long-term high-fat diet in the B6.129F2/J mouse model of insulin resistance. Mol Cell Biochem. doi: 10.1007/s11010-015-2597-6

Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52:302–10.

Buettner R, Parhofer KG, Woenckhaus M, et al (2006) Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. J Mol Endocrinol 36:485–501. doi: 10.1677/jme.1.01909

Campisi J, Leem TH, Fleshner M (2003) Stress-induced extracellular Hsp72 is a functionally significant danger signal to the immune system. Cell Stress Chaperones 8:272–86.

Cavalcanti CB dos S, Carvalho SCB do E, Barros MVG de (2009) Anthropometric indicators of abdominal obesity: review of the papers indexed on SciELO electronic library. Rev bras cineantropom desempenho hum 217–225.

Cho Y, Shore SA (2016) Obesity, Asthma, and the Microbiome. Physiology (Bethesda) 31:108–16. doi: 10.1152/physiol.00045.2015

Chung J, Nguyen A-K, Henstridge DC, et al (2008) HSP72 protects against obesity-induced insulin resistance. Proc Natl Acad Sci U S A 105:1739–44. doi: 10.1073/pnas.0705799105

Collins S, Martin TL, Surwit RS, Robidoux J (2004) Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics. Physiol Behav 81:243–8. doi: 10.1016/j.physbeh.2004.02.006

Cote I, Samet J, Vandenberg JJ (2008) U.S. air quality management: local, regional and global approaches. J Toxicol Environ Health A 71:63–73. doi: 10.1080/15287390701557917

Damiani R, Piva M (2012) Is cardiac tissue more susceptible than lung to oxidative effects induced by chronic nasotropic instillation of residual oil fly ash (ROFA)?

Davies KJA, Quintanilha AT, Brooks GA, Packer L (1982) Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 107:1198–1205. doi: 10.1016/S0006-291X(82)80124-1

Di Naso FC, Porto RR, Fillmann HS, et al (2015) Obesity depresses the anti-inflammatory HSP70 pathway, contributing to NAFLD progression. Obesity (Silver Spring) 23:120–9. doi: 10.1002/oby.20919

Dimauro I, Mercatelli N, Caporossi D (2016) Exercise-induced ROS in Heat Shock Proteins response. Free Radic Biol Med. doi: 10.1016/j.freeradbiomed.2016.03.028

Eljsvogels TMH, Thompson PD (2015) Exercise Is Medicine At Any Dose ? JAMA 314:1915–1916. doi: 10.1001/jama.2015.10858

Estadella D, Oyama LM, Dâmaso AR, et al (2004) Effect of Palatable Hyperlipidic Diet on Lipid Metabolism of Sedentary and Exercised Rats. Nutrition 20:218–224. doi: 10.1016/j.nut.2003.10.008

Febbraio MA, Ott P, Nielsen HB, et al (2002) Exercise induces hepatosplanchnic release of heat shock protein 72 in humans. J Physiol 544:957–62.

Ferreira JCB, Rolim NPL, Bartholomeu JB, et al (2007) Maximal lactate steady state in running mice: effect of exercise training. Clin Exp Pharmacol Physiol 34:760–5. doi: 10.1111/j.1440-1681.2007.04635.x

Fischer CP, Hiscock NJ, Penkowa M, et al (2004) Supplementation with vitamins C and E inhibits the release of interleukin-6 from contracting human skeletal muscle. J Physiol 558:633–45. doi: 10.1113/jphysiol.2004.066779

Fisher-Wellman K, Bloomer RJ (2009) Acute exercise and oxidative stress: a 30 year history. Dyn Med 8:1. doi: 10.1186/1476-5918-8-1

Furukawa S, Fujita T, Shimabukuro M, et al (2004) Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 114:1752–61. doi: 10.1172/JCI21625

Gao C, Chen X, Li J, et al (2014) Myocardial mitochondrial oxidative stress and dysfunction in intense exercise: regulatory effects of quercetin. Eur J Appl Physiol 114:695–705. doi: 10.1007/s00421-013-2802-9

Ghio AJ, Suliman HB, Carter JD, et al (2002) Overexpression of extracellular superoxide dismutase decreases lung injury after exposure to oil fly ash. Am J Physiol Lung Cell Mol Physiol 283:L211–8. doi: 10.1152/ajplung.00409.2001

Gobatto CA, de Barros Manchado-Gobatto F, Carneiro LG, et al (2009) Maximal lactate steady state for aerobic evaluation of swimming mice. Comp Exerc Physiol 6:99–103. doi: 10.1017/S1755254009990109

Gobatto CA, de Mello MA, Sibuya CY, et al (2001) Maximal lactate steady state in rats submitted to swimming exercise. Comp Biochem Physiol A Mol Integr Physiol 130:21–7.

Goettems-Fiorin PB, Grochanke BS, Baldissera FG, et al (2016) Fine particulate matter potentiates type 2 diabetes development in high-fat diet-treated mice: stress response and extracellular to intracellular HSP70 ratio analysis. J Physiol Biochem. doi: 10.1007/s13105-016-0503-7

Goodwin ML, Harris JE, Hernández A, Gladden LB (2007) Blood lactate measurements and analysis during exercise: a guide for clinicians. J Diabetes Sci Technol 1:558–69.

Hallal PC, Andersen LB, Bull FC, et al (2012) Global physical activity levels: surveillance progress, pitfalls, and prospects. Lancet 380:247–57. doi: 10.1016/S0140-6736(12)60646-1

Hariri N, Thibault L (2010) High-fat diet-induced obesity in animal models. Nutr Res Rev 23:270–99. doi: 10.1017/S0954422410000168

Heck, TG; Nunes, RB; Petry, MR; Maslinkiewicz, A; Saldiva, PHN; Dal Ago, P; Rhoden C (2014) Residual Oil Fly Ash (ROFA) Inhalation Promotes Lung and Heart Oxidative Stress without Hemodynamic Effects in Exercising Rats. J Exerc Physiol 17:11–25.

Heck, TG; Petry, MR; Maslinkiewicz, A; Goettems-Fiorin, PB; Ludwig, MS; Saldiva, PHN; Dal Ago, P; Rhoden C (2014) Effects of ambient particles inhalation on lung oxidative stress parameters in exercising rats. J Exerc Physiol 17:58–69.

Heck TG (2011) Razão entre o conteúdo extracelular e intracelular de HSP70 como um sinal de alerta imunológico e marcador de intensidade de exercício. Universidade Federal do Rio Grande do Sul - UFRGS

Heck TG, Schöler CM, de Bittencourt PIH (2011) HSP70 expression: does it a novel fatigue signalling factor from immune system to the brain? Cell Biochem Funct 29:215–226.

Henstridge DC, Forbes JM, Penfold SA, et al (2010) The relationship between heat shock protein 72 expression in skeletal muscle and insulin sensitivity is dependent on adiposity. Metabolism 59:1556–61. doi: 10.1016/j.metabol.2010.01.027

Homem de Bittencourt PI, Lagranha DJ, Maslinkiewicz A, et al (2007) LipoCardium: endothelium-directed cyclopentenone prostaglandin-based liposome formulation that completely reverses atherosclerotic lesions. Atherosclerosis 193:245–58. doi: 10.1016/j.atherosclerosis.2006.08.049

Hotamisligil GS (2006) Inflammation and metabolic disorders. Nature 444:860–7. doi: 10.1038/nature05485

Jones Q, Voegeli TS, Li G, et al (2011) Heat shock proteins protect against ischemia and inflammation through multiple mechanisms. Inflamm Allergy Drug Targets 10:247–59.

Keaney JF, Larson MG, Vasan RS, et al (2003) Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arterioscler Thromb Vasc Biol 23:434–9. doi: 10.1161/01.ATV.0000058402.34138.11

Keller C, Keller P, Giralt M, et al (2004) Exercise normalises overexpression of TNF-alpha in knockout mice. Biochem Biophys Res Commun 321:179–82. doi: 10.1016/j.bbrc.2004.06.129

Kido T, Bai N, Yatera K, et al (2011) Diesel exhaust inhalation induces heat shock protein 70 expression in vivo. Inhal Toxicol 23:593–601. doi: 10.3109/08958378.2011.595843

King V, Norman JE, Seckl JR, Drake AJ (2014) Post-weaning diet determines metabolic risk in mice exposed to overnutrition in early life. Reprod Biol Endocrinol 12:73. doi: 10.1186/1477-7827-12-73

Koya D, Kanasaki K (2011) Biology of obesity: Lessons from animal models of obesity. J Biomed Biotechnol. doi: 10.1155/2011/197636

Krause M, Heck TG, Bittencourt A, et al (2015) The chaperone balance hypothesis: the importance of the extracellular to intracellular HSP70 ratio to inflammation-driven type

2 diabetes, the effect of exercise, and the implications for clinical management. Mediators Inflamm 2015:249205. doi: 10.1155/2015/249205

Laemmli UK (1970) Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. Nature 227:680–685. doi: 10.1038/227680a0

Lollo PCB, Moura CS, Morato PN, Amaya-Farfan J (2013) Differential response of heat shock proteins to uphill and downhill exercise in heart, skeletal muscle, lung and kidney tissues. J Sports Sci Med 12:461–6.

Maatz LF, Wood GJA, Rivero DHRF, Saldiva PHN (2009) Tracheal instillation of urban PM2.5 suspension promotes acute cardiac polarization changes in rats. Brazilian J Med Biol Res 42:207–213. doi: 10.1590/S0100-879X2009000200009

Magalhães F de C, Amorim FT, Passos RLF, et al (2010) Heat and exercise acclimation increases intracellular levels of Hsp72 and inhibits exercise-induced increase in intracellular and plasma Hsp72 in humans. Cell Stress Chaperones 15:885–95. doi: 10.1007/s12192-010-0197-7

Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47:469–74.

Martín-Gallán P, Carrascosa A, Gussinyé M, Domínguez C (2003) Biomarkers of diabetes-associated oxidative stress and antioxidant status in young diabetic patients with or without subclinical complications. Free Radic Biol Med 34:1563–74.

Meier R, Cascio WE, Ghio AJ, et al (2014) Associations of short-term particle and noise exposures with markers of cardiovascular and respiratory health among highway maintenance workers. Environ Health Perspect 122:726–32. doi: 10.1289/ehp.1307100

Milne KJ, Noble EG (2002) Exercise-induced elevation of HSP70 is intensity dependent.

Morton JP, Kayani AC, McArdle A, Drust B (2009) The exercise-induced stress response of skeletal muscle, with specific emphasis on humans. Sports Med 39:643–62. doi: 10.2165/00007256-200939080-00003

Mukhopadhyay I, Nazir A, Saxena DK, Chowdhuri DK (2003) Heat shock response: hsp70 in environmental monitoring. J Biochem Mol Toxicol 17:249–54. doi: 10.1002/jbt.10086

Newsholme P, de Bittencourt PIH (2014) The fat cell senescence hypothesis: a mechanism responsible for abrogating the resolution of inflammation in chronic disease. Curr Opin

Clin Nutr Metab Care 17:295–305. doi: 10.1097/MCO.0000000000000077

Ng M, Fleming T, Robinson M, 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

Noble EG, Milne KJ, Melling CWJ (2008) Heat shock proteins and exercise: a primer. Appl Physiol Nutr Metab = Physiol Appl Nutr métabolisme 33:1050–65. doi: 10.1139/H08-069

Novelli ELB, Diniz YS, Galhardi CM, et al (2007) Anthropometrical parameters and markers of obesity in rats. Lab Anim 41:111–9. doi: 10.1258/002367707779399518

Ostrowski K, Rohde T, Asp S, et al (1999) Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol 515 (Pt 1:287–91.

Ostrowski K, Schjerling P, Pedersen BK (2000) Physical activity and plasma interleukin-6 in humans--effect of intensity of exercise. Eur J Appl Physiol 83:512–5. doi: 10.1007/s004210000312

Pearson JF, Bachireddy C, Shyamprasad S, et al (2010) Association between fine particulate matter and diabetes prevalence in the U.S. Diabetes Care 33:2196–201. doi: 10.2337/dc10-0698

Petersen AMW, Pedersen BK (2005) The anti-inflammatory effect of exercise. J Appl Physiol 98:1154–62. doi: 10.1152/japplphysiol.00164.2004

Prigol M, Luchese C, Nogueira CW (2009) Antioxidant effect of diphenyl diselenide on oxidative stress caused by acute physical exercise in skeletal muscle and lungs of mice. Cell Biochem Funct 27:216–22. doi: 10.1002/cbf.1559

Qin X-D, Qian Z, Vaughn MG, et al (2015) Gender-specific differences of interaction between obesity and air pollution on stroke and cardiovascular diseases in Chinese adults from a high pollution range area: A large population based cross sectional study. Sci Total Environ 529:243–8. doi: 10.1016/j.scitotenv.2015.05.041

Rhoden CR, Lawrence J, Godleski JJ, González-Flecha B (2004) N-acetylcysteine prevents lung inflammation after short-term inhalation exposure to concentrated ambient particles. Toxicol Sci 79:296–303. doi: 10.1093/toxsci/kfh122

Rodrigues-Krause J, Krause M, O'Hagan C, et al (2012) Divergence of intracellular and

extracellular HSP72 in type 2 diabetes: Does fat matter? Cell Stress Chaperones 17:293–302. doi: 10.1007/s12192-011-0319-x

Rosa JS, Heydari S, Oliver SR, et al (2011) Inflammatory cytokine profiles during exercise in obese, diabetic, and healthy children. J Clin Res Pediatr Endocrinol 3:115–21. doi: 10.4274/jcrpe.v3i3.23

Rubio E, Valenciano AI, Segundo C, et al (2002) Programmed cell death in the neurulating embryo is prevented by the chaperone heat shock cognate 70. Eur J Neurosci 15:1646–54.

Rummel C, Bredehöft J, Damm J, et al (2016) Obesity Impacts Fever and Sickness Behavior During Acute Systemic Inflammation. Physiology (Bethesda) 31:117–30. doi: 10.1152/physiol.00049.2015

Silveira EMS, Rodrigues MF, Krause MS, et al (2007) Acute exercise stimulates macrophage function: possible role of NF-κB pathways. Cell Biochem Funct 25:63–73. doi: 10.1002/cbf.1365

Slovic AD, de Oliveira MA, Biehl J, Ribeiro H (2015) How Can Urban Policies Improve Air Quality and Help Mitigate Global Climate Change: a Systematic Mapping Review. J Urban Heal. doi: 10.1007/s11524-015-0007-8

Smith KB, Smith MS (2016) Obesity Statistics. Prim Care 43:121–35. doi: 10.1016/j.pop.2015.10.001

Starkie R, Ostrowski SR, Jauffred S, et al (2003) Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. FASEB J 17:884–6. doi: 10.1096/fj.02-0670fje

Steensberg A, Fischer CP, Keller C, et al (2003) IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. Am J Physiol Endocrinol Metab 285:E433–7. doi: 10.1152/ajpendo.00074.2003

Surwit RS, Feinglos MN, Rodin J, et al (1995) Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. Metabolism 44:645–51.

Townsend KL, Lorenzi MM, Widmaier EP (2008) High-fat diet-induced changes in body mass and hypothalamic gene expression in wild-type and leptin-deficient mice. Endocrine 33:176–88. doi: 10.1007/s12020-008-9070-1

USA-FAO - Food and Agriculture Organization (1997) Food and Agriculture Organization.

Carbohydrates is Human Nutrition. http://www.fao.org/docrep/w8079e/w8079e00.htm#Contents. Accessed 15 Apr 2016

Walsh RC, Koukoulas I, Garnham A, et al (2001) Exercise increases serum Hsp72 in humans. Cell Stress Chaperones 6:386–93.

Wang B, Xu D, Jing Z, et al (2014) Effect of long-term exposure to air pollution on type 2 diabetes mellitus risk: a systemic review and meta-analysis of cohort studies. Eur J Endocrinol 171:R173–82. doi: 10.1530/EJE-14-0365

Whitham M, Fortes MB (2008) Heat shock protein 72: release and biological significance during exercise. Front Biosci 13:1328–39.

WHO WHO (2003) Diet, nutrition and the prevention of chronic diseases.

Xu Z, Xu X, Zhong M, et al (2011) Ambient particulate air pollution induces oxidative stress and alterations of mitochondria and gene expression in brown and white adipose tissues. Part Fibre Toxicol 8:20. doi: 10.1186/1743-8977-8-20

Yang X, Yuan J, Sun J, et al (2008) Association between heat-shock protein 70 gene polymorphisms and DNA damage in peripheral blood lymphocytes among coke-oven workers. Mutat Res 649:221–9. doi: 10.1016/j.mrgentox.2007.09.004

Ziotopoulou M, Mantzoros CS, Hileman SM, Flier JS (2000) Differential expression of hypothalamic neuropeptides in the early phase of diet-induced obesity in mice. Am J Physiol Endocrinol Metab 279:E838–45.

Tables

Table 1. Experimental Groups

Groups	***Diet***	***Instillation***	***Physical Exercise***
CTR; n=6	*Standard*	*10µL Physiological Solution*	*Rest*
CTR+MIE; n=10	*Standard*	*10µL Physiological Solution*	*20 min. Swimming*
PM; n=7	*Standard*	*10µL PM (50µg/10µL)*	*Rest*
PM+MIE; n=6	*Standard*	*10µL PM (50µg/10µL)*	*20 min. Swimming*
HFD; n=8	*HFD*	*10µL Physiological Solution*	*Rest*
HFD+MIE; n=9	*HFD*	*10µL Physiological Solution*	*20 min. Swimming*
HFD+PM; n=7	*HFD*	*10µL PM (50µg/10µL)*	*Rest*
HFD+PM+MIE; n=7	*HFD*	*10µL PM (50µg/10µL)*	*20 min. Swimming*

Subtitles

Figure 1 Feed intake by high-fat diet-treated mice for 16 weeks. CTR = Standard Chow (n = 29). HFD = High-fat Diet (n = 31). (A) Intake in grams (g) and (B) intake in kilocalories (kcal). Data expressed as mean ± standard deviation. *P = 0.04 compared to the control. Two-way ANOVA followed by post-hoc Tukey's test.

Figure 2 Body mass, body mass index, adiposity**,** and fasting blood glucose of high-fat diet-treated mice for 16 weeks. CTR = standard diet (n = 29). HFD = high-fat diet (n = 31). (A) Body mass in grams (g), (B) Body Mass Index, (C) Epididymal White Adipose Tissue Weight, and (D) Fasting Glucose. Data expressed as mean ± standard deviation. *P = 0.02 compared to the CTR. Two-way ANOVA followed by post-hoc Tukey's test in A, B and D. *P = 0.01 compared to CTR. Student *t* test in C.

Figura 3 Glucose Tolerance Test response in high-fat diet-treated mice for 16 weeks. CTR = standard diet (n = 29). HFD = high-fat diet (n = 31). GTT performed upon administration (i.p.) of glucose (1 g/kg) before high-fat diet intake (4) and every 4^{th} (B), 8^{th} (C), 12^{th} (D) and 16^{th} (E) week. Glucose intolerance has based from AUC calculation after 16 weeks of treatment (F). Data expressed as mean ± standard deviation. *P = 0.01; and **P = 0.01, both compared to CTR. Two-way ANOVA followed by post-hoc Tukey's test.

Figura 4 Lipid peroxidation and activity of antioxidant enzymes of the mice lung after treatment. CTR = standard diet; PM = instillation of 50µg/10µL of $PM_{2.5}$ suspension intranasally; HFD = high-fat diet for 16 weeks; HFD+PM = high-fat diet and $PM_{2.5}$ instillation. RES = Animals maintained at rest for 20 min in shallow water (30±1ºC). MIE = Animals submitted to acute moderate exercise (4% of load, water at 30±1ºC). (A) Lipid peroxidation evaluated by TBARS. *P < 0.05 compared to HFD+MIE. #P < 0.05 compared to HFD+PM+MIE; (B) Enzymatic activity of SOD. *P < 0.05 compared to CTR. #P<0.05 compared to PM and (C) enzymatic activity of CAT (P = 0.355). Data expressed as mean ± standard deviation, n = 5 – 9 per group. One-way ANOVA followed by post-hoc Tukey's test.

Figura 5 Lipid peroxidation and activity of antioxidant enzymes of the mice heart after treatment. CTR = standard diet; PM = instillation of 50µg/10µL of $PM_{2.5}$ suspension intranasally; HFD = high-fat diet for 16 weeks; HFD+PM = high-fat diet and $PM_{2.5}$ instillation. RES = Animals maintained at rest for 20 min in shallow water (30±1ºC). MIE = Animals submitted to acute moderate exercise (4% of load, water at 30±1ºC). (A) Lipid peroxidation evaluated by TBARS. *P < 0.05 compared to CTR. #P < 0.05 compared to CTR+MIE; (B) Enzymatic activity of SOD (P = 0.946) and (C) enzymatic activity of CAT. *P<0,05 compared to CTR. # P < 0.05 compared to CTR+MIE. Data expressed as mean ± standard deviation, n = 5 – 9 per group. One-way ANOVA followed by post-hoc Tukey's test.

Figura 6 HSP70 concentration in the heart and lung and eHSP72 plasmatic content of mice after treatment. CTR = standard diet; PM = instillation of 50µg/10µL of $PM_{2.5}$ suspension intranasally; HFD = high-fat diet for 16 weeks; HFD+PM = high-fat diet and $PM_{2.5}$ instillation. RES = Animals maintained at rest for 20 min in shallow water (30±1ºC). MIE = Animals submitted to acute moderate exercise (4% of load, water at 30±1ºC). iHSP70 content in the (A) heart (P = 0.721) and (B) lung (P = 0.115). (C) Plasmatic concentration of eHSP72. *P < 0.05

compared to CTR. Data expressed as mean ± standard deviation, n = 4 – 6 per group. One-way ANOVA followed by post-hoc Tukey's test.

Figura 7 eHSP72/iHSP70 ratio upon plasmatic vs heart, and plasmatic vs lung of mice after treatment. CTR = standard diet; PM = instillation of 50µg/10µL of $PM_{2.5}$ suspension intranasally; HFD = high-fat diet for 16 weeks; HFD+PM = high-fat diet and $PM_{2.5}$ instillation. RES = Animals maintained at rest for 20 min in shallow water (30±1ºC). MIE = Animals submitted to acute moderate exercise (4% of load, water at 30±1ºC). (A) eHSP72/iHSP70 ratio of the heart, *P < 0.05 compared to CTR. (B) eHSP72/iHSP70 ratio of the lung, *P < 0.05 compared to CTR. Data expressed as mean ± standard deviation, n = 4 – 6 per group. One-way ANOVA followed by post-hoc Tukey's test.

Illustration

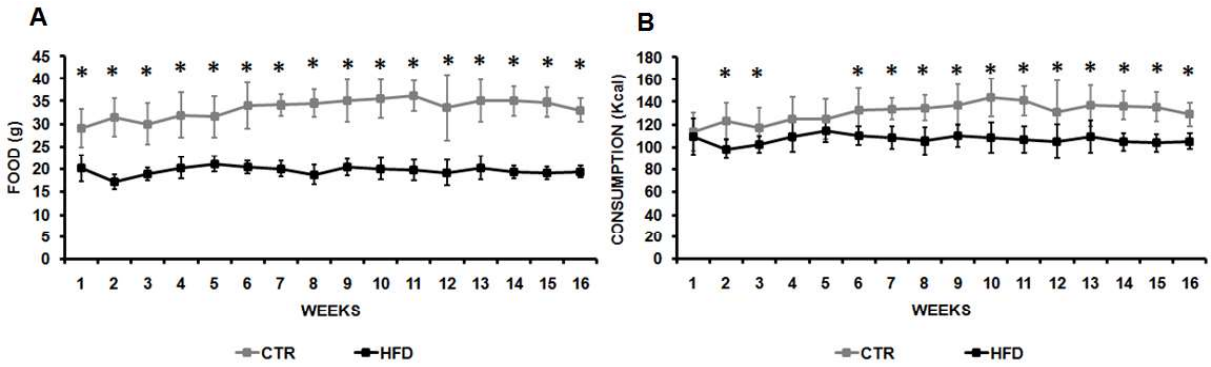

Figure 1

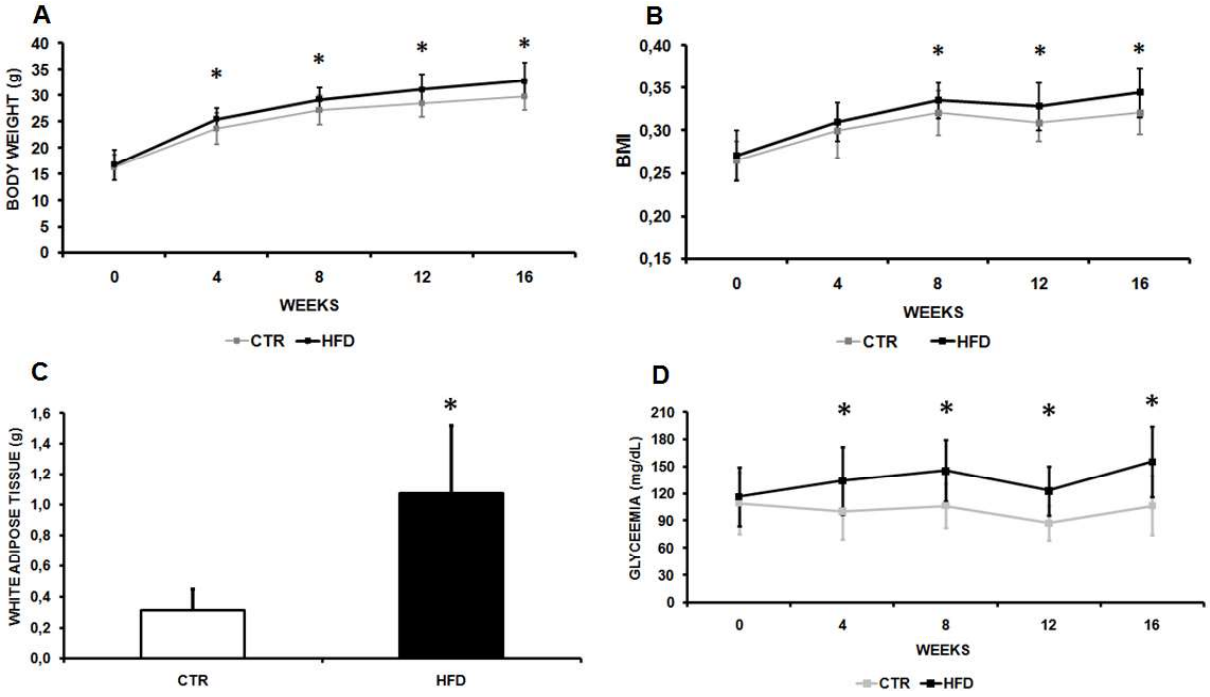

Figure 2

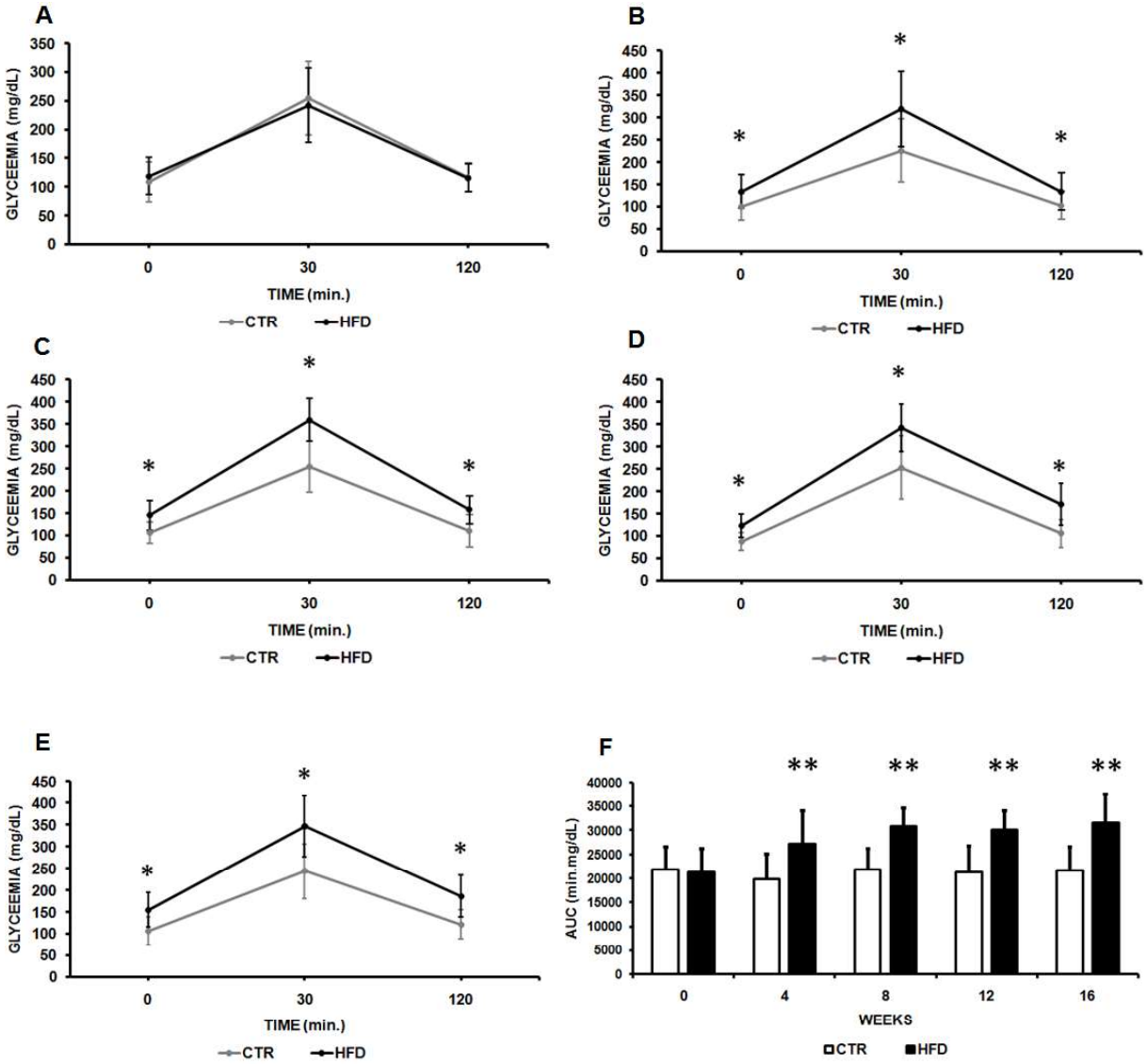
A
B
C
D
E
F
GLYCEEMIA (mg/dL)
TIME (min.)
CTR
HFD
AUC (min.mg/dL)
WEEKS
0
30
120
4
8
12
16

Figure 3

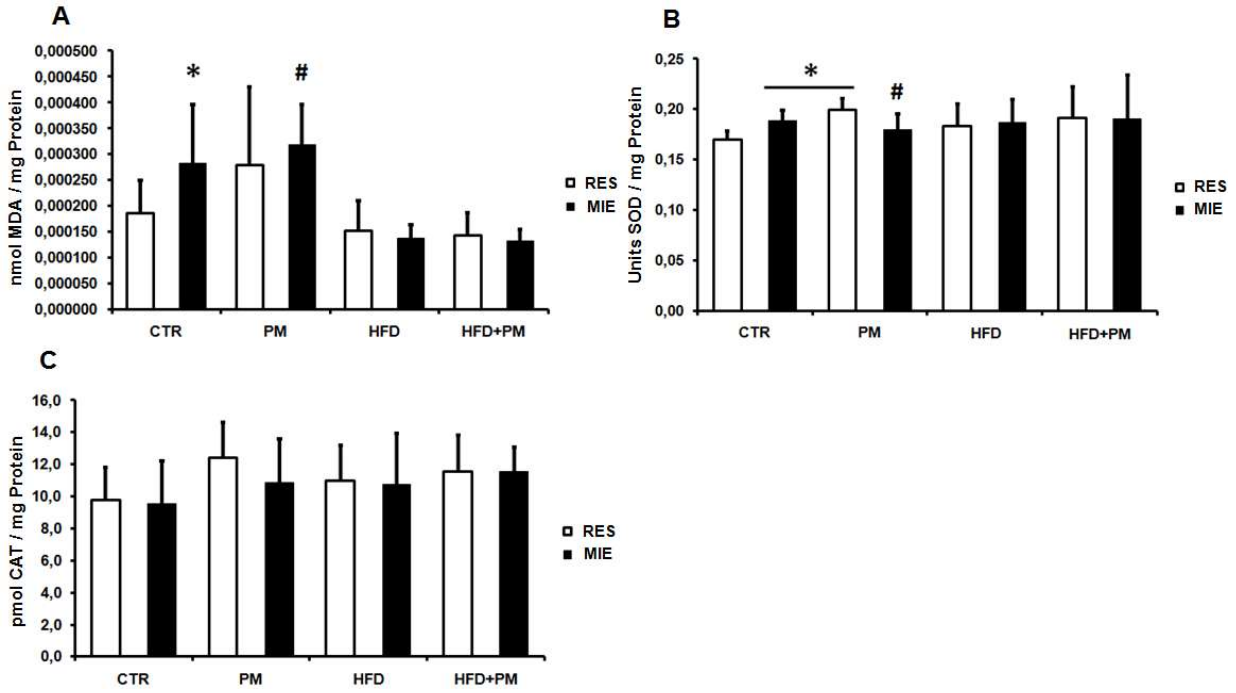

Figure 4

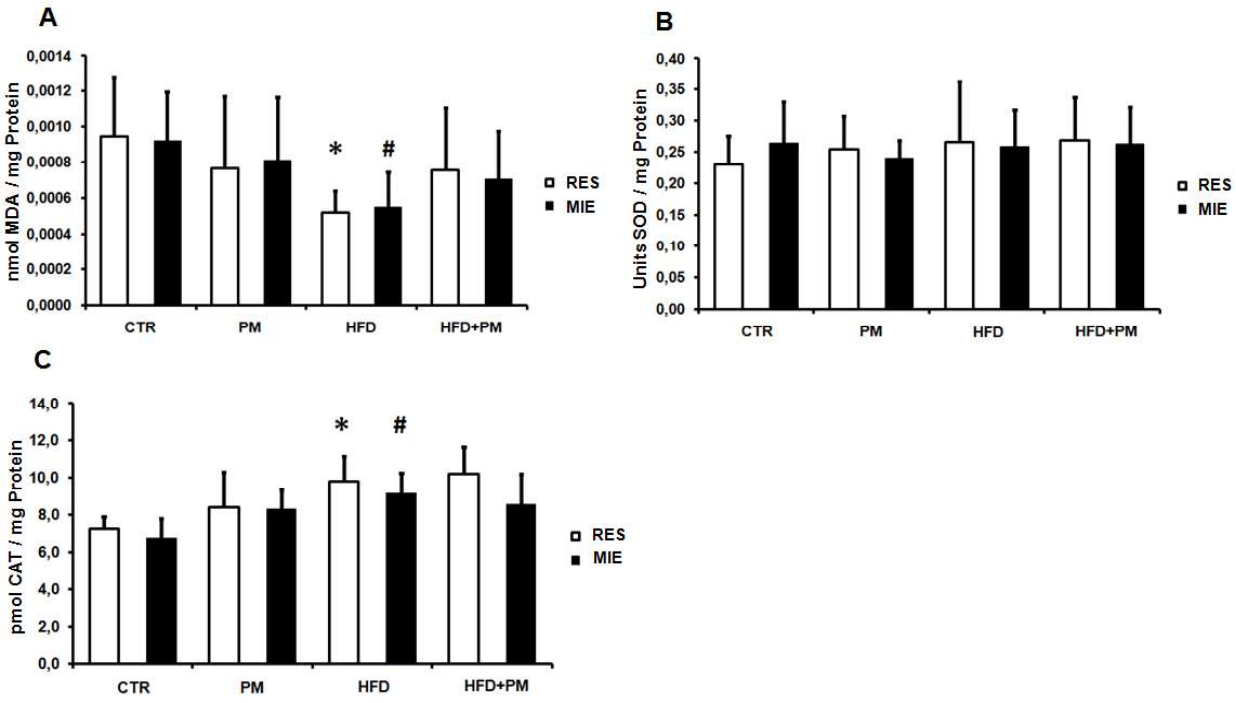

Figure 5

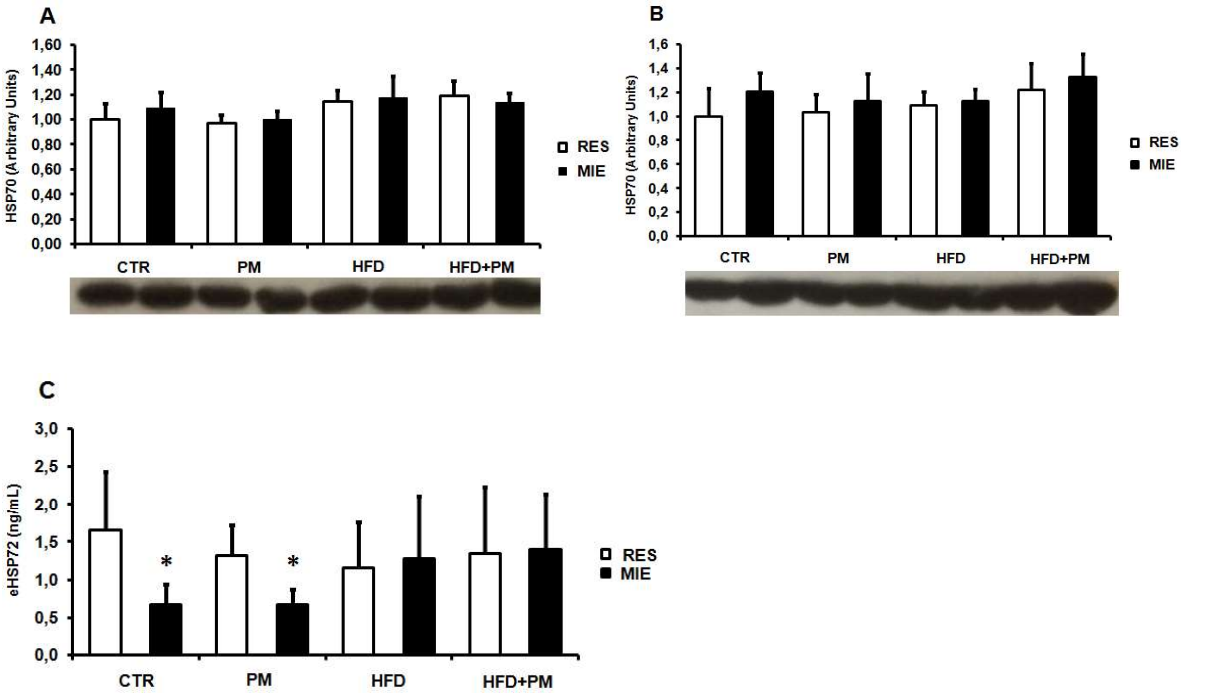

Figure 6

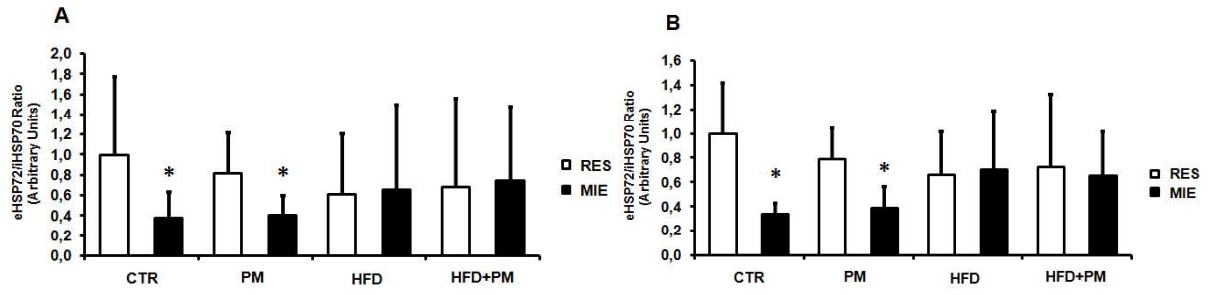

Figure 7

ANEXOS

1. Normas do Periódico European Journal of Applied Physiology

2014 - Fator de Impacto 2.187

Instructions for Authors

Types of Papers

The journal publishes Original Articles, Editorials, Invited Reviews, Letters to the Editor, and Replies. Instructions for submitting Review Proposals, and for the preparation of Invited Reviews can be obtained directly from the Reviews Editors Nigel Taylor (ntaylor@uow.edu.au) and Michael I. Lindinger (mi.lindinger@gmail.com).

Article Requirements

To facilitate rapid publication manuscripts should be prepared carefully in accordance with the following requirements. The Conflict of interest is mandatory for all articles types.

Original articles and Invited Reviews should be composed as follows.

- Title page (numbered as page 1)
- Abstract (except for Editorials, Letters to the Editor and Replies)
- Introduction
- Keywords
- Abbreviations
- Methods (also Technique or Case histories)
- Results
- Discussion (comments and comparison with other published results)
- Conclusions
- Acknowledgements
- Conflict of interest
- References
- Tables
- Legends

● Illustrations

Special Issues

• Contributions that are part of a Special Issue must include the following footnote on the title page: "This article is published as part of the Special Issue on [title of the Special Issue]"

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all coauthors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink “Submit online” on the right and upload all of your manuscript files following the instructions given on the screen.

Title Page

The title page should include:

The name(s) of the author(s)

A concise and informative title

The affiliation(s) and address(es) of the author(s)

The email address, telephone and fax numbers of the corresponding author

Abstract

Please provide a structured abstract of 150 to 250 words which should be divided into the following sections: Purpose (stating the main purposes and research question)

Methods

Results

Conclusions

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Specific remarks on Abstract

The sections should describe briefly and concisely the background and aim/hypothesis of the investigation, the most important methods, the major results and the conclusions drawn. Major results should be presented quantitatively where appropriate, and changes reported must be expected to be statistically significant (e.g. write "endurance time increased from a ± b to c ± d min" and not "endurance time increased (P ‹ 0.01)"). The conclusion should highlight the physiological significance of the study and not be a repetition of the results. The abstract should not contain any undefined abbreviations and references may not be cited.

Text Formatting

Manuscripts should be submitted in Word.

Use a normal, plain font (e.g., 10point Times Roman) for text.

Use italics for emphasis.

Use the automatic page numbering function to number the pages.

Do not use field functions.

Use tab stops or other commands for indents, not the space bar.

Use the table function, not spreadsheets, to make tables.

Use the equation editor or Math Type for equations.

Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should

also not contain any figures or tables. Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lowercase letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols. Always use footnotes instead of endnotes.

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Abbreviations

While authors are encouraged to use abbreviations when appropriate, these should be used sparingly and should conform to the convention of the subject area, e.g. for cardiovascular physiology, thermophysiology and respiratory physiology.

Abbreviations should be defined at first mention in the text and supplied as a list, in alphabetical order, to be included after the keywords , e.g.

Result section

The text in the Results section should be written in a way so that it can be understood by physiologically interested scientists who are not specialists in the particular field of the investigation. Excessive usage of fieldspecific abbreviations should be avoided. The outcome of statistical analysis should be reported in the text only when it was used to test a specified hypothesis. Detailed descriptions of aspects such as subjects involved in the study, including statistical analyses, are better presented in tables. Authors are encouraged to include as figures original records obtained during experiments, and not simply groupmean responses; for instance, representative original records can often be used to illustrate complex differences between groups.

Conclusions section

Authors should provide concise conclusions to their work and are encouraged to put their results into a wider physiological context. The conclusions must not just be a repetition of the results.

REFERENCES

Citation

Cite references in the text by name and year in parentheses. Some examples: Negotiation research spans many disciplines (Thompson 1990).

This result was later contradicted by Becker and Seligman (1996).

This effect has been widely studied (Abbott 1991; Barakat et al. 1995a, b; Kelso and Smith 1998; Medvec et al. 1999, 2000).

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

Reference list entries should be alphabetized by the last names of the first author of each work. Order multi-author publications of the same first author alphabetically with respect to second, third, etc. author. Publications of exactly the same author(s) must be ordered chronologically.

Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731738. doi: 10.1007/s0042100809558

Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

Article by DOI

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. doi:10.1007/s001090000086

Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230257

Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing Physics Web. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

Dissertation

Trent JW (1975) Experimental acute renal failure. Dissertation, University of California.

Always use the standard abbreviation of a journal’s name according to the ISSN List of Title. Word Abbreviations, see If you are unsure, please use the full journal title.

For authors using EndNote, Springer provides an output style that supports the formatting of intext citations and reference list.

TABLES

All tables are to be numbered using Arabic numerals.

Tables should always be cited in text in consecutive numerical order.

For each table, please supply a table caption (title) explaining the components of the table.

Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.

Footnotes to tables should be indicated by superscript lowercase letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Electronic Figure Submission

Supply all figures electronically.

Indicate what graphics program was used to create the artwork.

For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.

Vector graphics containing fonts must have the fonts embedded in the files.

Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Definition: Black and white graphic with no shading.

Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.

All lines should be at least 0.1 mm (0.3 pt) wide.

Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.

Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art

Definition: Photographs, drawings, or paintings with fine shading, etc.

If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.

Halftones should have a minimum resolution of 300 dpi.

Color Art

Color art is free of charge for online publication.

If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent. If the figures will be printed in black and white, do not refer to color in the captions. Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

To add lettering, it is best to use Helvetica or Arial (sans serif fonts).

Keep lettering consistently sized throughout your finalsized artwork, usually about 2–3 mm (8–12 pt). Variance of type size within an illustration should be minimal, e.g., do not use 8pt type on an axis and 20pt type for the axis label.Avoid effects such as shading, outline letters, etc. Do not include titles or captions within your illustrations.

Figure Numbering

All figures are to be numbered using Arabic numerals.

Figures should always be cited in text in consecutive numerical order.

Figure parts should be denoted by lowercase letters (a, b, c, etc.).

If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures, "A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

Figure Captions

Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.

Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.

No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.

Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.

Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

Figures should be submitted separately from the text, if possible.

When preparing your figures, size figures to fit in the column width.

For most journals the figures should be 39 mm, 84 mm, 129 mm, or 174 mm wide and not higher than 234 mm.

For books and book sized journals, the figures should be 80 mm or 122 mm wide and not higher than 198 mm.

Permissions

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that.

All figures have descriptive captions (blind users could then use a textto speech software or a textto Braille hardware)

Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)

Any figure lettering has a contrast ratio of at least 4.5:1

ELECTRONIC SUPPLEMENTARY MATERIAL

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Submission

Supply all supplementary material in standard file formats.

Please include in each file the following information: article title, journal name, author names; affiliation and email address of the corresponding author.

To accommodate user downloads, please keep in mind that larger sized files may require very long download times and that some users may experience other problems during downloading.

Audio, Video, and Animations

Aspect ratio: 16:9 or 4:3

Maximum file size: 25 GB

Minimum video duration: 1 sec

Supported file formats: avi, wmv, mp4, mov, m2p, mp2, mpg, mpeg, flv, mxf, mts, m4v, 3gp

Text and Presentations

Submit your material in PDF format; .doc or .ppt files are not suitable for longterm viability.

A collection of figures may also be combined in a PDF file.

Spreadsheets

Spreadsheets should be converted to PDF if no interaction with the data is intended.

If the readers should be encouraged to make their own calculations, spreadsheets should be submitted as .xls files (MS Excel).

Specialized Formats

Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

It is possible to collect multiple files in a .zip or .gz file.

Numbering

If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.

Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.

Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

Captions

For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

Electronic supplementary material will be published as received from the author without any conversion, editing, or reformatting.

Accessibility

In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

The manuscript contains a descriptive caption for each supplementary material

Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

DOES SPRINGER PROVIDE ENGLISH LANGUAGE SUPPORT?

Manuscripts that are accepted for publication will be checked by our copyeditors for spelling and formal style. This may not be sufficient if English is not your native language and substantial editing would be required. In that case, you may want to have your manuscript edited by a native speaker prior to submission. A clear and concise language will help editors and reviewers concentrate on the scientific content of your paper and thus smooth the peer review process.

The following editing service provides language editing for scientific articles in all areas Springer publishes in:

Edanz English editing for scientists

Use of an editing service is neither a requirement nor a guarantee of acceptance for publication.

Please contact the editing service directly to make arrangements for editing and payment.

ETHICAL RESPONSIBILITIES OF AUTHORS

This journal is committed to upholding the integrity of the scientific record. As a member of the

Committee on Publication Ethics (COPE) the journal will follow the COPE guidelines on how to deal with potential acts of misconduct.

Authors should refrain from misrepresenting research results which could damage the trust in the journal, the professionalism of scientific authorship, and ultimately the entire scientific endeavour. Maintaining integrity of the research and its presentation can be achieved by following the rules of good scientific practice, which include:

The manuscript has not been submitted to more than one journal for simultaneous consideration.

The manuscript has not been published previously (partly or in full), unless the new work concerns an expansion of previous work (please provide transparency on the reuse of material to avoid the hint of textrecycling (“selfplagiarism”)).

A single study is not split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (e.g. “salamipublishing”).

No data have been fabricated or manipulated (including images) to support your conclusions

No data, text, or theories by others are presented as if they were the author’s own (“plagiarism”). Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks are used for verbatim copying of material, and permissions are secured for material that is copyrighted.

Important note: the journal may use software to screen for plagiarism.

Consent to submit has been received explicitly from all coauthors, as well as from the responsible authorities tacitly or explicitly at the institute/organization where the work has been carried out, before the work is submitted.

Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

In addition:

Changes of authorship or in the order of authors are not accepted after acceptance of a manuscript.

Requesting to add or delete authors at revision stage, proof stage, or after publication is a serious matter and may be considered when justifiably warranted.

Justification for changes in authorship must be compelling and may be considered only after receipt of written approval from all authors and a convincing, detailed explanation about the role/deletion of the new/deleted author. In case of changes at revision stage, a letter must accompany the revised manuscript. In case of changes after acceptance or publication, the request and documentation must be sent via the Publisher to the EditorinChief.

In all cases, further documentation may be required to support your request. The decision on accepting the change rests with the EditorinChief of the journal and may be turned down. Therefore authors are strongly advised to ensure the correct author group, corresponding author, and order of authors at submission.

Upon request authors should be prepared to send relevant documentation or data in order to verify the validity of the results. This could be in the form of raw data, samples, records, etc.

If there is a suspicion of misconduct, the journal will carry out an investigation following the COPE guidelines. If, after investigation, the allegation seems to raise valid concerns, the accused author will be contacted and given an opportunity to address the issue. If misconduct has been established beyond reasonable doubt, this may result in the EditorinChief's implementation of the following measures, including, but not limited to:

If the article is still under consideration, it may be rejected and returned to the author.

If the article has already been published online, depending on the nature and severity of the infraction, either an erratum will be placed with the article or in severe cases complete retraction of the article will occur. The reason must be given in the published erratum or retraction note.

The author's institution may be informed.

COMPLIANCE WITH ETHICAL STANDARDS

To ensure objectivity and transparency in research and to ensure that accepted principles of ethical and professional conduct have been followed, authors should include information regarding sources of funding, potential conflicts of interest (financial or nonfinancial), informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals.

Authors should include the following statements (if applicable) in a separate section entitled "Compliance with Ethical Standards" when submitting a paper:

Disclosure of potential conflicts of interest

Research involving Human Participants and/or Animals

Informed consent

Please note that standards could vary slightly per journal dependent on their peer review policies (i.e. single or double blind peer review) as well as per journal subject discipline.

Before submitting your article check the instructions following this section carefully.

The corresponding author should be prepared to collect documentation of compliance with ethical standards and send if requested during peer review or after publication.

The Editors reserve the right to reject manuscripts that do not comply with the abovementioned guidelines. The author will be held responsible for false statements or failure to fulfill the abovementioned guidelines.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

Authors must disclose all relationships or interests that could have direct or potential influence or impart bias on the work. Although an author may not feel there is any conflict, disclosure of relationships and interests provides a more complete and transparent process,

leading to an accurate and objective assessment of the work. Awareness of a real or perceived conflicts of interest is a perspective to which the readers are entitled. This is not meant to imply that a financial relationship with an organization that sponsored the research or compensation received for consultancy work is inappropriate. Examples of potential conflicts of interests **that are directly or indirectly related to the research** may include but are not limited to the following:

Research grants from funding agencies (please give the research funder and the grant number)

Honoraria for speaking at symposia

Financial support for attending symposia

Financial support for educational programs

Employment or consultation

Support from a project sponsor

Position on advisory board or board of directors or other type of management relationships

Multiple affiliations

Financial relationships, for example equity ownership or investment interest

Intellectual property rights (e.g. patents, copyrights and royalties from such rights)

Holdings of spouse and/or children that may have financial interest in the work

In addition, interests that go beyond financial interests and compensation (nonfinancial interests) that may be important to readers should be disclosed. These may include but are not limited to personal relationships or competing interests directly or indirectly tied to this research, or professional interests or personal beliefs that may influence your research.

The corresponding author collects the conflict of interest disclosure forms from all authors. In author collaborations where formal agreements for representation allow it, it is sufficient for the corresponding author to sign the disclosure form on behalf of all authors. Examples of forms can be found here:

The corresponding author will include a summary statement in the text of the manuscript in a separate section before the reference list, that reflects what is recorded in the potential conflict of interest disclosure form(s).

See below examples of disclosures:

Funding: This study was funded by X (grant number X).

Conflict of Interest: Author A has received research grants from Company A. Author B has received a speaker honorarium from Company X and owns stock in Company Y. Author C is a member of committee Z.

If no conflict exists, the authors should state:

Conflict of Interest: The authors declare that they have no conflict of interest.

RESEARCH INVOLVING HUMAN PARTICIPANTS AND/OR ANIMALS

1) Statement of human rights

When reporting studies that involve human participants, authors should include a statement that the studies have been approved by the appropriate institutional and/or national research ethics committee and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

If doubt exists whether the research was conducted in accordance with the 1964 Helsinki

Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that the independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study.

The following statements should be included in the text before the References section:

Ethical approval: "All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

For retrospective studies, please add the following sentence:

"For this type of study formal consent is not required."

2) Statement on the welfare of animals

The welfare of animals used for research must be respected. When reporting experiments on animals, authors should indicate whether the international, national, and/or institutional guidelines for the care and use of animals have been followed, and that the studies have been approved by a research ethics committee at the institution or practice at which the studies were conducted (where such a committee exists).

For studies with animals, the following statement should be included in the text before the

References section:

Ethical approval: "All applicable international, national, and/or institutional guidelines for the care and use of animals were followed."

If applicable (where such a committee exists): "All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted."

If articles do not contain studies with human participants or animals by any of the authors, please select one of the following statements:

"This article does not contain any studies with human participants performed by any of the authors."

"This article does not contain any studies with animals performed by any of the authors."

"This article does not contain any studies with human participants or animals performed by any of the authors."

INFORMED CONSENT

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. Hence it is important that all participants gave their informed consent in writing prior to inclusion in the study. Identifying details (names, dates of birth, identity numbers and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scientific purposes and the participant (or parent or guardian if the participant is incapable) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases, and informed consent should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort scientific meaning.

The following statement should be included:

Informed consent: "Informed consent was obtained from all individual participants included in the study."

If identifying information about participants is available in the article, the following statement should be included:

"Additional informed consent was obtained from all individual participants for whom identifying information is included in this article."

AFTER ACCEPTANCE

Upon acceptance of your article you will receive a link to the special Author Query Application at Springer's web page where you can sign the Copyright Transfer Statement

online and indicate whether you wish to order Open Choice, offprints, or printing of figures in color.

Once the Author Query Application has been completed, your article will be processed and you will receive the proofs.

Open Choice

In addition to the normal publication process (whereby an article is submitted to the journal and access to that article is granted to customers who have purchased a subscription),

Springer provides an alternative publishing option: Springer Open Choice. A Springer Open Choice article receives all the benefits of a regular subscription based article, but in addition is made available publicly through Creative Commons Attribution Non Commercial 4.0 International License.

Copyright transfer

Authors will be asked to transfer copyright of the article to the Publisher (or grant the Publisher exclusive publication and dissemination rights). This will ensure the widest possible protection and dissemination of information under copyright laws.

Open Choice articles do not require transfer of copyright as the copyright remains with the author. In opting for open access, the author(s) agree to publish the article under the Creative

Commons Attribution License.

Creative Commons Attribution Non Commercial 4.0 International License

Offprints

Offprints can be ordered by the corresponding author.

Color illustrations

Online publication of color illustrations is free of charge. For color in the print version, authors will be expected to make a contribution towards the extra costs.

Proof reading

The purpose of the proof is to check for typesetting or conversion errors and the completeness and accuracy of the text, tables and figures. Substantial changes in content, e.g., new results, corrected values, title and authorship, are not allowed without the approval of the Editor.

After online publication, further changes can only be made in the form of an Erratum, which will be hyperlinked to the article.

Online First

The article will be published online after receipt of the corrected proofs. This is the official first publication citable with the DOI. After release of the printed version, the paper can also be cited by issue and page numbers.

2. Parecer da Comissão de Ética no Uso de Animais (CEUA)

Comissão de Ética no Uso de Animais da UNIJUÍ
CEUA/UNIJUÍ

PARECER CONSUBSTANCIADO Nº. 011/2013

Protocolo de Pesquisa nº. 009/2013 de 01/10/2013.

Projeto: "HSP70 COMO BIOMARCADOR DO "PONTO CRÍTICO" DA REALIZAÇÃO DE EXERCÍCIO FÍSICO EXPOSTO À POLUIÇÃO ATMOSFÉRICA NO DIABETES"

Finalidade: Pesquisa.

Duração: Início: 06/01/2014- Término: 30/12/2015.

Pesquisador Responsável: Prof. Thiago Gomes Heck

Equipe: Claudia Ramos Rhoden, , Paulo Ivo Homem de Bittencourt Mirna Stela Ludwig, Bethânia Salamoni, Pauline Brendler Goettems, Fernanda Giesel Baldissera, Maicon Machado Sulzbacher, Analu Bender dos Santos, Eloisa Gabriela de Pelegrin Basso, Renan Daniel Bueno Basso e Iberê Kostrycki Machado.

Área do conhecimento: Ciências da Saúde

Cronograma de utilização de Animais:
Data: 06 de Janeiro de 2014
Espécie: *Mus musculus* - B6129SF2/J (B6)
Sexo: Machos
Quantidade: 96 animais
Período da Manutenção do(s) Animai(s): 24 semanas
Data: 04 de Agosto de 2014
Espécie: *Mus musculus* - B6129SF2/J (B6)
Sexo: Machos
Quantidade: 96 animais
Período da Manutenção do(s) Animai(s): 24 semanas

Avaliação do Protocolo de Pesquisa, segundo orientações da Lei Nº 11.794 de outubro de 2008.

Os pesquisadores apresentaram a Comissão de Ética no Uso de Animais UNIJUÍ, em 14/11/13 o projeto de pesquisa gerado pelo SIE, de acordo com as exigências de documentação necessária para submissão de projetos.

A apresentação da documentação necessária atendeu todas as recomendações do parecer consubstanciado nº 010/2013 da CEUA/UNIJUÍ.

PARECER DO COMITÊ

Assim, mediante a importância social e científica que o projeto apresenta a sua aplicabilidade e conformidade com os requisitos éticos, somos de parecer favorável à realização do projeto classificando-o como **APROVADO**, pois o mesmo atende aos Requisitos Fundamentais das Normas de Conduta para a Utilização de Animais no Ensino, Pesquisa e Extensão da UNIJUI, assim como as responsabilidades do pessoal envolvido no uso de animais da Resolução Normativa Nº1 do CONCEA, de 09 de julho de 2010, descreve no seu artigo 9º Aos pesquisadores, docentes e responsáveis técnicos por atividades experimentais, pedagógicas ou de criação de animais compete:

I – assegurar o cumprimento das normas de criação e uso ético de animais;

II – submeter à CEUA proposta de atividade, especificando os protocolos a serem adotados;

III – apresentar à CEUA, antes do início de qualquer atividade, as informações e a respectiva documentação, na forma e conteúdo definidos nas Resoluções Normativas do CONCEA;

IV – assegurar que as atividades serão iniciadas somente após decisão técnica favorável da CEUA e, quando for o caso, da autorização do CONCEA;

V – solicitar a autorização prévia à CEUA para efetuar qualquer mudança nos protocolos anteriormente aprovados;

VI – assegurar que as equipes técnicas e de apoio envolvidas nas atividades com animais recebam treinamento apropriado e estejam cientes da responsabilidade no trato dos mesmos;

VII – notificar à CEUA as mudanças na equipe técnica;

VIII – comunicar à CEUA, imediatamente, todos os acidentes com animais, relatando as ações saneadoras porventura adotadas;

IX – estabelecer junto à instituição responsável mecanismos para a disponibilidade e a manutenção dos equipamentos e da infra-estrutura de criação e utilização de animais para ensino e pesquisa científica;

X – fornecer à CEUA informações adicionais, quando solicitadas, e atender a eventuais auditorias realizadas.

Solicita-se ao (à) pesquisador (a) o envio a esta CEUA, de relatórios parciais sempre quando houver alguma alteração no projeto, bem como o relatório final.

Ijuí, 20 de novembro de 2013.

Prof. Daniel Curvello de Mendonça Müller
Coordenador da CEUA/UNIJUÍ