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

Bruna Marmett

Efeito da suplementação de picolinato de cromo associada ao exercício aeróbio sobre estresse oxidativo em ratos expostos à poluição.

Porto Alegre
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Dissertação 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

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Porto Alegre
2017

Catalogação na Publicação

Marmett, Bruna
Efeito da suplementação de picolinato de cromo associada ao exercício aeróbio sobre estresse oxidativo em ratos expostos à poluição. / Bruna Marmett. -- 2017.
94 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, 2017.

Orientador(a): Cláudia Ramos Rhoden ; coorientador(a): Ramiro Barcos Nunes.

1. Poluição Atmosférica. 2. Suplmentação Dietética. 3. Exercício Aeróbio. 4. Estresse Oxidativo . I. Título.

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

DEDICATÓRIO

Dedico, com todo meu amor, esta dissertação à minha família, minha mãe, Tânia, minha irmã, Débora e meu pai, Gerson (*in memorian)* por sempre estarem ao meu lado e terem me ensinado tanto. Vocês são meu maior exemplo, eu os admiro muito.

AGRADECIMENTOS

Agradeço à

Minha família pelo seu amor incondicional, carinho, apoio, conselhos e por fazerem de mim o que sou, devo tudo a vocês.

Ao Luís, meu namorado, por sempre estar ao meu lado tornando minha vida mais leve e feliz, sempre me apoiando, confortando e me fazendo sorrir. Aprendo muito com sua personalidade e caráter, você é uma grande inspiração para mim.

À minha orientadora Prof.ª Drª Cláudia Ramos Rhoden e ao meu coorientador Prof. Dr. Ramiro Barcos Nunes pela confiança, incentivo, amizade e excelente orientação. Aprendi e espero aprender muito ainda com vocês, são grandes exemplos profissionais à serem seguidos.

À minha amiga e colega Rose pela companhia nessa jornada que foi o mestrado. Só tenho a te agradecer pelo companheirismo, experiências, aprendizado e pela sua grande amizade.

À Kellen, minha IC, pelo seu comprometimento, responsabilidade e cuidado com os animais, além da companhia nos nossos inúmeros dias em experimentos.

À Camila pelo seu empenho em me auxiliar nas técnicas e por sempre estar disponível para dúvidas e incertezas. Muito obrigada por toda a dedicação e amizade!

À Carol, minha companheira das coletas, sua ajuda foi muito importante para o desenvolvimento do trabalho.

À Elo e à Júlia pelo auxilio na realização das coletas, análises e pelo comprometimento e dedicação de vocês com o trabalho.

Ao laboratório de Poluição Atmosférica e a todos os colegas pela ajuda e por deixarem o laboratório sempre em um clima alegre, divertido, e o transformar em minha segunda casa.

À Secretaria dos Programas de Pós-Graduação e especialmente a Cris por sempre estarem solícitos a ajudar e esclarecer dúvidas, tornando tudo mais fácil.

À Joana e à Inês pela disposição e auxilio no biotério e por todo o cuidado e carinho que têm pelos animais do experimento.

À CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior pela oportunidade e a concessão da bolsa para o desenvolvimento desse mestrado.

RESUMO

A exposição à poluição atmosférica é associada ao desenvolvimento de diversas doenças e à mortalidade precoce. Enquanto a suplementação de picolinato de cromo (CrPic) pode atuar como antioxidante reduzindo os danos a saúde causados pela poluição. O exercício realizado em um ambiente poluído acarreta em aumento da exposição a poluentes, no entanto, sua prática regular é responsável por reduzir estresse oxidativo atenuando os efeitos nocivos causados por esta condição ambiental. No presente estudo foi investigado os efeitos da suplementação de CrPic associado ao exercício aeróbio em ratos expostos a poluição atmosférica sobre medidas de estresse oxidativo. Ratos Wistar machos (n=64) foram instilados com uma suspensão de 50µg de *residual oil fly ash* (ROFA), suplementados com CrPic (1mg/kg/dia) e submetidos a um protocolo de corrida por 50 minutos, 5 dias/semana por 90 dias. A massa corporal foi avaliada semanalmente e o teste de tolerância ao exercício foi realizado antes e após os protocolos, além da avaliação da atividade das enzimas superoxido dismutase (SOD) e catalase (CAT) e a concentração de malondialdeído (MDA) no pulmão, coração e gastrocnêmico. Os grupos treinados demonstraram menor ganho de massa corporal (p<0.05), aumento da distância (p<0.0001), tempo (p<0.0001) e velocidade (p<0.0001) da realização do exercício. No pulmão não houve diferença nas análises da SOD (p=0.2756), CAT (p=0.1198) e MDA (p=0.7189). No coração a atividade das enzimas SOD (p=0.0763), CAT (p=0.4999) e a concentração de MDA (p=0.8656) não foram diferentes. No gastrocnêmico, os grupos treinados apresentam maior atividade da SOD (p<0.0001) e menor concentração de MDA (p=0.0014), enquanto a atividade da CAT (p=0.4487) não apresentou diferença. Concluindo, o ROFA induziu uma adaptação no pulmão e coração prevenindo o dano oxidativo, enquanto o treinamento aeróbio foi responsável pela melhora das defesas antioxidantes reduzindo o estresse oxidativo no gastrocnêmico.

Palavras-chave: Poluição do Ar; Exercício; Tolerância ao Exercício; Cromo; Estresse oxidativo.

ABSTRACT

Air pollution is strongly associated with premature deaths and increased susceptibility to development of diseases. Chromium picolinate (CrPic) supplementation lead to positive effects associated with decrease of oxidative stress, reducing adverse health effects caused by pollution. Exercising in a polluted environmental could increase pollution exposure, however exercise training could protect against oxidative stress induced by pollution, attenuating the harmful effects caused by this environmental condition. So, the aim of this study was investigated the effects of CrPic supplementation associated with aerobic exercise in rats exposed to air pollution in measures of oxidative stress. Therefore, sixty-four Male Wistar rats were instilled with 50μg of residual oil fly ash (ROFA) suspension, supplemented with CrPic (1mg/kg/day) and underwent to a treadmill running protocol for 50 minutes, 5days/week for 90 days. Body mass was evaluated weekly, exercise tolerance test were performed before and after experimental protocols and oxidative stress analyses of SOD and CAT activity and MDA concentration were analyzed in lung, heart and gastrocnemius. As result, trained groups demonstrated lower gain of body mass (p=0.0006), increase distance (p<0.0001), time (p<0.0001) and velocity (p<0.0001) of the running when compared to sedentary groups. Oxidative stress analyses in lungs showed no differences in SOD (p=0.2756), CAT (p=0.1198) and MDA (p=0.7189). In heart activity of SOD (p=0.0763), CAT (p=0.4999) and MDA (p=0.8656*)* concentration showed no difference. In gastrocnemius, trained groups demonstrated an increased SOD (p<0.0001) activity and decrease concentration of MDA (p=0.0014) compared to sedentary groups, although CAT (p=0.4487) activity demonstrate no differences among groups. In conclusion, air pollution exposure in a healthy sample could lead to an adaptation in lungs and heart preventing oxidative damage, while exercise training is responsible for improvement in antioxidant defense decreasing oxidative stress of gastrocnemius.

Keyword: Air pollution; Exercise; Exercise Tolerance; Chromium; Oxidative stress.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS

ATP	Adenosina trifosfato
AVC	Acidente vascular cerebral
CAT	Catalase
CO	Monóxido de carbono
CO_2	Dióxido de carbono
COX	Ciclooxigenase
Cr	Cromo
CrPic	Picolinato de cromo
DM2	Diabetes mellitus tipo 2
ERON	Espécies reativas de oxigênio e nitrogênio
GPx	Glutationa peroxidase
GSH	Glutationa reduzida
GSH/GSSG	Razão de glutationa reduzida/oxidada
H_2O_2	Peróxido de hidrogênio
HPA	Hidrocarbonetos policíclicos aromáticos
IL-6	Interleucina-6
LOX	Lipo-oxigenase
MDA	Malondialdeído
MP	Material particulado
NADPH	Fosfato de dinucleótido de nicotinamida e adenina
NF-Kβ	Fator nuclear-kβ
NO	Oxido nítrico
NO_2	Dióxido de nitrogênio
NOS	Oxido nítrico sintase
$O_2^{\bullet -}$	Ânion radical superóxido
O_3	Ozônio troposférico
OH·-	Radical hidroxil
OMS	Organização Mundial da Saúde
PGC-1α	Receptor ativado por proliferador de peroxissoma coativador-1α
PLA2	Enzima fosfolipase A2
PPAR-γ	Receptor ativado por proliferador de peroxissoma

ROFA	*Residual Oil Fly Ash*
SO_2	Dióxido de enxofre
SOD	Superóxido dismutase
TNF-α	Fator de necrose tumoral α
$VO_{2máx}$	Consumo máximo de oxigênio
XO	Xantina oxidase

SUMÁRIO

1. INTRODUÇÃO

1.1. POLUIÇÃO ATMOSFÉRICA

A poluição atmosférica é uma condição ambiental definida como a contaminação do ar por qualquer agente químico, físico ou biológico que seja capaz de modificar as características naturais da atmosfera (WHO, 2006). A poluição do ar representa no cenário mundial um risco para a saúde da população, uma vez que está associada à aproximadamente 3,3 milhões de mortes precoces por ano em todo o mundo. Mais alarmantes são as projeções realizadas com base no atual cenário de emissões de poluentes, as quais indicam que a contribuição da poluição para mortalidade irá duplicar até 2050 (Lelieveld *et al.*, 2015).

A Organização Mundial da Saúde (OMS) apresenta valores de referencia para o limite máximo de emissão de poluentes para que a qualidade do ar seja mantida. No entanto, estes valores não são respeitados, já está confirmado que 92% da população está exposta diariamente a valores muito acima daqueles preconizados pela OMS para a média anual de material particulado fino (MP2,5) (WHO, 2006). Estudos comprovam que intervenções governamentais visando à redução da poluição atmosférica apresentaram benefícios diretos na qualidade do ar e na saúde, reduzindo a mortalidade decorrente da poluição, bem como risco de mortalidade por doenças cardiovasculares e morbidade cardiorrespiratória (Henschel *et al.*, 2012; Roberts, 2013).

O aumento da emissão de poluentes ocorreu juntamente com o crescimento econômico e o desenvolvimento industrial (Miraglia e Gouveia, 2014). Estudos epidemiológicos mostraram que durante a crise econômica e o período de recessão na Europa ocorreu a redução da emissão de todos os poluentes primários, aproximadamente 35% de redução de dióxido de nitrogênio (NO_2), 25% de dióxido de carbono (CO_2) e 48% de dióxido de enxofre (SO_2) (Chanel *et al.*, 2016; Malico *et al.*, 2016). No Brasil, mais precisamente em regiões metropolitanas, estima-se uma mortalidade de 20.500 óbitos anuais decorrentes dos níveis de poluição atmosférica, o que causa um significativo impacto econômico apresentando grande magnitude em gastos na saúde publica, sendo a estimativa de US$ 1,7 bilhões por ano (Miraglia e Gouveia, 2014).

A exposição aos poluentes que compõe a poluição do ar impacta de forma direta a saúde, aumentando a incidência de diversas doenças e causando o agravamento do quadro de condições clínicas já instauradas (Burnett *et al.*, 2014). A inalação dos poluentes afeta diversos órgãos e sistemas, o sistema respiratório é alvo desta exposição, tanto de forma aguda quanto de forma crônica, causando sintomas como irritação de vias aéreas, inflamação e redução da função pulmonar, que a longo prazo resulta em asma, enfisema e câncer de pulmão (Kuo *et al.*, 2006; Kampa e Castanas, 2008). O sistema cardiovascular é igualmente afetado, ocorrendo inflamação sistêmica, efeitos sobre coagulação sanguínea e disponibilidade de oxigênio, obstrução de veias, angina, e até mesmo culminando em doenças cardíacas isquêmicas e infarto (Burnett *et al.*, 2014; Pope *et al.*, 2016). A poluição também contribui para o inicio e progressão de hipertensão, aterosclerose, diabetes mellitus tipo 2, obesidade, síndrome metabólica e infertilidade (Kampa e Castanas, 2008; Checa Vizcaíno *et al.*, 2016; Pope *et al.*, 2016; Wolf *et al.*, 2016).

1.1.1. Composição e Fontes da Poluição Atmosférica

A poluição é constituída por diversos compostos, entre eles, pode-se citar o material particulado (MP), dióxido de nitrogênio (NO_2), dióxido de enxofre (SO_2), hidrocarbonetos policíclicos aromáticos (HPA), monóxido de carbono (CO) e o ozônio troposférico (O_3). Aqueles poluentes emitidos diretamente das fontes são os chamados poluentes primários. Liberados na atmosfera, os poluentes primários podem interagir entre si ou sofrer fotólise resultando na formação dos poluentes secundários (WHO, 2006).

Esses compostos são emitidos por diferentes fontes, dentre elas as fontes naturais e antropogênicas. As fontes naturais são, principalmente, os eventos naturais do ambiente, como a atividade vulcânica, erosões e desastres naturais, enquanto as fontes antropogênicas são as resultantes de atividades humanas. As fontes dos poluentes ainda podem ser classificadas de acordo com a sua dispersão, em fontes fixas e móveis. As fontes emissoras fixas correspondem principalmente a indústrias, devido aos processos de geração de energia, fabricação e transformação dos produtos. As fontes móveis correspondem principalmente aos veículos

automotores, os quais emitem poluentes oriundos da queima de combustível para a geração de energia. A contribuição das fontes para a emissão de poluentes varia de acordo com a região. Nas capitais brasileiras, a principal fonte de emissão até a década de 80 eram as indústrias. Atualmente, as indústrias migraram para o interior e a principal fonte emissora passou a ser os veículos automotores (Toledo e Nardocci, 2011).

A poluição do ar ainda pode ser caracterizada por poluição *outdoor* e *indoor.* A poluição *outdoor* corresponde a contaminação do ar presente em ambientes abertos. Sendo esta, derivada, principalmente, da queima de combustíveis fosseis pelos veículos automotores e pelas indústrias. A poluição *indoor* representa a concentração de poluentes nos ambientes fechados, como em residências, escritório, escolas, instalações de saúde, edifícios públicos e privados. As principais fontes de emissão de poluentes *indoor* são equipamentos presentes nos ambientes internos e as atividades cotidianas realizadas nestes ambientes, como a combustão de gás para aquecer ou cozinhar, sendo que estes poluentes quando emitidos permanecem mais concentrados por estarem presentes em ambientes fechados (WHO, 2006; 2010b).

A poluição *outdoor* e *indoor* apresentam concentração diferente de contaminantes em sua composição e acredita-se que a poluição *outdoor* seja mais prejudicial à saúde. No entanto a poluição *indoor* pode ser tão nociva quanto, pois está presente nos ambientes onde as pessoas passam a maior parte das suas vidas, sendo determinante para a saúde e bem estar da população. Estima-se que, em 2012, 11,6% da mortalidade mundial ocorreu decorrente da poluição *outdoor* e *indoor* associadas (WHO, 2010b; Challoner *et al.*, 2015).

1.1.2. Poluição Atmosférica X Estresse oxidativo

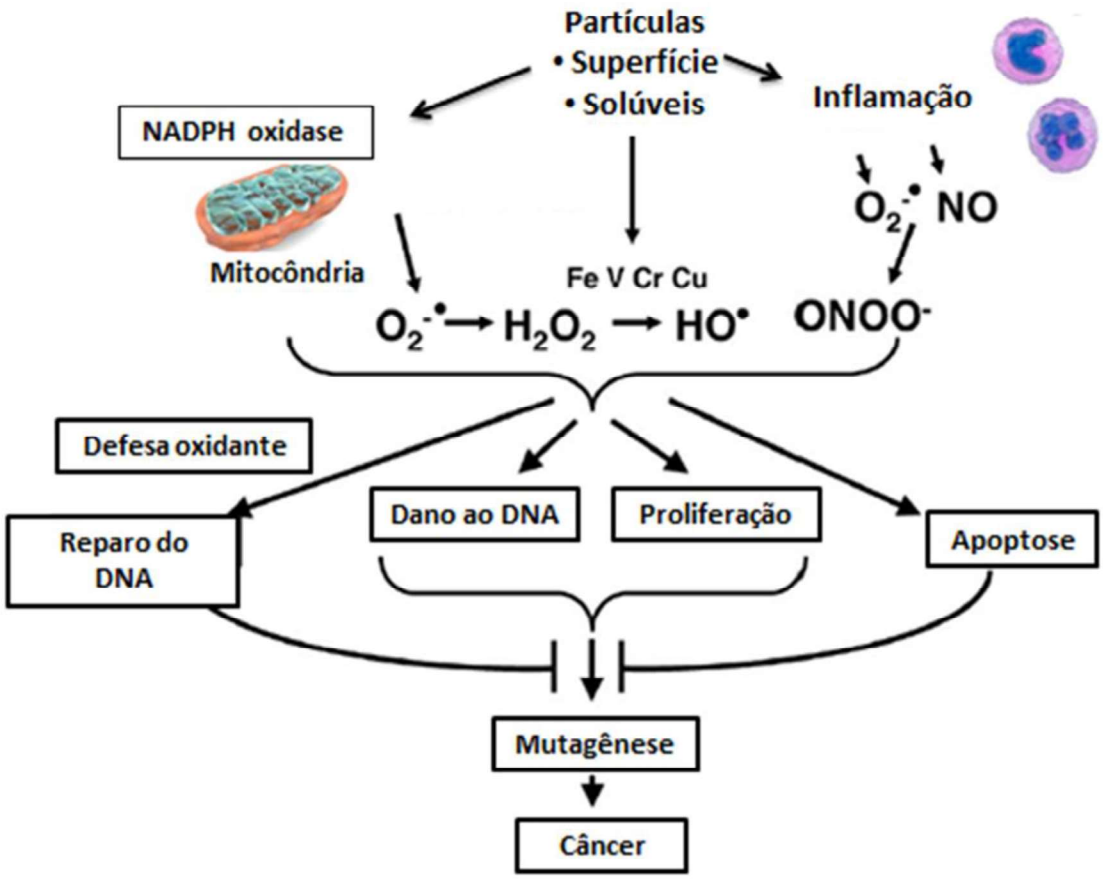
O estresse oxidativo é considerado um potencial fator responsável por desencadear os efeitos nocivos causados pela poluição atmosférica. Os poluentes que compõe a poluição do ar são capazes de aumentar a formação das espécies reativas de oxigênio e nitrogênio (ERON) ocorrendo uma sobreposição destas às defesas antioxidantes do organismo, caracterizando o estresse oxidativo. Uma vez instaurado, inicia-se uma série de alterações como oxidação de proteínas,

peroxidação lipídica, alterações em sinalização celular, dano ao DNA e pode ser desencadeada uma resposta inflamatória e produção de citocinas pró-inflamatórias (González-Flecha, 2004; Risom *et al.*, 2005; Pope *et al.*, 2016).

O estresse oxidativo mediado pelos poluentes pode surgir de diferentes fontes de formação de espécies reativas de oxigênio (ERO), podendo envolver: a geração direta de ERO a partir das partículas; os compostos solúveis como metais de transição e compostos orgânicos; alterações da função mitocondrial e da enzima NADPH-oxidase; e ativação de células inflamatórias capazes de formar ERO. Na superfície das partículas de poluentes encontram-se metais de transição, como ferro, cobre e vanádio, os quais podem gerar ERO através da reação de Fenton ou agir como catalisadores da reação de Harber–Weiss, ambas reações que realizam a redução de peróxido de hidrogênio (H_2O_2) em radical hidroxil ($OH^{\bullet}$) através de metais de transição (Fraunberger *et al.*, 2016; Pope *et al.*, 2016).

Além dos mecanismos envolvidos com a formação de radicais livres, os metais presentes na poluição são capazes de gerar outros danos, como a substituição de íons utilizados em reações fisiológicas por metais inalados, interferir em canais iônicos e levar a inibição enzimática. Ainda, pode ocorrer acúmulo de metais nas organelas celulares prejudicando sua função e ligar-se ao DNA afetando a expressão gênica. Neste caso, quando ocorrem alterações no DNA, as quais não são reparadas, essas células proliferam-se e pode-se resultar em carcinogênese. Os possíveis mecanismos de indução do estresse oxidativo e dano em DNA pela exposição às partículas presentes na poluição atmosférica e seu papel na carcinogênese estão ilustrados na Figura 1 (Risom *et al.*, 2005; Kampa e Castanas, 2008; Mehta *et al.*, 2008).

Figura 1. Mecanismos de indução do estresse oxidativo e dano em DNA pela exposição às partículas presentes na poluição atmosférica e seu papel na carcinogênese.



Fonte: Adaptada de Risom *et al*., 2005.

Quando inaladas, as partículas que compõe a poluição, como o MP2,5, pode ocasionar a liberação de mediadores pró-inflamatórios pelos macrófagos alveolares e células epiteliais, portanto a exposição a esta partícula libera citocinas pró-inflamatórias como a interleucina-6 (IL-6) e o fator de necrose tumoral α (TNF-α). Possivelmente essa liberação nos pulmões pode originar e sustentar uma condição de inflamação sistêmica. Todos estes fatores são colaboradores cruciais para o desenvolvimento e agravamento de doenças respiratórias e cardiovasculares, mostrando uma correlação positiva entre a concentração dos poluentes e a ocorrência destas doenças. As altas concentrações dos metais presentes nos poluentes são capazes de induzir estresse oxidativo, aumentando os níveis de peroxidação lipídica e a razão de glutationa reduzida/oxidada (GSH/GSSG). Todos

estes fatores contribuem para os mecanismos patológicos desencadeados pela exposição à poluição, aumentando a susceptibilidade da população em desenvolver doenças crônicas (Risom *et al.*, 2005; Kampa e Castanas, 2008).

1.1.3. Poluição Atmosférica e suplementação antioxidante

A utilização de suplementos antioxidantes associada com a exposição à poluição do ar tem ganhado notoriedade por reduzir os efeitos adversos à saúde causados pela poluição, tendo em vista que a exposição à poluição do ar acarreta o aumento da formação de ERO, as quais geram uma cascata de efeitos nocivos e estão associadas com o desencadeamento da resposta inflamatória. Neste contexto, as defesas antioxidantes estão reduzidas e a suplementação antioxidante pode trazer resultados benéficos, causando um aumento das defesas antioxidantes, atenuando a ação das substancias oxidativas, e consequentemente o dano causado por elas (Romieu *et al.*, 2002; Sienra-Monge *et al.*, 2004).

A primeira linha de defesa antioxidante aos poluentes atua ainda no pulmão, os fluidos que revestem o trato respiratório são compostos por antioxidantes semelhantes aos encontrados na corrente sanguínea, incluindo glutationa reduzida, acido ascórbico, acido úrico e α-tocoferol. Estão presentes também os antioxidantes enzimáticos como a superóxido dismutase (SOD), glutationa peroxidase (GPx) e catalase (CAT). Uma exposição a baixas concentrações de poluição leva a formação de ERO, ativando a resposta antioxidante, seguida da transcrição de enzimas responsáveis pela detoxificação, citoproteção e resposta antioxidante. Enquanto a exposição a elevadas concentrações de poluentes acarretará na expressão do fator nuclear-kβ (NF-kβ), alterações na função mitocondrial e da enzima NADPH, bem como aumento da expressão de citocinas pró-inflamatórias. Os antioxidantes são compostos que neutralizam as ERO através da redução da sua reatividade, portanto a suscetibilidade a danos oxidativos depende amplamente da habilidade dos sistemas antioxidantes de neutralizar as ERO e da velocidade de recuperação da defesa antioxidante (Kelly, 2004; Romieu *et al.*, 2008).

Muitos antioxidantes são provenientes da dieta, tanto antioxidantes não enzimáticos quanto substratos e cofatores de antioxidantes enzimáticos, sendo assim os fatores dietéticos devem ser considerados e a suplementação de

antioxidantes pode modular o impacto causado pela poluição do ar. Estudo realizado com crianças asmáticas que receberam suplementação antioxidante de vitamina C e E mostrou que aquelas que receberam placebo tiveram uma maior expressão de citocinas pró-inflamatórias (IL-6 e IL-8) em resposta a exposição ao ozônio quando comparadas com as que receberam suplementação antioxidante (Sienra-Monge *et al.*, 2004). Já estudo experimental de Husari e colaboradores (2016) demonstrou que com suplementação antioxidante a expressão de mediadores inflamatórios, apoptose e estresse oxidativo foram atenuados (Husari *et al.*, 2016).

No entanto, a suplementação antioxidante quando utilizada em indivíduos saudáveis não demonstrou os mesmos efeitos, onde não foi observada alteração em marcadores inflamatórios nos indivíduos que receberam a suplementação quando comparados com o grupo placebo (Samet *et al.*, 2001; Mudway *et al.*, 2006). Assim, os suplementos com propriedades antioxidantes e anti-inflamatórias têm o potencial de proteger os efeitos adversos causados pela poluição na saúde nos indivíduos que estão mais suscetíveis, como aqueles que têm uma doença respiratória ou cardíaca pré-existente (Tong, 2016). Portanto, o estado nutricional, a presença de doença crônica e fatores genéticos podem ser determinantes para a suscetibilidade ao estresse oxidativo, o qual é causado pela poluição (Romieu *et al.*, 2008; Tong, 2016).

1.1.4. Poluição Atmosférica e exercício físico

Poluição do ar e exercício físico são tópicos controversos, sendo que o primeiro ocasiona malefícios à saúde, enquanto o outro é responsável por uma séria de adaptações benéficas no organismo. Tendo em vista que grande parte dos exercícios físicos é realizada em ambientes abertos, há uma maior exposição á poluição do ar, emergindo o questionamento de que a realização do exercício físico em um ambiente poluído poderia ser prejudicial a saúde, ao invés de causar efeitos protetores. Portanto, o impacto da realização de exercício em ambientes poluídos vem sendo estudada com o objetivo de elucidar este tema (Lovinsky-Desir *et al.*, 2016; Matt *et al.*, 2016; Wang, 2016).

Durante a realização do exercício físico ocorre o aumento da frequência respiratória e broncodilatação, ambas as adaptações ultrapassam o período da

realização do exercício, com isso o volume de ar inalado é maior do que em repouso. Estudo realizado por Int Panis e colaboradores (2010) comparou a frequência ventilatória de indivíduos praticando ciclismo e outros dirigindo carro, mostrando que durante o ciclismo as quantidades referentes à inalação de poluentes são de 400% a 900% maiores do que durante a condução do carro. Ainda, se no ar há elevadas concentrações de poluentes, estes serão inaladas e irão depositar-se no trato respiratório, podendo reduzir o consumo máximo de oxigênio ($VO_{2máx}$) devido a redução da transferência de oxigênio para o sangue e resultar na redução do desempenho no exercício. Assim, os indivíduos que realizam exercícios em áreas urbanas estariam mais propensos aos riscos gerados pela poluição do que aqueles sedentários na mesma região ou aqueles indivíduos que realizam exercícios em ambientes fechados ou rurais (Löndahl *et al.*, 2007; Int Panis *et al.*, 2010; Scichilone *et al.*, 2010; Matt *et al.*, 2016).

Se assumirmos que os efeitos adversos à saúde são proporcionais a quantidade de ar poluído inalado, espera-se que seja mais nociva para os indivíduos que se exercitam. No entanto, avaliando a razão risco/benefício da prática do exercício em ambientes poluídos, o consenso geral é de que qualquer efeito nocivo proveniente da inalação do ar poluído é atenuado pelos efeitos positivos do exercício (Hartog *et al.*, 2011). Tendo em vista que o exercício regular é capaz de melhorar mecanismos fisiológicos e desfechos de doenças que a exposição à poluição pode exacerbar (Giles e Koehle, 2014).

A prática regular de exercício é capaz de reduzir pressão sanguínea, inflamação sistêmica, coagulação sanguínea, além de melhorar a função endotelial, todos estes fatores atuando em conjunto podem causar a redução do risco de doenças cardiovasculares (Desouza *et al.*, 2000; Warburton *et al.*, 2006; Giles e Koehle, 2014). Estudos experimentais mostraram que, após protocolo de exercício aeróbio, ocorreu a redução de inflamação pulmonar, da resposta pró-inflamatória e do estresse oxidativo em ratos expostos à poluição. Contribuindo com estes resultados, o estudo de Matt e colaboradores (2016) concluiu que o exercício físico reduz o impacto imediato das altas concentrações de MP sobre as vias aéreas (Matt *et al.*, 2016).

Em São Paulo, Carneiro e colaboradores (2011) utilizaram diferentes biomarcadores para avaliar a qualidade do ar em uma região próxima a uma rodovia

com intenso tráfego veicular e em locais próximos, porém afastando-se da rodovia, e concluiu que todos os marcadores utilizados apresentaram o mesmo padrão, conforme aumentava a distância do tráfego melhorava a qualidade do ar. A exposição a poluentes advindos do tráfego automotivo pode contrapor os efeitos protetores do exercício sobre inflamação das vias aéreas, e consequentemente outras complicações associadas à inalação de poluentes (Carneiro *et al.*, 2011; Lovinsky-Desir *et al.*, 2016).

A intensidade, o tipo e a frequência do exercício, assim como o local onde será realizado, são fatores que podem interferir na exposição à poluição e devem ser levados em conta no momento de avaliação dos riscos e benefícios envolvidos com esta prática. Outro aspecto que deve ser considerado é a elaboração de estratégias para reduzir possíveis danos que a poluição possa causar durante o exercício, como planejar os treinamentos em locais o mais distante possível do tráfego, e horários onde a concentração de poluentes esteja menor, como por exemplo, no período da manhã, minimizando a exposição ao ozônio que é mais expressiva no final da tarde (Giles e Koehle, 2014).

1.2. *RESIDUAL OIL FLY ASH* (ROFA)

Residual Oil Fly Ash (ROFA) é a denominação dada ao resíduo inorgânico que permanece após a oxidação de derivados de carbono, ou seja, é resultado da combustão incompleta destes materiais. O ROFA é um dos componentes da poluição urbana presentes no MP, e em sua composição estão metais de transição como o ferro, níquel e vanádio, sendo o poluente mais utilizado para avaliar a contribuição destes metais na toxicidade da poluição do ar. As partículas de ROFA estão presentes em um tamanho aerodinâmico de ≤2.5 μm, o qual quando inalado é capaz de alcançar a corrente sanguínea, estando fortemente associado aos efeitos nocivos da poluição. Inclusive, o ROFA é associado a alterações cardíacas agudas e aumento da mortalidade (Chen e Lippmann, 2009; Brook *et al.*, 2010; Magnani *et al.*, 2013).

Dentre os mecanismos responsáveis pelos efeitos nocivos advindos da inalação de ROFA, pode-se citar que este poluente desencadeia a ativação de células fagocíticas, as quais produzem ânion radical superóxido ($O_2^{\bullet -}$), através da ativação do complexo da família da enzima NADPH oxidase, uma importante fonte

não mitocondrial de ERO. A produção de $O_2^{\bullet-}$ desencadeia o recrutamento de leucócitos e ativação de macrófagos culminando em um quadro inflamatório. O $O_2^{\bullet-}$ ainda pode ser dismutado em H_2O_2 pela enzima SOD, sendo capaz de difundir-se através da membrana e regular vias de sinalização intracelular. O que foi demonstrado em estudo experimental de Magnani e colaboradores (2013) onde a exposição aguda ao ROFA gerou a ativação da NADPH oxidase no pulmão de camundongos (Magnani *et al.*, 2013). Ainda, no estudo de Orana e colaboradores (2016) foi observado aumento da produção de $O_2^{\bullet-}$ no pulmão após a exposição ao ROFA e um consequente aumento da expressão da atividade da enzima antioxidante SOD, mostrando uma resposta adaptativa (Orona *et al.*, 2016).

A exposição ao ROFA também causa impacto na função mitocondrial, levando ao aumento da taxa de transporte de elétrons na cadeia respiratória, despolarização da membrana mitocondrial, redução da produção de ATP e consequente deficiência da fosforilação oxidativa. Sendo que estes efeitos são desencadeados visando prevenir a formação de $O_2^{\bullet-}$, o qual poderia causar danos na organela. Entre os metais que compõe o ROFA, pode-se destacar o ferro, este, quando em forma de íon ferroso, é capaz de catalisar a reação de Fenton, a qual é responsável pela produção de ERO (Pattanaik *et al.*, 2016).

Ao avaliar exposição aguda ao ROFA, estudos experimentais observaram alterações como o aumento do fator de necrose tumoral α (TNF-α) e IL-6 após 5 horas da instilação de doses de ROFA (Magnani *et al.*, 2013). Além disso, é capaz de causar aumento na pressão pulmonar, pressão resistiva, pressão viscoelástica, elastância dinâmica e elastância estática, assim como estimular citocinas pró-inflamatórias no pulmão, desencadeando inflamação sistêmica apenas 24h após a exposição permanecendo por 4 dias (Carvalho *et al.*, 2014).

Portanto, a inalação do ROFA causa prejuízo nos mecanismos pulmonares, colapso alveolar, influxo de células inflamatórias no pulmão e na corrente sanguínea (Carvalho *et al.*, 2014). Com isso, órgãos como o pulmão e o coração têm seu metabolismo oxidativo prejudicado como uma resposta a exposição aguda ao ROFA, iniciando um dano tecidual local, que leva ao estresse oxidativo sistêmico e a inflamação (Magnani *et al.*, 2013).

1.3. PICOLINATO DE CROMO

O Cromo (Cr) é um elemento traço amplamente utilizado para o controle da glicose e aumento da sensibilidade à insulina (Anderson, 2003; Wang e Cefalu, 2010; Peng e Yang, 2015). Este mineral é encontrado em duas formas de valências, o cromo hexavalente e o cromo trivalente, sendo que a primeira representa sua forma tóxica e a segunda a orgânica. Os suplementos que apresentam Cr em sua composição normalmente utilizam a associação do cromo trivalente com algum ligante. Dentre os ligantes mais utilizados pode-se destacar o acido picolínico, formando assim o picolinato de cromo (CrPic) (Vincent, 2000; Anderson, 2003).

O CrPic é amplamente utilizado com o objetivo de atenuar alterações no metabolismo de carboidrato, como a resistência a insulina e diabetes mellitus tipo 2 (DM2) (Press *et al.*, 1990; Wang e Cefalu, 2010; Suksomboon *et al.*, 2014). As doses utilizadas apresentam grande variação, sendo de 25 µg/kg a 1000 µg/kg, e estima-se que 0,4 a 2,5% do total ingerido de Cr seja absorvido. No entanto, a absorção do Cr em forma de CrPic é maior do que outros compostos, sendo a taxa de absorção de 0,7 até 5,2% (Press *et al.*, 1990; Suksomboon *et al.*, 2014).

A ação mais conhecida do Cr envolve a modulação da glicose, a qual ocorre através do influxo de Cr na célula, ativando a apocromoulina em cromodulina, desencadeando mecanismo que culmina no aumento da captação de glicose e sensibilidade a insulina. Outra ação da suplementação de CrPic, demonstrada por Abebe e colaboradores (2010), é a melhora na função endotelial e aumento da vasodilatação, atribuídas ao aumento do relaxamento da aorta pelo oxido nítrico (NO) endotelial, que resultou na melhora da circulação, e na recuperação após insulto de isquemia/reperfusão em animais hipertensos. Além destes, o CrPic pode apresentar uma série de efeitos positivos, como a melhora dos níveis de colesterol e doenças cardiovasculares, propriedades antioxidantes e anti-inflamatórias, assim como modulação de padrões comportamentais como depressão e ansiedade, além de alterações na composição corporal (Volek *et al.*, 2006; Abebe *et al.*, 2010; Al-Rasheed *et al.*, 2013; Dubey *et al.*, 2015).

Abordando mais especificamente as propriedades antioxidantes demonstradas por este suplemento, pode-se destacar estudos realizados com animais diabéticos, onde a suplementação de CrPic foi capaz de aumentar a atividade das enzimas antioxidantes CAT, SOD e GPx tanto no sangue quanto no

cérebro, coração e fígado, assim como os antioxidantes não-enzimáticos plasmáticos. Além de diminuir as concentrações de malondialdeído (MDA), indicando a redução da lipoperoxidação, de forma sistêmica e em órgãos como cérebro, fígado e rim. Estes resultados indicam uma capacidade de prevenção ao dano oxidativo proveniente da suplementação de CrPic. A melhora no estresse oxidativo observada com a suplementação está relacionada com a redução dos níveis de TNF-α e inibição de NF-kB (Sahin *et al.*, 2012; Al-Rasheed *et al.*, 2013; Sundaram *et al.*, 2013).

A ação antioxidante e anti-inflamatória do CrPic observada pode ser atribuída a diferentes mecanismos, como a recuperação dos níveis de GSH, possivelmente pela suplementação levar ao aumento da glicose-6-fosfato desidrogenase, resultando em maior geração de NADPH. Capacidade em atenuar os níveis de MDA e aumentar a atividade das enzimas SOD e CAT, quando estes estão alterados. Além de promover a redução dos níveis séricos de nitrito, levando ao bloqueio da reação do nitrito com o $O_2^{\bullet -}$, reduzindo a produção de peroxinitrito (Cefalu *et al.*, 2010; Sahin *et al.*, 2013; Sundaram *et al.*, 2013; Seif, 2015).

A utilização da suplementação de CrPic desencadeia uma série de efeitos positivos associados a redução de estresse oxidativo e, consequentemente, da resposta inflamatória. Essas ações benéficas ocorrem especialmente em casos onde há uma alteração nestes marcadores já instaurada. Tendo em vista suas ações, o CrPic pode ser administrado visando contribuir na melhora do controle metabólico e oxidativo, podendo auxiliar no tratamento de diferentes condições clínicas (Suksomboon *et al.*, 2014; Peng e Yang, 2015).

1.4. EXERCÍCIO FÍSICO

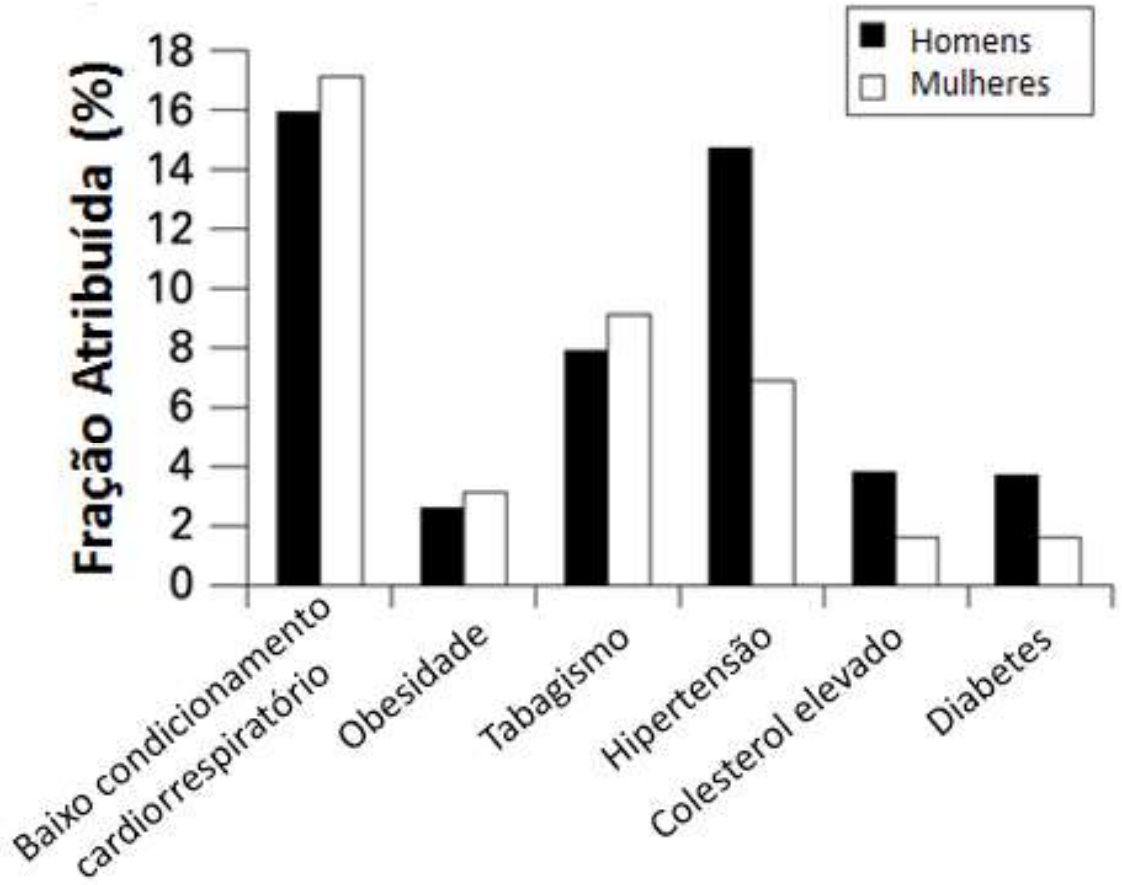
A atividade física inclui realização de atividades de lazer, tempo de locomoção, esportes e exercícios planejados. A OMS, na Recomendação Global de Atividade Física de 2010, orienta a realização de, no mínimo, 150 minutos por semana de exercício físico aeróbio com intensidade moderada ou 75 minutos de exercício de intensidade vigorosa, em sessões de pelo menos 10 minutos de duração. Com relação aos exercícios de fortalecimento da musculatura a recomendação é de 2 vezes ou mais por semana. Esta recomendação é para

melhorar o condicionamento cardiorrespiratório e muscular, reduzir risco de doenças crônicas e depressão. Para que ocorram benefícios adicionais, a recomendação é de 300 minutos de exercício moderado ou 150 minutos de exercício vigoroso por semana (WHO, 2010a).

Os benefícios oriundos da prática do exercício físico estão bem estabelecidos e são diversos, entre eles estão melhora da qualidade de vida, prevenção e tratamento de condições clínicas como doenças cardiovasculares, obesidade, diabetes, câncer, depressão, doença de Alzheimer e Parkinson. É possível observar estudos demonstrando que a intervenção com exercício físico por um ano foi capaz de reduzir a obesidade abdominal, pressão sanguínea e acúmulo de gordura hepática. Além de causar impacto positivo na capacidade física e mental de pacientes com Parkinson, enquanto que a frequência reduzida da realização de exercício está associada com maior índice de ansiedade (Finucane *et al.*, 2011; Lauzé *et al.*, 2016; Stubbs *et al.*, 2016).

A inatividade física é uma pandemia e pode ser considerada a quarta causa de morte no mundo. O sedentarismo representa um risco significativo para a saúde, já que aumenta a probabilidade de desenvolver doenças cardíacas, diabetes, câncer e acidente vascular cerebral (AVC). Além disso, a inatividade física é um dos problemas de saúde publica mais importantes do século 21, como é possível observar no estudo de Blair e colaboradores (2009), onde foi observado que o baixo condicionamento cardiorrespiratório, comum em indivíduos sedentários apresentou maior fração atribuída a causa de morte entre os indivíduos estudados, ultrapassando valores de obesidade, tabagismo, hipertensão, colesterol elevado e diabetes. Esse resultado está ilustrado na Figura 2 (Blair, 2009; Williams, 2009; Kohl *et al.*, 2012).

Figura 2. Fração atribuída (%) das causas de morte em homens e mulheres no ‘Aerobics Center Longitudinal Study’.



Fonte: Adaptada de Blair, 2009.

O hábito de vida sedentário é caracterizado pela elevação crônica da produção basal de ERO e redução da capacidade antioxidante, o que causa um desequilíbrio em favor dos oxidantes, tendo assim o estresse oxidativo, levando a uma ruptura do controle e sinalização redox e dano molecular. Esta condição é responsável por inúmeros efeitos nocivos que acarretam no desenvolvimento de doenças. O exercício físico está entre os fatores conhecidos por proteger contra estresse oxidativo, sendo que a resposta causada por ele estimula a defesa antioxidante e pode aumentar a síntese de proteínas específicas, atenuando o estresse oxidativo (Buresh e Berg, 2015).

1.4.1. Exercício físico e sinalização redox

Evidências sobre os efeitos benéficos do exercício físico surgiram na década de 1950, no entanto, apenas em 1978 foi identificada a primeira relação entre a função muscular e a formação de radicais livres. Sendo observado um aumento na formação de ERO após a contração muscular de forma repetida, e aumento de 2 a 3 vezes na formação de radicais livres no músculo esquelético após a realização de corrida até a exaustão (Koren *et al.*, 1980; Davies *et al.*, 1982; Powers *et al.*, 2016). Esses resultados sendo associados ao aumento da lipoperoxidação, distúrbios na respiração mitocondrial e perda da integridade do retículo sarcoplasmático (Gomez-Cabrera *et al.*, 2013). Atualmente esta relação está bem estabelecida, a atividade muscular contrátil intensa resulta na formação de estresse oxidativo, evidenciadas também em humanos, ao observar um aumento na concentração de MDA no plasma em atletas após provas de ciclismo e corridas de maratonas (Gómez-Cabrera *et al.*, 2003; Gomez-Cabrera *et al.*, 2006).

O estresse oxidativo está envolvido em diferentes estados fisiopatológicos causados através do dano celular e tecidual, incluindo proteína, lipídeo e DNA. Por outro lado, a formação de ERO é necessária para ações fisiológicas como sinalização e adaptação celular. Durante a realização do exercício físico há a formação de ERO, neste caso agindo como importantes moduladores da contração muscular, proteção antioxidante e reparo de dano oxidativo. As ERO formadas durante o exercício físico atuam como mediadores principais na regulação das moléculas antioxidantes, tendo em vista o aumento dos níveis de glutationa redutase após o exercício (Powers e Jackson, 2008; Powers *et al.*, 2010; Gomez-Cabrera *et al.*, 2013).

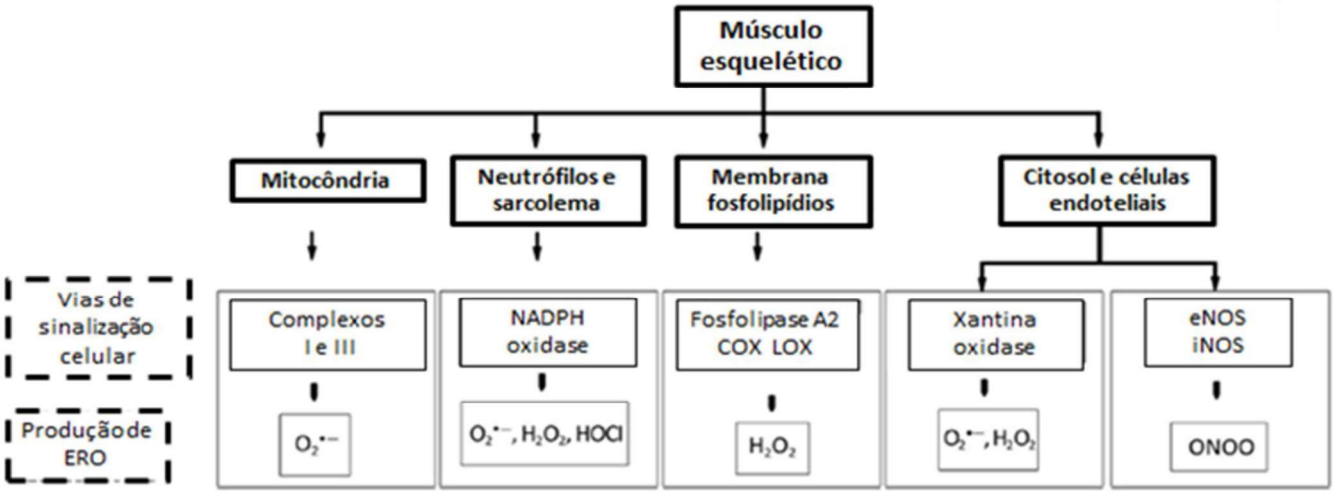
A formação de ERO durante o exercício é proveniente de diferentes fontes. A primeira a ser citada pode ser a mitocôndria, geralmente considerada a principal geradora de ERO, sendo os complexos I e III os principais locais de formação de $O_2^{\bullet -}$. O aumento da atividade contrátil durante o exercício é diretamente relacionada com maior consumo de oxigênio e elevada atividade mitocondrial, causando um aumento de 50 a 100 vezes na formação de $O_2^{\bullet -}$ durante exercícios aeróbios. No entanto, estudos mais recentes sugerem que a mitocôndria não é a principal fonte de formação das ERO durante o exercício, tendo em vista que a mitocôndria produz

mais ERO durante seu estado de repouso, estágio 4 da respiração (basal), do que na sua fase ativa, estágio 3 da respiração (Jackson *et al.*, 2007; Gomez-Cabrera *et al.*, 2008; Powers e Jackson, 2008; Gomez-Cabrera *et al.*, 2013).

Outra fonte de ERO, a mais provável de ser a principal, seria a enzima NADPH oxidase, localizada no retículo sarcoplasmático, túbulo T e sarcolema. O $O_2^{\bullet -}$ gerado por esta enzima é capaz de regular a função contrátil através da liberação de cálcio tanto no músculo esquelético quanto no cardíaco. A enzima fosfolipase A2 (PLA2) presente na membrana é responsável por aumentar ERO durante contrações repetitivas e fatigantes, sendo que as enzimas ciclooxigenase (COX) e lipo-oxigenase (LOX) agem em conjunto com a PLA2. Além da enzima xantina oxidase (XO), a qual é responsável pela formação de ERO durante exercícios intermitentes de alta intensidade (Jackson *et al.*, 2007; Powers e Jackson, 2008; Powers *et al.*, 2011).

Ainda, a enzima oxido nítrico sintase (NOS) é responsável pela formação do NO no músculo esquelético, o qual tem como função regular o tônus vascular. A contração muscular extenuante pode aumentar a produção de NO através da ativação das isoformas endotelial e induzida da NOS (eNOS e iNOS, respectivamente), podendo formar peroxinitrito, um radical livre extremamente reativo. O aumento da formação das ERON apresenta uma função chave na regulação de vias de sinalização sensíveis ao estado redox, as quais são essenciais para a adaptação muscular em resposta ao treinamento físico. A formação de ERO pode ser observada na Figura 3 (Gomez-Cabrera *et al.*, 2013).

Figura 3. Potenciais sítios de formação de ERO no músculo esquelético.



Fonte: Adaptada de Gomez-Cabrera *et al.,* 2013.

As principais ERO produzidas nas fibras musculares são o $O_2^{\bullet-}$ e o NO, ambos reagem com outras moléculas e formam uma gama de outras ERO e ERN, respectivamente. O $O_2^{\bullet-}$ quando dismutado, pela SOD ou de forma espontânea, produz H_2O_2, um oxidante fraco e com meia-vida relativamente longa, o que permite que se difunda pelas células e atravesse membrana celular. Além disso, o H_2O_2 reage com diferentes moléculas e ativa vias de sinalização, sendo uma importante molécula sinalizadora. Enquanto o NO pode reagir com o $O_2^{\bullet-}$ e formar peroxinitrito, agente altamente oxidativo, o qual altera o estado redox pela depleção do grupo tiol na célula, além de reduzir a biodisponibilidade do $O_2^{\bullet-}$ e NO, influenciando na sinalização redox. Com isso, a produção de ERO intracelular é necessária para o remodelamento celular que ocorre no músculo esquelético em resposta a repetidas sérias de exercício (Powers e Jackson, 2008; Powers *et al.*, 2010; Powers *et al.*, 2011; Goncalves *et al.*, 2015; Powers *et al.*, 2016).

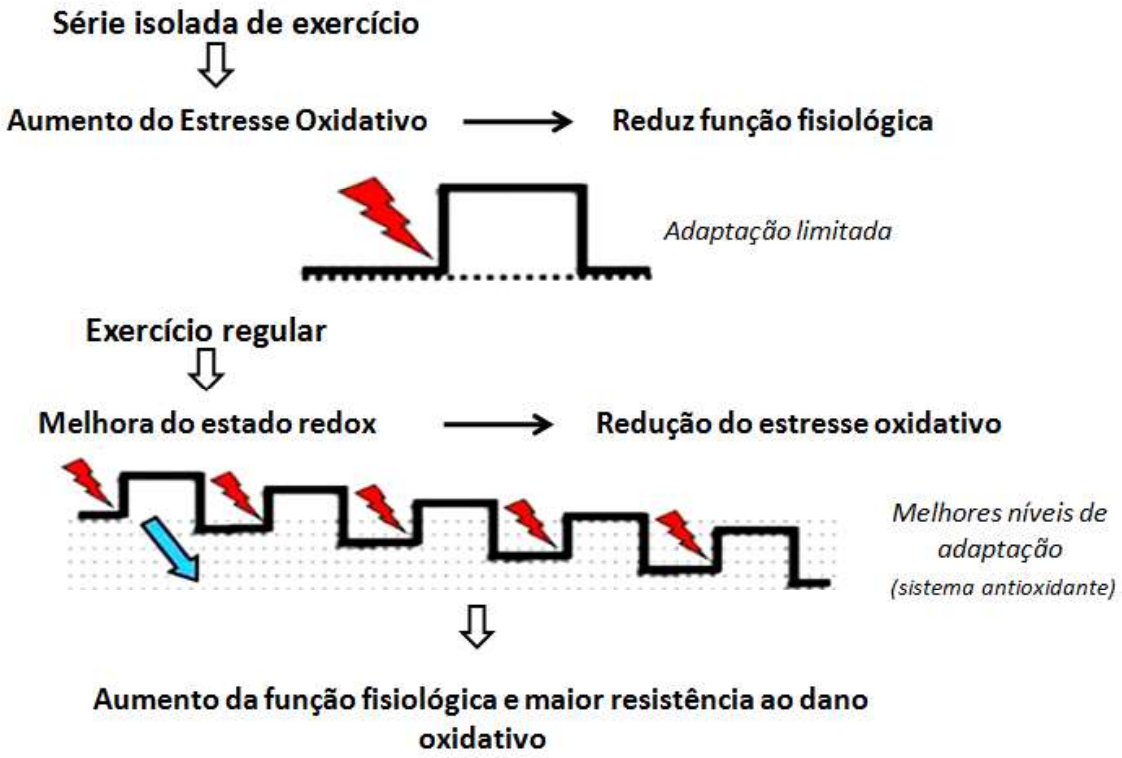
Uma importante relação entre sinalização por vias sensíveis ao estado redox, a produção de ERO induzidas pelo exercício e o remodelamento do músculo envolve a regulação do estado redox pela família NF-kβ, a qual é ativada pelo exercício e essencial para a expressão das enzimas antioxidantes. Outra importante adaptação celular após o exercício é o aumento da biogênese mitocondrial, estando associada a efeitos benéficos como aumento da capacidade oxidativa e tolerância ao exercício. O receptor ativado por proliferador de peroxissoma (PPAR-γ) coativador-1α (PGC-

1α) é um regulador da biogênese mitocondrial, sendo que já demonstrou ser responsável por regular processos celulares, incluindo a diferenciação das fibras musculares. A expressão da PGC-1α, no músculo, pode ser associada a uma variedade de estímulos associadas ao exercício (Russell *et al.*, 2003; Powers *et al.*, 2011; Powers *et al.*, 2016).

O exercício físico realizado regularmente e em intensidade moderada é responsável por uma séria de regulações celulares, como controle de expressão genica, regulação de vias de sinalização celular e modulação da produção de força pelo músculo esquelético. Sendo capaz de reverter as mudanças desfavoráveis induzidas pelo estresse oxidativo, promovendo um estilo de vida saudável. Além disso, o exercício físico habitual está relacionado com redução do risco de desenvolvimento de doenças cardiovasculares e morte. Inversamente, o exercício extenuante realizado de forma aguda pode causar uma produção excessiva de radicais livres, causando dano a composição celular, reduzindo a síntese proteína e ativando proteases, o que irá promover uma disfunção na contratilidade do músculo levando a fraqueza e fadiga muscular. Inclusive, em indivíduos sedentários com doença vascular pré-existente, a realização de exercício vigoroso pode aumentar o risco de morte súbita (Gomez-Cabrera *et al.*, 2006; Powers e Jackson, 2008; Radak *et al.*, 2013; Giles e Koehle, 2014).

Na Figura 4 é possível visualizar a diferença das respostas adaptativas resultantes do exercício. A resposta adaptativa a uma única série de exercício é limitada, e o dano oxidativo ocorre frequentemente. Enquanto níveis moderados de dano oxidativo podem ser importantes para a indução do sistema de reparo ao dano oxidativo, permitindo a indução das defesas antioxidantes, o que resulta na melhora da proteção ao estresse oxidativo (Radak *et al.*, 2013).

Figura 4. Diferença entre a resposta adaptativa após uma única série de exercício e a adaptação resultante do exercício regular.



Fonte: Adaptada de Radack *et al.,* 2013.

A relação entre estresse oxidativo e exercício é extremamente complexa, considerando que dependendo do modo, intensidade, duração do exercício e condições clínicas de indivíduos que irão praticar o exercício as respostas oxidativas serão diferentes. Esses desfechos contraditórios podem ser explicados pela teoria de “hormesis”, a qual conclui que baixas doses de um agente que em altas doses é prejudicial, pode induzir um efeito adaptativo benéfico na célula ou organismo (Calabrese e Baldwin, 2003; Gomez-Cabrera *et al.*, 2008; Boccatonda *et al.*, 2016).

2. JUSTIFICATIVA

A poluição atmosférica é responsável por uma série de efeitos nocivos tanto para o ambiente quanto para a saúde da população exposta. Os poluentes presentes na poluição do ar contribuem para o início e progressão de diversas doenças respiratórias, cardiovasculares e, inclusive, o câncer. O mecanismo mais provável pelo qual esses efeitos são causados é o estresse oxidativo, sendo responsável por alterações como oxidação de proteínas, peroxidação lipídica, alterações em sinalização celular e dano ao DNA. Enquanto a suplementação antioxidante e o exercício físico regular apresentam efeitos opostos ao causados pela poluição, ambos podendo desencadear processos de adaptação e sinalização que atenuam o estresse oxidativo, reduzem resposta inflamatória e aumentam as defesas antioxidantes do organismo.

Tendo em vista o aumento das emissões de poluentes, e os danos causados por estes, cada vez mais torna-se essencial a descoberta de meios para reduzir os danos causados pela poluição. Portanto, a associação de uma suplementação antioxidante, como a de picolinato de cromo, com o exercício aeróbio realizado de forma programada e regular, poderia ser uma alternativa que beneficia a saúde da população.

3. OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar o efeito da suplementação de picolinato de cromo associada ao treinamento aeróbio sobre os efeitos causados pela exposição subcrônica à poluição atmosférica através da utilização do teste de capacidade máxima ao exercício e de marcadores de estresse oxidativo.

3.2 OBJETIVOS ESPECÍFICOS

- Avaliar o efeito da suplementação de picolinato de cromo associada com treinamento aeróbio em ratos expostos à poluição atmosférica por 12 semanas sobre a massa corporal.

- Avaliar o efeito da suplementação de picolinato de cromo associada com treinamento aeróbio em ratos expostos à poluição atmosférica por 12 semanas sobre o teste de tolerância ao exercício, utilizando como marcadores o tempo, a distância percorrida e a velocidade.

- Avaliar o efeito da suplementação de picolinato de cromo associada com treinamento aeróbio em ratos expostos à poluição atmosférica por 12 semanas sobre estresse oxidativo através da mensuração da atividade das enzimas antioxidantes superóxido dismutase e catalase, além da concentração de malondialdeído no pulmão, no coração e no gastrocnêmio.

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5. ARTIGO CIENTÍFICO

Aerobic training reduces lipoperoxidation and increases SOD activity in skeletal muscle of rats exposed subchronically to Residual Oil Fly Ash and supplemented with chromium picolinate

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Running title: Exercise benefits in exposure to air pollution

Acknowledgment: Bruna Marmett received grant support from the Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for her master degree.

The authors declare they have no actual or potential competing financial interests.

Abstract

Background: Air pollution exposure is closely related to premature mortality and increased risk for a range of diseases. Exercising in polluted ambient could lead to adverse effects caused by a higher exposure. However, exercise could protect against oxidative stress induced by air pollution. As well as, supplementation of chromium picolinate (CrPic) could act as an antioxidant, reducing the negative health impact of pollution.

Objectives: We investigate the effects of CrPic supplementation associated with aerobic exercise in measures of oxidative stress in rats exposed to air pollution.

Methods: Male Wistar rats were instilled with 50µg of residual oil fly ash (ROFA) suspension, supplemented with CrPic (1mg/kg/day) and underwent to a treadmill running protocol for 50 min, 5 days/week for 90 days. Body mass was evaluated weekly; exercise tolerance test was performed before and after experimental protocols; superoxide dismutase (SOD) and catalase (CAT) activity and malondialdehyde (MDA) concentration were analyzed in lung, heart and gastrocnemius.

Results: Trained groups demonstrated lower gain of body mass, increased distance, time and velocity of the running when compared to sedentary groups ($p<0.05$). Oxidative stress analyses in lungs and heart showed no differences ($p>0.05$). In gastrocnemius, trained groups demonstrated an increased SOD activity and decrease concentration of MDA compared to sedentary groups ($p<0.05$), although CAT activity demonstrate no differences among groups.

Conclusion: Air pollution exposure could lead to an oxidative adaptation in lungs and heart in a healthy sample, while exercise training is responsible for improvement in antioxidant defense decreasing oxidative stress of gastrocnemius.

Keywords: Air Pollution; Exercise; Exercise Tolerance; Chromium; Oxidative Stress.

Introduction

Air pollution is currently classified as a leading environmental cause of cancer and ranked as one of the top ten causes of disability (IARC 2013). Beyond, the pollutants present in air are already recognized by increase risks for a range of diseases, such as chronic obstructive pulmonary disease (COPD), cerebrovascular disease (CEV), atherosclerotic and ischemic heart disease (IHD) (Aguilera et al. 2016; IARC 2013; WHO 2006). Also, polluted air is related to premature mortality, being estimated a global mortality burden of more than 3 million premature deaths/year. The projections of emission scenario indicate that these values could double by 2050, if any measure of air quality control be made (Lelieveld et al. 2015; Silva et al. 2016).

There is strong evidence that pollutants present in air triggers oxidative stress and systemic inflammation, which are responsible for the detrimental effects of air pollution (Aguilera et al. 2016; Fraunberger et al. 2016; Pope et al. 2016). These particles directly generate reactive oxygen species (ROS), alter mitochondrial function and NADPH-oxidase enzyme and activate inflammatory cells that form ROS. All these factors contribute to the pathological mechanism, increasing the susceptibility of the population to develop chronic diseases (Fraunberger et al. 2016; Pope et al. 2016).

Physical inactivity is one of the most significant problems of public health in 21st century and is the fourth leading cause of death worldwide (Blair 2009; Kohl et al. 2012). While, physical activity demonstrates well established health benefits (Finucane et al. 2011; Lauzé et al. 2016; Stubbs et al. 2016). Exercise training induces the formation of ROS that act as important mediators of physiologic signaling and cellular adaptations, modulating muscle contraction, regulation of antioxidant protection and repair of oxidative damage (Giles and Koehle 2014; Lovinsky-Desir et al. 2016; Matt et al. 2016). Therefore, exercising could also protect against oxidative stress induced by air pollution, attenuating the harmful effects caused by this environmental condition (Buresh and Berg 2015; Gomez-Cabrera et al. 2013; Powers and Jackson 2008; Powers et al. 2010).

Chromium picolinate (CrPic) is a trivalent chromium complex largely used to control glucose and improve insulin sensitivity. Furthermore, CrPic supplementation demonstrated antioxidant activity in animals with established oxidative stress, being responsible for increasing glutathione (GSH) levels, catalase (CAT) and superoxide dismutase (SOD) activity, besides decrease of lipid peroxidation (Al-Rasheed et al. 2013; Refaie et al. 2009;

Sahin et al. 2013; Sundaram et al. 2013b). The mechanism pointed to be responsible for antioxidant actions of CrPic involves blocking peroxynitrite formation and restoring GSH levels by increasing glucose-6-phosphate dehydrogenase activity (G6PD) (Sundaram et al. 2013a). Furthermore, CrPic antioxidant activity could decrease the damage effects that air pollution cause on health, attenuating the action of oxidative molecules and subsequently the damage induced by them (Tong 2016).

Thus, exercise and nutrition interventions are both strategies that may reduce the negative health impact from exposure to air pollution. The majority of studies investigating air pollution with exercise demonstrate controversial findings, besides an additional investigation with the association of antioxidant supplement remains still unclear (Andersen et al. 2015; Giles et al. 2011; Giles and Koehle 2014).

Therefore, the aim of the present study was to examine the effects of CrPic supplementation associated with aerobic exercise in subchronical air pollution exposure in measures of oxidative stress. In addition, we investigate the adaptation of aerobic exercise in groups exposed to air pollution and supplemented with CrPic.

Methods

Animals and treatment

Male Wistar rats (forty-five-days old) were obtained from Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). The animals were housed under standard conditions (food and water *ad libitum*, temperature between 22 and 24 °C, light – dark cycle of 12 hours). The handling of the animals obeyed Law nº 11.794 of 10/08/2008, Law nº 6,899 of 07/15/2009 and Resolution nº 879 of 02/15/2008 (CFMV), as well as resolutions of the National Council on Animal Experimentation and all procedures were in accordance with the Guide for the Care and Use of Laboratory Animals adopted by National Institute of Health (NIH-USA). This study was approved by CEUA/UFCSPA, under the protocol number 159/15.

Sixty four animals were randomly distributed into eight experimental groups: ROFA-SED, instilled with ROFA and sedentary rats (n=8); ROFA-SED-CrPic, instilled with ROFA, sedentary and supplemented with CrPic rats (n=7); ROFA-AT, instilled with ROFA and trained rats (n=8); ROFA-AT-CrPic, instilled with ROFA, trained and supplemented with CrPic rats (n=6); Sal-SED, instilled with saline and sedentary rats (n=8); Sal-SED-CrPic,

instilled with saline, sedentary and supplemented with CrPic rats (n=8); Sal-AT, instilled with saline and trained rats (n=8); Sal-AT-CrPic, instilled with saline, trained and supplemented with CrPic rats (n=8).

Rats exposed to air pollution received via intranasal instillation 50µg of ROFA, which was applied as a recognized particulate matter (PM). This dose represents the concentration of 29µg/m³, which is the value found in a polluted city (Medeiros et al. 2004). ROFA particle were collected from electrostatic precipitator installed in one of the chimneys of a large steel plant in São Paulo, Brazil. Characterization of ROFA is included in Supplemental Material, Table S1. Particles were prepared by suspending 50µg of ROFA in 10µl steril saline solution, sonicated for 20 min in an ultrasonic water bath. The volume was increased to 20µl and, after, to 30µl when rats were 60 and 90 days old, aiming to ensure that the suspension will reach the lungs as the development of the rat respiratory system (Southam et al. 2002). Supplemented groups received 1mg/kg of CrPic in 1mL steril saline solution (presentation form: powder, with purity of ≥ 98%, Pharma Nostra®, Brazil) by oral gavage for 90 days (Sundaram et al. 2013a). Control groups underwent the same instillation and supplementation protocols however received vehicle.

Animals from control and exercised groups were submitted to exercise tolerance test before and after the aerobic training protocol. First, animals were submitted to adaptation period to running on a motor driven treadmill for 5 days for 10 min/day (Marton et al. 2016). The test consists in running on the electric treadmill with an inclination of 15°, starting with speed of 5 m/min and increment of 5m/min every 3min until exhaustion. Exhaustion was established as the time that the animal was unable to run for at least 15 s, even while receiving an electrical stimulus (1.5 µA) (Ferreira et al. 2007). Trained animals underwent aerobic exercise training sessions, 5 times per week, at morning, during 12 weeks. The sessions lasted 50 min with an intensity corresponding to 70% of exercise tolerance test, as described previously. The running time was extended by 10 min/week, from the adaptation time, until 50 min/day. Body mass were measured weekly at morning. Animals were euthanized after 12 weeks of treatment and training. Lung, heart and gastrocnemius were dissected and stored in -80ºC for forward analyses of oxidative stress.

Oxidative stress analysis

To tissue preparation, lung, heart and gastrocnemius were defrosted, weighed in an analytical balance and homogenized in KPi buffer (KCl 1.15%), pH 7.4, containing protein

inhibitors (1µg/mL pepstatin, 1µg/mL aprotein, 1µg/mL leupeptin and 0,5 mM PMSF) with proportion of 5 ml/1 g, 7 ml/1 g and 5 ml/1 g of tissue, respectively. The homogenization was performed in a tissue homogenizer (CT-136.1, Cientec®). The homogenized samples were centrifuged at 3000 rpm for 10 minutes at 4°C to remove nuclei and cell debris and the supernatants was kept at -80ºC until oxidative stress analyses. The supernatant was used to determine SOD and CAT activities and malondialdehyde (MDA) concentration.

Protein concentration of the tissues homogenates was measured by Bradford's method (Schleicher and Wieland 1978) using bovine serum albumin as standard. To assess the dosage of protein in the tissue, 10 µl of homogenized sample was diluted in 190 µl of distilled water. Sixty microliters of this solution were placed in plastic cuvettes, containing 2,9 ml of Bradford reagent. The sample absorbance was determined at 595 nm, in a Lambda 35 spectrophotometer (Perkin-Elmer of Brazil, SP, Brazil).

SOD activity was determined based on the inhibition of pyrogallol auto-oxidation by the enzyme (Marklund and Marklund 1974). One unit of SOD is defined as the enzyme quantity capable of inhibiting 50% of the reaction. A total of 930 µl of TRIS buffer, 4 µl of 30 µM catalase and 50 µl of homogenized tissue was placed into cuvettes. Then, 16 µl of 24 mM pyrogallol in 10 mMHCl was added to the solution. The sample absorbance were determined in a Lambda 35 spectrophotometer (Perkin-Elmer of Brazil, SP, Brazil), at 420 nm after 60 and 120 s. The results were expressed as USOD/mg of total protein.

CAT activity was determined through the decomposition of hydrogen peroxide at 25°C, according to Aebi (1984). The reaction mixture contained 2865 µl of phosphate buffer 50 mM (pH 7.0) and 30 µl of homogenized tissue, then, 35 µl of 0.02 M hydrogen peroxide was added to the solution. The samples absorbance were determined in a Lambda 35 spectrophotometer (Perkin-Elmer of Brazil, SP, Brazil), at 240 nm for 120 sec and the results are expressed in pmol/mg of total protein.

To determine MDA concentration was used the technique according to Esterbauer and Cheeseman (1990). Briefly, to promote the precipitation of proteins, 250 µl of tissue homogenate supernatant was added to 500µl of 10% trichloroacetic acid solution. After cooling in ice for 15 min, the samples were centrifuged 3200 rpm for 15 minutes at 4°C, then 500 µl of 0.670% thiobarbituric acid was added to 500 µl of supernatant. The solution was agitated and heated at 100°C in a water-bath for 10 minutes. Then, the stained supernatant was placed in glass microcuvettes to determine the absorbance at 535 nm in a Lambda 35 spectrophotometer (Perkin-Elmer of Brazil, SP, Brazil). MDA concentration was expressed in

nmol/mg of total proteins. To calculate the MDA concentration, the standard curve generated from the known concentrations of 1, 1, 3, 3-Tetrametoxypropane 100 nmol/ml in 1% H_2SO_4 solution was utilized.

Statistic analysis

The statistic analysis was performed using Kolmogorov-Smirnov test to evaluate normality of all variables. Two-way repeated measures analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple comparison test were performed for comparisons between treatment and control groups in body mass variable. Exercise tolerance test and oxidative stress variables were analyzed using one-way analysis of variance (ANOVA) followed by Tuckey multiple comparison test for comparisons between treatment and control groups. For statistical analysis and graphics creation it was used SigmaPlot version 12.0 for Windows (Systat Software, Inc.) and GraphPad Prism version 6.0 for Windows (Prism 6; GraphPad Software, Inc.). A p-value ≤ 0.05 was considered statistically significant.

Results

Mortality and body mass

During the study, there were 3 losses of animals due to causes not related to the experiments (data not showed). The initial body mass was similar among groups ($p>0.05$) and at the end of the study, all groups showed increase of body mass when compared to the initial measure ($p<0.001$). Also, when final body mass was compared among groups, the trained ones demonstrated lower gain of body mass compared to Sal-SED group ($p<0.05$). The results are presented in Figure 1.

Exercise tolerance test

Exercise tolerance test performed at the beginning of the study demonstrated similar distance ($p=0.9231$), time ($p=0.9368$) and velocity ($p=0.7889$) between groups. In the final exercise tolerance test was observed an increase on distance, time and velocity of the running in the trained groups when compared to sedentary groups ($p<0.0001$), as shown in Figure 2.

Oxidative stress

There were no differences in SOD (p=0.2756) and CAT (p= 0.1198) activity, likewise MDA concentration (p=0.7189) in lung, as presented in Figure 3. In heart, no differences were observed among groups in SOD (p=0.0763) and CAT (p=0.4999) activities and in MDA (p=0.8656) concentration (Figure 4).

Considering gastrocnemius analysis, reported in Figure 5, SOD activity was higher in the groups with aerobic training, ROFA-AT, Sal-AT and Sal-AT-CrPic when compared to all sedentary groups ROFA-SED, ROFA-SED-CrPic, Sal-SED, Sal-SED-CrPic (p<0.0001). While, ROFA-AT-CrPic demonstrated higher activity of SOD when compared to ROFA-SED (p<0.0001). In relation to CAT activity no differences were observed among groups (p=0.4487). The concentration of MDA was lower in trained groups in comparison with ROFA+SED and Sal-SED-CrPic groups (p=0.0014).

Discussion

The main findings of the present study were the improvement of exercise tolerance and the reduction of oxidative stress in gastrocnemius muscle. These findings were evidenced by the increase in distance of run, time to exhaustion and maximal velocity of the exercise tolerance test, as well as increase of SOD activity and decrease in concentration of MDA in the gastrocnemius muscle. However, there was no difference among groups in oxidative stress parameters evaluated in lung and heart tissues.

Regarding to body mass, all groups increased body mass during the study, which was expected due to the growth process. In addition, aerobic exercise could attenuate the mass gain, probably due to increase energy expenditure, resulting in a negative energy balance and consequently reduction of mass gain. The same outcome was observed in Cigarroa experimental study (2016), which demonstrated that a treadmill intervention could counterbalance the mass gain of animals fed with cafeteria diet (Cigarroa et al. 2016).

Our findings suggest that aerobic training leads to exercise tolerance, evidenced by increase of run distance, time to exhaustion and maximal velocity, as demonstrated in experimental studies that use aerobic training (Ferreira et al. 2007; Marton et al. 2016). The repetitive muscle contraction during exercise training lead to a variety of responses including: activation of mitochondrial biogenesis, fiber type adaptation and angiogenesis. These responses increase aerobic metabolism capacity and muscular resistance (Powers et al. 2011;

Radak et al. 2013; Radák et al. 2000; Steinbacher and Eckl 2015). Therefore, the 12 weeks of training could adapt animals to exercise and lead to the beneficial effects of exercise.

In the present study, it was not observed any difference in the lung SOD and CAT activity and MDA concentration among groups, indicating that dose used of ROFA did not alter the activity of these antioxidant enzymes nor promoted lipid peroxidation. Besides antioxidant supplementation and exercise, alone or in association, did not demonstrated effects on SOD and CAT activity, nor in concentration of MDA. Controversy to our results, an acute exposure of high doses of pollutants (500µg, 750µg and 1mg) could induced functional cardiopulmonary changes in rats and be involved in the oxidant inflammatory response (Rhoden et al. 2005; Rhoden et al. 2008; Rivero et al. 2005). While, lower doses administrated chronically (50µg and 250µg) did not lead to oxidant lipid damage in the rat lungs nor increased activity of SOD and CAT enzymes (Damiani et al. 2012).

In contribution to the understanding of our result, when evaluating the progression of a systemic oxidative response after acute exposure to ROFA, it was firstly observed an increase in TBARS levels, a lipid peroxidation end-product, which occur in 1h and 3h after exposure to ROFA. In addition, it was observed a depletion of plasma GSH, along with increased GSSG levels indicating a decrease GSH/GSSG ratio in 1h after exposure to ROFA. Also, carbonyl groups from oxidatively modified plasma proteins were accumulated after 3 hours. In relation to SOD activity, it was decreased after 3h of the treatment, being inversely associated with ROFA exposure, in view that the activity of this enzyme often decreased in oxidative stress conditions. However, when SOD activity was assed 5h after ROFA exposure, it was increased along with a reduction of TBARS levels, indicating an adaptive response to the systemic oxidative stress triggered by ROFA at earlier time points**.** So, at 3h, it was observed an increase in systemic oxidative markers in association with decrease of plasma antioxidant status, which indicate a plasmatic oxidative damage. However, at 5h after ROFA exposure can be observed an adaptatively response, such as increased SOD activity and reduced TBARS content (Magnani et al. 2011; Marchini et al. 2014).

The same systemics alterations were observed after acute exposure to ROFA in lung tissues, suggesting that the oxidative damage markers in plasma may result from translocation of oxidation end-products from the lung to systemic circulation (Magnani et al. 2011). Therefore, ROFA exposure in our study possibly induced oxidative alterations, decreasing antioxidant enzymes and lead to lipid peroxidation, however, as the exposure was subchronic, these parameters may have return to basal level, indicating an adaptation of lungs to the

ROFA exposure. So, the oxidative stress could have been reversed and had occurred a pulmonary adaptation from ROFA exposure.

Regarding to heart oxidative variables was not found difference among groups, indicating that long-term exposure to inhalation of ROFA, antioxidant supplement and exercise training had no effect in activity of antioxidant enzymes and lipid peroxidation in heart. Our study was performed with healthy animals without cardiac conditions, while in studies using myocardial infarction model occurs inflammatory migration to sites where a pre-established inflammation is increased, such as the heart, which is an outcome that was not observed in healthy animals. Moreover, when assessed effect of ROFA in hypertensive rats, it had increased heart vulnerability in developing arrhythmia and demonstrated that an underlying condition, like cardiovascular disease, represent greatest risk after ROFA exposure (Farraj et al. 2009; Hazari et al. 2009; Knuckles and Dreher 2007; Marchini et al. 2016; Meng et al. 2016).

Additional investigation demonstrated that acute intratracheal instillation of 750µg induced changes in heart rate variability simultaneously with increases in oxidant levels (Rhoden et al. 2005). While, in consistency with our results, a study reporting exposure to 50 and 250µg of ROFA for 90 days, also observed no change in oxidative stress markers, such as SOD, CAT and MDA (Damiani et al. 2012). Interestingly, Gurgueira and colleagues demonstrated that acute inhalation of concentrated ambient particle (CAP) led to potential oxidant injuries followed by an up-regulation of antioxidant defenses, indicated by the increased SOD and CAT activities in lung and heart. Furthermore, after 24 h of inhalation period, occurred the reversibility of oxidative stress suggesting that oxidants mediated by pollution exposure may trigger adaptive responses and the lung and heart can adapt to the increase levels of oxidants induced by pollutants (Gurgueira et al. 2002).

Regarding to gastrocnemius evaluations of oxidative stress, we found an increased SOD activity together with decreased MDA concentration in trained groups, suggesting a positive effect of aerobic exercise in oxidative stress on gastrocnemius muscle. Contributing to our results, it is well known that muscle activity during exercise increases ROS formation and simultaneously promotes an increase in antioxidant defense system, as well as improves the resistance to oxidative stress (Powers et al. 2016; Radak et al. 2008; Steinbacher and Eckl 2015). Moreover, a higher activity of SOD in skeletal muscle are related with the intensity and duration of exercise, noticed by studies reporting that higher intensities and longer durations of exercise was associated with increased SOD activity (Powers and Jackson 2008).

Furthermore, aerobic exercise is already known for promote an increase of 20-112% of SOD activity in exercised muscles, this adaptation could be due to the endurance exercise recruitment patterns of muscle fibers, which recruit highly oxidative fibers (type I) rather than less oxidative fibers (type IIb) during the exercise. Therefore, the induced increase of SOD activity mediated by exercise is greatest in skeletal muscles composed of highly oxidative fibers (Powers and Jackson 2008; Powers et al. 2011; Powers et al. 2016). In our study the exercise model used was a treadmill running training that is classified as an endurance exercise, possibly occur the recruitment of type I fibers resulting in an increased SOD activity.

However, we found no significant difference in CAT activity among groups, indicating no alteration in this enzyme through the exercise in skeletal muscle. Probably, CAT activity did not demonstrate changes due to higher concentrations of GPx in highly oxidative fibers. Identical to SOD, GPx during to endurance exercise promotes the increase of 20–177% in GPX activity in skeletal muscles. Therefore, instead of CAT be recruited to transform hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2), GPx was responsible for H_2O_2 neutralization (Powers and Jackson 2008; Powers et al. 2016).

Notably, the reduced MDA concentration in trained groups observed in our study could be associated with increased SOD activity, which might prevent against lipid peroxidation, knowing that ROS generation induced by exercise is a stimulus to activate the expression of antioxidant enzymes. More specifically, the superoxide anion ($O_2^{-\bullet}$), a primary free radical generated, can rapidly form H_2O_2 and subsequently can be reduced into hydroxyl radicals ($OH^{\bullet}$), which could interact with lipids leading to its oxidation that result in lipid peroxidation (Emerit et al. 2004; Fraunberger et al. 2016; Powers and Jackson 2008). Once SOD activity dismutate $O_2^{-\bullet}$ into H_2O_2, GPx and CAT can neutralize H_2O_2, preventing the formation of $OH^{\bullet}$. In view that CAT activity was not increased in our study, possibly GPx act converting H_2O_2 into water. Naturally, a decreased in MDA concentration is a consequence of SOD activity and a possible GPx action. Considering that an acute session of exercise increased lipid peroxidation while measures of lipoperoxidation was not increased after periodic training, it indicates that the ROS generated by exercise also improve antioxidant system protecting muscle from oxidation of lipids (Radak et al. 2008; Steinbacher and Eckl 2015).

In our study were evaluated healthy animals exposed to ROFA instillation, which could lead to an adaptive response against inhalation of this polluted, therefore the oxidative

analyze of lung and heart might have no significant difference among groups due to health state of the animals. In healthy animals and individuals, exercise may act as a preventive strategy, which occur due to attenuation of inflammation, decrease of diastolic blood pressure, increase glucose control, antioxidant defense and aerobic capacity, while in animals or individuals with a disease it could be used as a non-pharmacological auxiliary therapy (Gielen et al. 2010; Kilic-Erkek et al. 2016; Powers et al. 2014; Sloan et al. 2007; Swain and Franklin 2006)

In relation to the supplementation lack of effect in these analyses, CrPic can play an antioxidant effect when there is an oxidative disruption, since CrPic supplementation is a method to preserve antioxidant status whereas is present a depletion of antioxidant enzymes and an increase in oxidative stress (Cefalu et al. 2010; Marmett and Nunes 2016; Wang et al. 2007). In the present study, was not able to observe a positive action of CrPic, since the oxidative scenario caused by the ROFA was probably reversed in the early days after exposure, in view of chronicity of ROFA exposure lead to an adaptation of oxidative metabolism in organs (Magnani et al. 2011; Marchini et al. 2014).

The present study has some limitations that warrant discussion. First, GPx activity was not assessed in organs that evaluated other antioxidants enzymes, such as SOD and CAT. In view that CAT was not different among groups in organs where were performed the analyses it is possible that GPx has played a role in antioxidant defense and reduction of lipid peroxidation. Second, antioxidant enzymes and MDA concentration was not evaluated in a systemic via, which would provide a full scenario of oxidative stress.

Conclusion

In conclusion, exercise training could decrease body mass gain and increase exercise tolerance, as well as lead to beneficial adaptation in oxidative stress through increasing antioxidant SOD enzyme activity and decreasing MDA concentration, indicating reduced lipid peroxidation in skeletal muscle. In this context, the dose of CrPic supplementation used in this study was not related to beneficial effects, probably because there was no depletion of antioxidant enzymes and increased oxidative stress in our sample. It is important to highlight that subchronic exposure to ROFA, as used in our study, did not had same detrimental results found in studies evaluating an acute exposure to this pollutant. Moreover, the emission of pollutants is rising and if any measured was made to control it, the new and increased rate of

emissions may cause more damage than the concentration used in this study. Therefore, more studies are required, investigating the quantity of pollutants emitted, as well as, the damage that the new increases rate of pollution will cause in health.

Nevertheless, our study showed that in a healthy sample, ROFA could lead to an adaptation in lungs and heart preventing a lasting oxidative damage, while exercise training was responsible for improvement in SOD activity and decrease lipid peroxidation of skeletal muscle, such as gastrocnemius.

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Figures

Figure 1. Body mass previous and after 12 weeks of CrPic supplementation and aerobic exercise intervention in rats exposed to ROFA. Values in mean ± SE. ROFA-SED, ROFA-AT, Sal-SED, Sal-SED-CrPic, Sal-AT, Sal-AT-CrPic n=8; ROFA-SED-CrPic n=7; ROFA-AT-CrPic n=6; †† $p< 0.05$ *vs.* Initial Body Mass; * $p< 0.05$ *vs.* ROFA+SED; ** $p< 0.05$ *vs.* ROFA-SED-CrPic; # $p< 0.05$ *vs.* Sal-SED; † $p< 0.05$ *vs.* Sal-SED-CrPic.

Figure 2. Exercise tolerance test before and after 12 weeks of CrPic supplementation and aerobic exercise intervention in rats exposed to ROFA. (A) Initial and final distance of exercise tolerance test; (B) Initial and final time of exercise tolerance test; (C) Initial and final velocity of exercise tolerance test. Values in mean ± SE. ROFA-SED, ROFA-AT, Sal-SED, Sal-SED-CrPic, Sal-AT, Sal-AT-CrPic n=8; ROFA-SED-CrPic n=7; ROFA-AT-CrPic n=6; * $p< 0.05$ vs. ROFA+SED; ** $p< 0.05$ vs. ROFA-SED-CrPic; # $p< 0.05$ vs. Sal-SED; † $p< 0.05$ vs. Sal-SED-CrPic.

Figure 3. Oxidative stress in lung after 12 weeks of CrPic supplementation and aerobic exercise intervention in rats exposed to ROFA. Analysis of (A) SOD activity in lung; (B) CAT activity in lung; (C) MDA concentration in lung. Values in mean ± SE. ROFA-SED, ROFA-AT, Sal-SED, Sal-SED-CrPic, Sal-AT, Sal-AT-CrPic n=8; ROFA-SED-CrPic n=7; ROFA-AT-CrPic n=6.

Figure 4. Oxidative stress in heart after 12 weeks of CrPic supplementation and aerobic exercise intervention in rats exposed to ROFA. Analysis of (A) SOD activity in heart; (B) CAT activity in heart; (C) MDA concentration in heart. Values in mean ± SE. ROFA-SED, ROFA-AT, Sal-SED, Sal-SED-CrPic, Sal-AT, Sal-AT-CrPic n=8; ROFA-SED-CrPic n=7; ROFA-AT-CrPic n=6.

Figure 5. Oxidative stress in gastrocnemius muscle after 12 weeks of CrPic supplementation and aerobic exercise intervention in rats exposed to ROFA. Analysis of (A) SOD activity in gastrocnemius; (B) CAT activity in gastrocnemius; (C) MDA concentration in gastrocnemius. Values in mean ± SE. ROFA-SED, ROFA-AT, Sal-SED, Sal-SED-CrPic, Sal-AT, Sal-AT-CrPic n=8; ROFA-SED-CrPic n=7; ROFA-AT-CrPic n=6; *$p< 0.05$ vs. ROFA+SED; **$p< 0.05$ vs. ROFA-SED-CrPic; #$p< 0.05$ vs. Sal-SED; †$p< 0.05$ vs. Sal-SED-CrPic.

Figure 1.

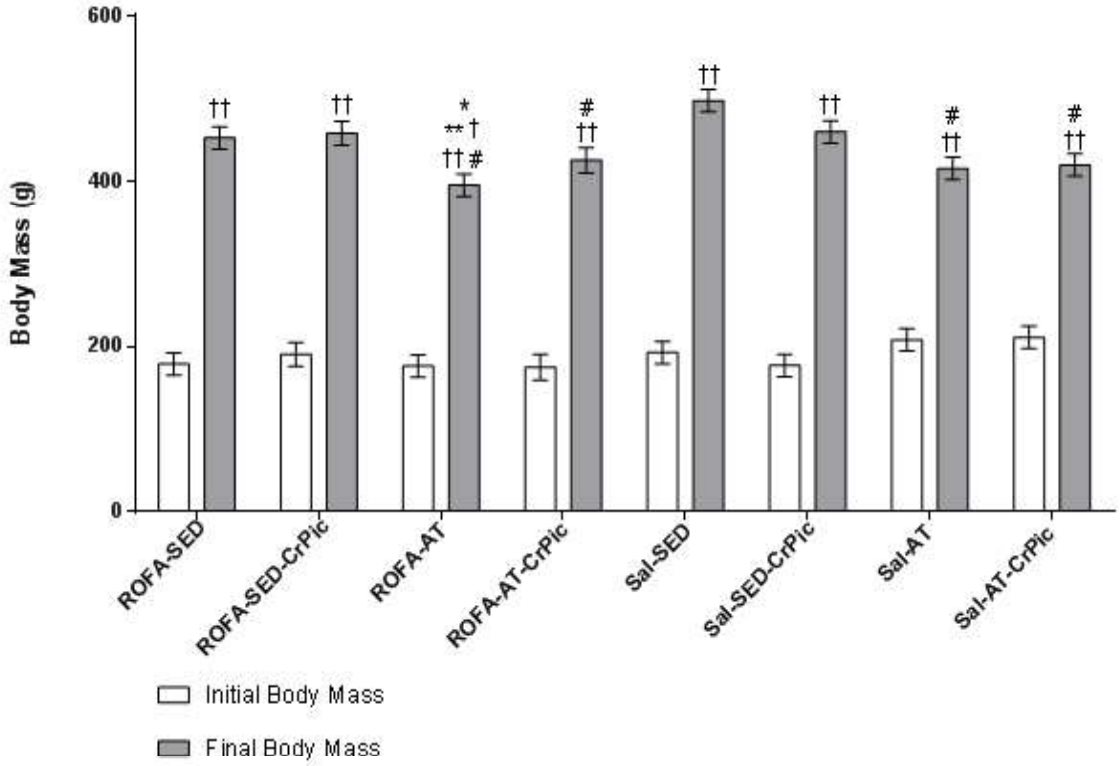

600
400
200
0
Body Mass (g)
††
††
*
**†
††#
#
††
††
#
††
#
††
ROFA-SED
ROFA-SED-CrPic
ROFA-AT
ROFA-AT-CrPic
Sal-SED
Sal-SED-CrPic
Sal-AT
Sal-AT-CrPic
Initial Body Mass
Final Body Mass

Figure 2.

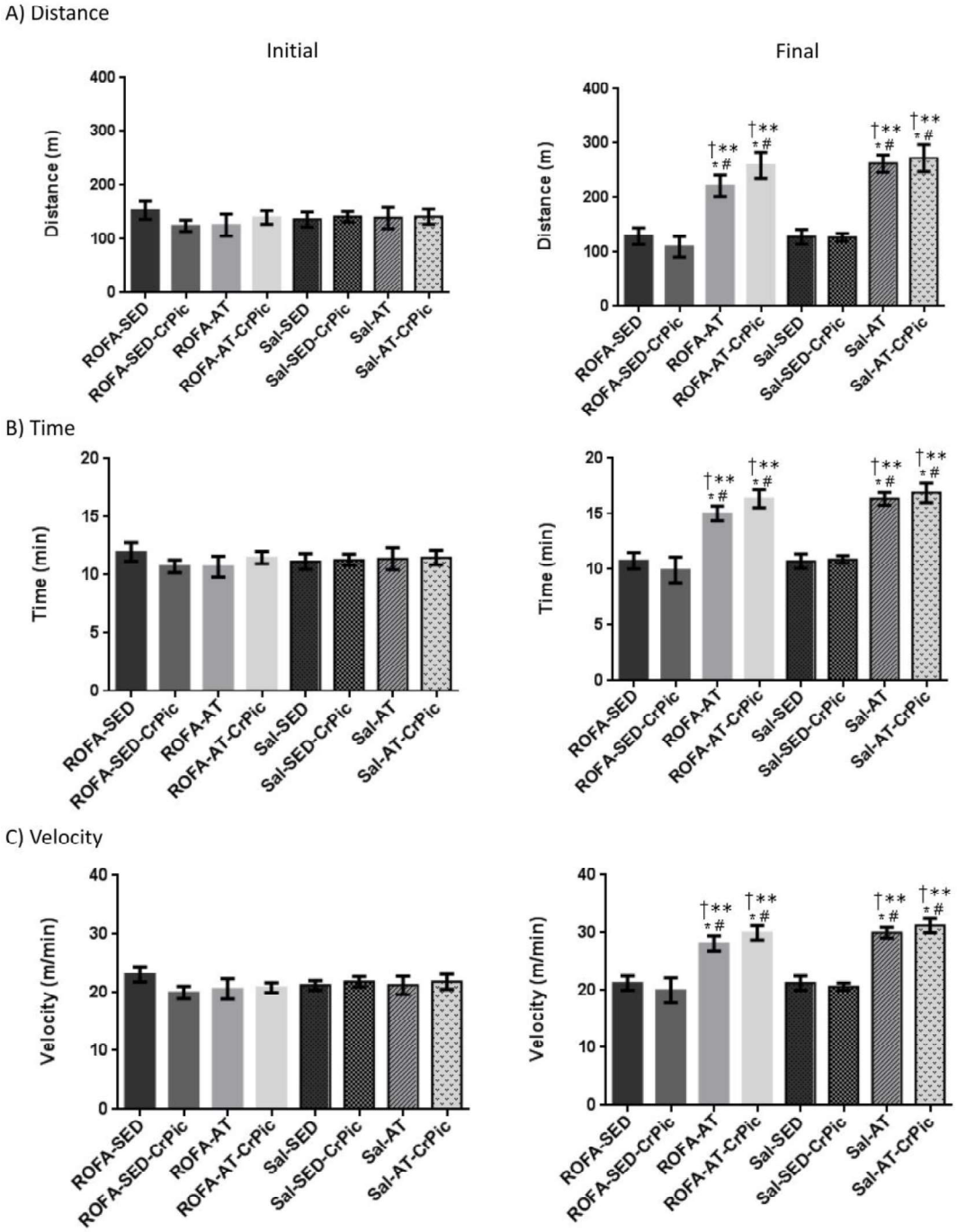

A) Distance
Initial
Final
Distance (m)
B) Time
Time (min)
C) Velocity
Velocity (m/min)
ROFA-SED
ROFA-SED-CrPic
ROFA-AT
ROFA-AT-CrPic
Sal-SED
Sal-SED-CrPic
Sal-AT
Sal-AT-CrPic

Figure 3.

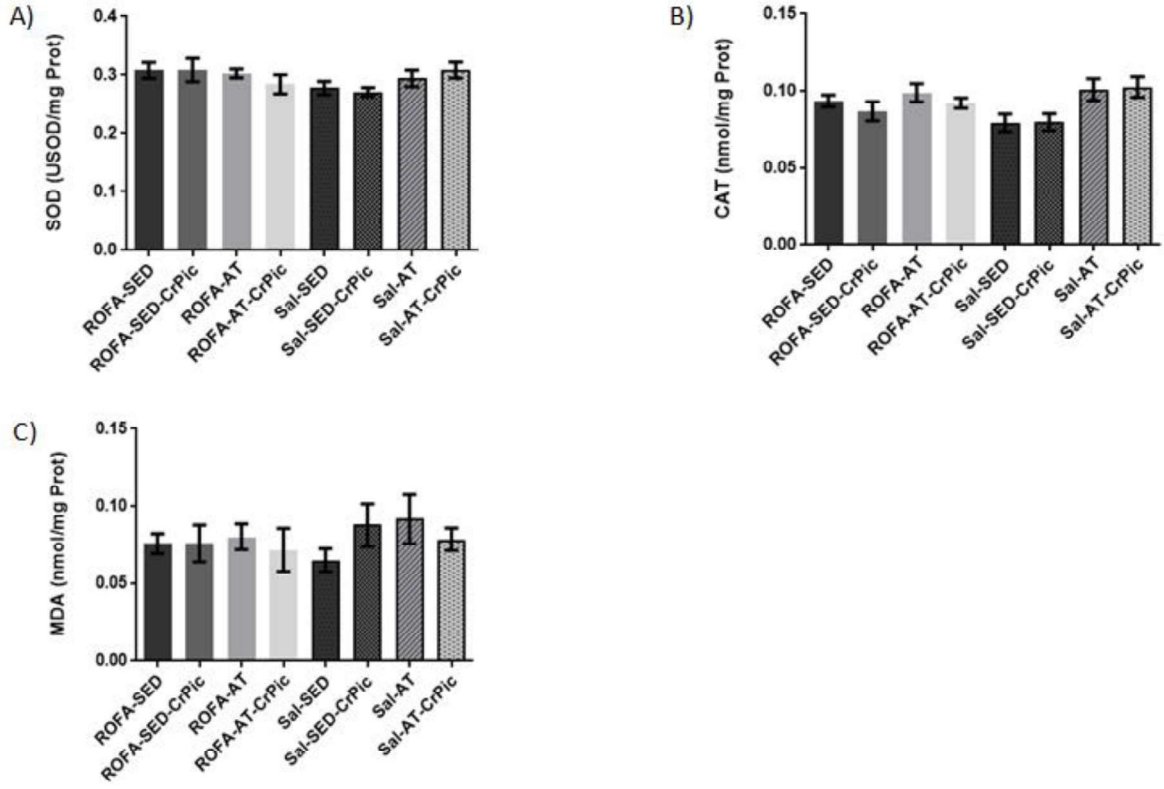

A)
SOD (USOD/mg Prot)
0.4
0.3
0.2
0.1
0.0
ROFA-SED
ROFA-SED-CrPic
ROFA-AT
ROFA-AT-CrPic
Sal-SED
Sal-SED-CrPic
Sal-AT
Sal-AT-CrPic
B)
CAT (nmol/mg Prot)
0.15
0.10
0.05
0.00
ROFA-SED
ROFA-SED-CrPic
ROFA-AT
ROFA-AT-CrPic
Sal-SED
Sal-SED-CrPic
Sal-AT
Sal-AT-CrPic
C)
MDA (nmol/mg Prot)
0.15
0.10
0.05
0.00
ROFA-SED
ROFA-SED-CrPic
ROFA-AT
ROFA-AT-CrPic
Sal-SED
Sal-SED-CrPic
Sal-AT
Sal-AT-CrPic

Figure 4.

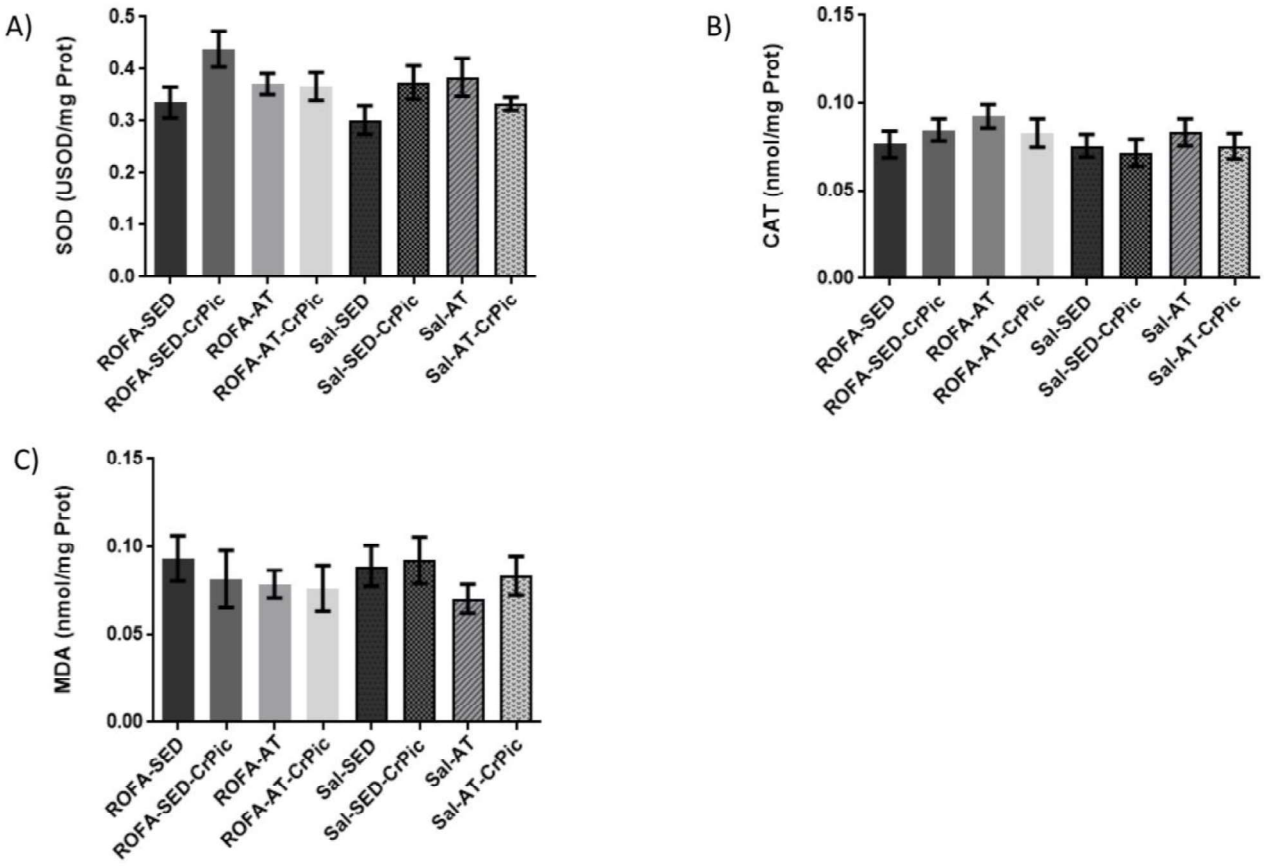
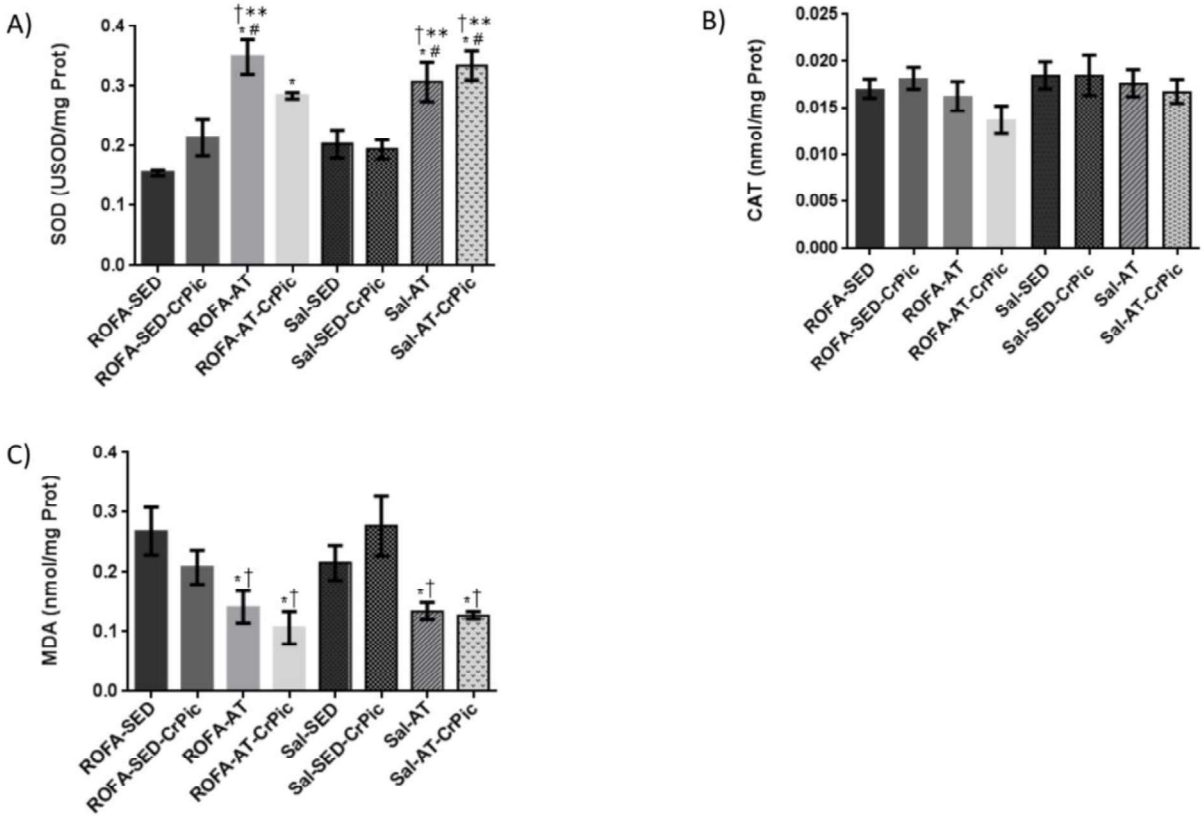

A)
SOD (USOD/mg Prot)
0.5
0.4
0.3
0.2
0.1
0.0
ROFA-SED
ROFA-SED-CrPic
ROFA-AT
ROFA-AT-CrPic
Sal-SED
Sal-SED-CrPic
Sal-AT
Sal-AT-CrPic
B)
CAT (nmol/mg Prot)
0.15
0.10
0.05
0.00
ROFA-SED
ROFA-SED-CrPic
ROFA-AT
ROFA-AT-CrPic
Sal-SED
Sal-SED-CrPic
Sal-AT
Sal-AT-CrPic
C)
MDA (nmol/mg Prot)
0.15
0.10
0.05
0.00
ROFA-SED
ROFA-SED-CrPic
ROFA-AT
ROFA-AT-CrPic
Sal-SED
Sal-SED-CrPic
Sal-AT
Sal-AT-CrPic

Figure 5.


A)
SOD (USOD/mg Prot)
0.0
0.1
0.2
0.3
0.4
ROFA-SED
ROFA-SED-CrPic
ROFA-AT
ROFA-AT-CrPic
Sal-SED
Sal-SED-CrPic
Sal-AT
Sal-AT-CrPic
B)
CAT (nmol/mg Prot)
0.000
0.005
0.010
0.015
0.020
0.025
C)
MDA (nmol/mg Prot)

Supplemental Material

Table S1. ROFA characterization of metals.

Metal	µg/g (mean ± SD)
Pb	3.1 ± 0.09
Al	789.9 ± 23.28
Zn	20.3 ± 0.04
Cd	0.04 ± 0.002
Ba	30.2 ± 0.31
Cu	9.7 ± 0.15
Ni	287.0 ± 10.8
As	4.1 ± 0.05
Se	7.5 ± 0.20
Mn	48.3±0.98
Sr	8.4 ± 0.16
Sb	2.3 ± 0.57
Fe	20,397.2 ± 283.3
Mg	372.5 ± 1.93
P	388.5 ± 255.8
Cr	7.6 ± 0.23

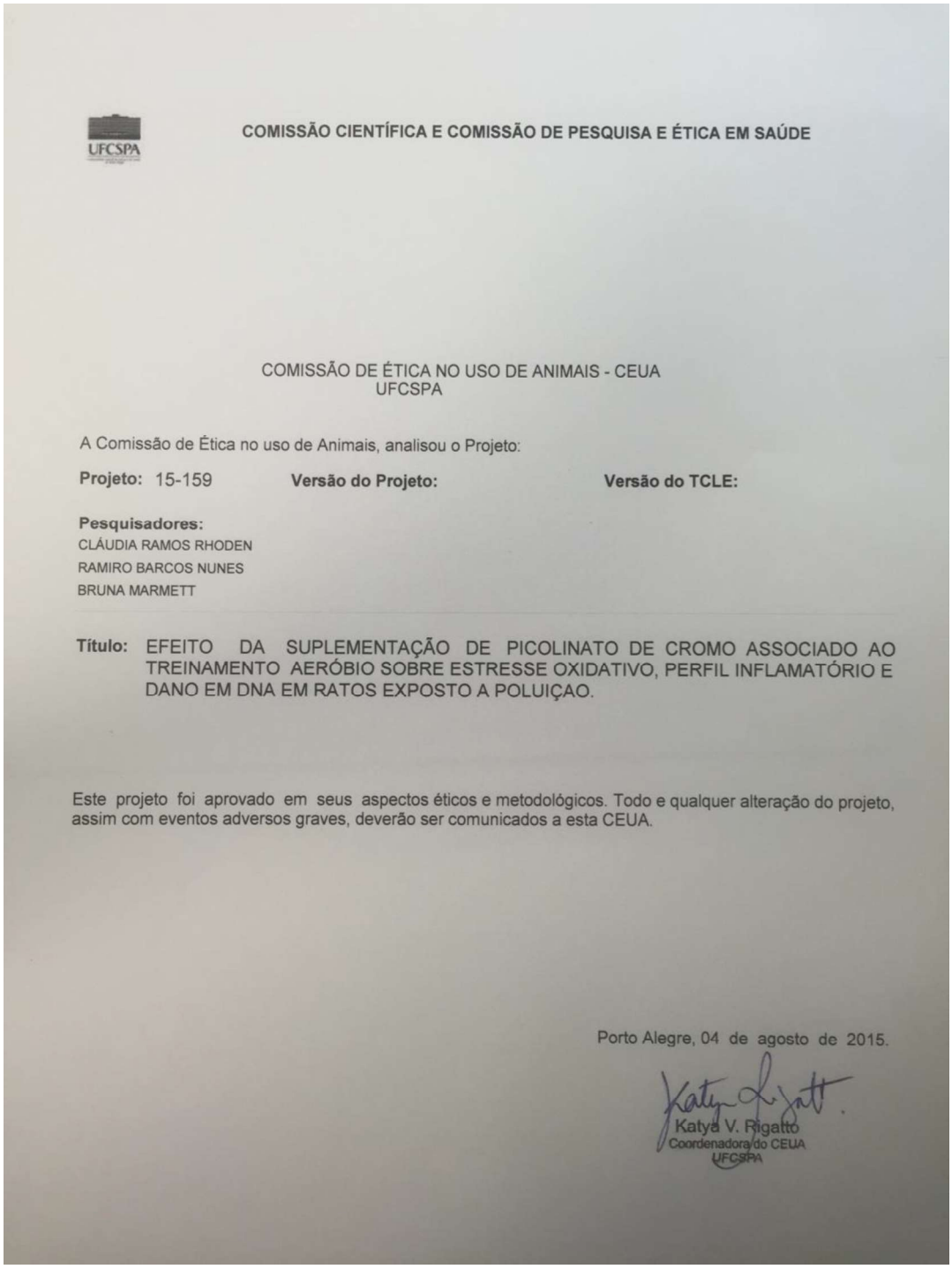
ROFA: Residual Oil Fly Ash; Pb: Lead; Al: Aluminium; Zn: Zinc; Cd: Cadmium; Ba: Barium; Cu: Copper; Ni: Nickel; As: Arsenic; Se: Selenium; Mn: Manganese; Sr: Strontium; Sb:Antimony; Fe: Iron; Mg: Magnesium; P: Phosphorus; Cr:Chromium.

6. CONCLUSÃO

A realização do presente estudo mostrou que a exposição a poluição do ar, mais especificamente o ROFA, de forma subcrônica e na concentração encontrada em cidades poluídas pode causar uma adaptação no pulmão e coração o que evita um dano oxidativo prolongado. Enquanto o exercício aeróbio levou a adaptações positivas no estresse oxidativo no músculo esquelético através do aumento da enzima antioxidante SOD e redução de lipoperoxidação. Neste estudo, a suplementação de CrPic não apresentou efeitos benéficos provavelmente devido a amostra não apresentar depleção das enzimas antioxidantes nem aumento de dano oxidativo a lipídios. Ainda, é importante destacar que os níveis de emissões de poluentes estão crescendo progressivamente, sendo assim essas maiores taxas de emissões podem causar danos mais pronunciados do que a concentração utilizada em nosso estudo. Portanto, mais estudos são necessários a fim de quantificar o aumento na taxa de emissão de poluentes assim como avaliar os danos à saúde causados por essa maior concentração de poluentes.

7. ANEXOS

7.1. ANEXO 1. PARECER DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA)



UFCSPA

COMISSÃO CIENTÍFICA E COMISSÃO DE PESQUISA E ÉTICA EM SAÚDE

COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA
UFCSPA

A Comissão de Ética no uso de Animais, analisou o Projeto:

Projeto: 15-159 **Versão do Projeto:** **Versão do TCLE:**

Pesquisadores:
CLÁUDIA RAMOS RHODEN
RAMIRO BARCOS NUNES
BRUNA MARMETT

Título: EFEITO DA SUPLEMENTAÇÃO DE PICOLINATO DE CROMO ASSOCIADO AO TREINAMENTO AERÓBIO SOBRE ESTRESSE OXIDATIVO, PERFIL INFLAMATÓRIO E DANO EM DNA EM RATOS EXPOSTO A POLUIÇAO.

Este projeto foi aprovado em seus aspectos éticos e metodológicos. Todo e qualquer alteração do projeto, assim com eventos adversos graves, deverão ser comunicados a esta CEUA.

Porto Alegre, 04 de agosto de 2015.

Katya V. Rigatto
Coordenadora do CEUA
UFCSPA

7.2. ANEXO 2. NORMAS DE PUBLICAÇÃO DA REVISTA "*ENVIRONMENTAL HEALTH PERSPECTIVES*"

All papers submitted to *EHP* are evaluated by the editors to determine whether the topic is within the scope of the journal. Papers also are assessed for originality, scientific quality, environmental health significance, clarity of presentation, and conciseness. Before papers are sent for peer review, they are screened for possible plagiarism (see Scientific Integrity below), and authors must submit a Competing Financial Interests Declaration form on behalf of all authors (see Competing Financial Interests below). Papers selected for review are assigned to an Associate Editor, who identifies reviewers and makes recommendations to the Editor-in-Chief. Members of the Editorial Review Board serve as a pool of potential reviewers of papers. Both the Board of Associate Editors and the Editorial Review Board are composed of leading scientists from all segments of the environmental health sciences. The overall acceptance rate of papers submitted to the journal is approximately 15%.

Types of Manuscripts

Manuscripts in the categories below are considered for publication. All manuscripts are peer reviewed except Brief Communications.

Commentaries (recommend length ≤ 5,000 words, excluding references and tables) present information and personal insight on a particular topic. Commentaries should not be extended critiques of single articles appearing in *EHP* or elsewhere. Factual data should be included to substantiate arguments. *EHP* reserves the right to reject Commentaries without review if they are perceived as being too polemic or personal in nature. *EHP* also reserves the right to propose that Commentaries be reviewed as one side of a point/counterpoint debate. Assuming the original author agrees, *EHP* will ask another author to address the opposite side of an argument. If both papers are accepted, *EHP* will publish them together. Manuscripts on ethical, legal, social, or policy issues may also be accepted in this category.

Research Articles (suggested length ≤ 7,000 words, excluding references, tables, figure legends, acknowledgments, and supplementary material) report original scientific research and discovery. Research Articles may come from any field of scientific research with direct relevance to the study of human health and the environment.

Substantive Reviews (suggested length ≤ 10,000 words, excluding references, tables, and figures) provide an overview, integration of information, and critical analysis of a particular field of research or theme related to environmental health sciences. Previous research should be comprehensively reviewed regardless of whether the findings are consistent with expectations or the review authors' hypotheses. It is appropriate for authors to discuss the strengths and weaknesses of individual studies, focus on high-quality studies that add to the weight of the evidence on the topic under review, identify information gaps, and make recommendations for future research. Lengthy historical perspectives generally are not appropriate.

Quantitative Reviews and Meta-Analyses (recommended length ≤ 10,000 words, excluding references, tables, and figures) present, contrast, and (when appropriate) combine data across studies to address a specific study question related to environmental health. Inclusion criteria and strategies used to search the literature systematically should be explicitly described, along with analytic methods used to evaluate or combine data. The potential for publication bias and heterogeneity among studies should be investigated, and graphical displays of data contributed by individual studies are encouraged. The strengths and weaknesses of individual studies and potential causes of discordant findings among studies also should be discussed. As with Substantive Reviews, authors should integrate and critically analyze information from previous research, identify information gaps, and make recommendations for future research.

Reviews Based on Meetings or Conferences (suggested length ≤ 7,000 words, excluding references, tables, and figures) should review the state of the science for a particular area, identify research gaps and needs, and explain how the outcome of the meeting or conference addresses those gaps and needs. These reviews should focus on the science or theme but not on the conference or meeting itself. *De novo* data, participant lists, dialogue of workgroups or committees, and discussion of the internal organization of the meeting are not allowed. These papers

should be submitted to *EHP* no more than 1 year after the meeting or conference takes place. Prospective authors should consult with the Editor-in-Chief before submitting a review based on a meeting or conference.

Brief Communications (≤ 3,000 words, excluding references, tables, and figures) are short scholarly reports that provide timely information of interest to the broad environmental health community. They may be used to highlight the importance of new environmental health programs or agencies or the advantages of new research approaches in the context of knowledge gaps; or to raise awareness of and make recommendations for addressing contemporary or emerging environmental health problems. A Brief Communication may take the form of a statement from an organization or group concerning the need for action on an environmental health issue (typically with recommendations). Authors should contact the Editor-in-Chief in advance for permission to submit. Brief Communications are reviewed internally for relevance, importance, and clarity, and are published in the Perspectives section of *EHP*. They are assigned a DOI number and indexed in PubMed/MEDLINE. Formatting requirements, including references and any tables or figures, are consistent with those for *EHP* Research Articles, with the exception of the abstract, which must be unstructured (without subheadings) and ≤ 200 words. In addition, Supplemental Material is not allowed.

Originality of Submission

Contributions submitted to *EHP* must be original works of the author(s) and must not have been previously published in print or online or simultaneously submitted to another publication. Previously published material (e.g., figures, tables) may be included in Commentaries and Reviews, assuming the original authors have given permission to reproduce the material and all copyright issues have been resolved. For original Research Articles, previously published schemata or illustrative figures are acceptable with the proper attribution and permission. Text or narrative from guidance documents, technical reports, and position papers by various government and nongovernmental organizations may be considered if they include new information. *EHP* will consider papers from dissertations that have been published in their entirety by a university in partial fulfillment of a degree. Manuscripts presented at a scientific meeting but not published in full or under review for

publication elsewhere also will be considered. As indicated in *Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication* [International Committee of Medical Journal Editors (http://www.icmje.org/urm_full.pdf)], it is the responsibility of the author to make a full statement to the editor concerning materials in a manuscript that might be considered redundant or duplicative. For additional clarification, please contact the Editor-in-Chief.

Scientific Integrity

EHP requires assurances (both in submission comments and in the methods section of the manuscript) that animals used in a study have been treated humanely and with regard for the alleviation of suffering, and that the protocol was approved by an institutional animal care and use committee. Research involving humans must have been conducted according to the Common Rule (http://ori.dhhs.gov/education/products/ucla/chapter2/page04b.htm). Research involving humans also must be approved by an appropriate institutional review board and comply with all relevant national, state, and local regulations. For research conducted outside the United States and thus exempt from U.S. federal regulations, authors must perform the research in accordance with principles of the Declaration of Helsinki (http://www.wma.net/en/30publications/10policies/b3/). Approval and compliance with research requirements regarding human subjects must be noted, and information regarding informed consent procedures must be described in the "Methods" section of manuscripts concerning human subjects research.

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EHP anticipates receiving submissions on research that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health and safety, agriculture, plants, animals, or the environment (also known as dual-use research). Papers flagged for dual-use issues by *EHP* editors will undergo an additional level of review concerning the implications to society of publishing such a paper, and *EHP* reserves the right to seek expert advice in such cases. Authors should be aware that *EHP* could determine that the risks to public health and safety of publishing the paper outweigh the benefits of publishing, even if the paper has otherwise been deemed acceptable for publication.

Suggested Guidelines

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Competing Financial Interests

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All research papers, reviews, and commentaries must include a structured abstract of ≤ 250 words, which should not contain references. No information should be reported in the abstract that does not appear in the text of the manuscript. In general we recommend that authors indicate study names or sources of data that are integral to the study in the title or abstract. Conclusions should mention the relevance of the work to environmental health science. Headings to be used in the structured abstracts vary by article type as described below:

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EHP covers all disciplines engaged in the broad field of environmental health sciences. Therefore, authors should write in a clear and simple manner, in the active

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Whenever possible, provide an estimate of variability or precision when reporting measures of association or central tendency (e.g., confidence intervals, standard deviations, interquartile ranges), regardless of whether *p*-values are also reported for these estimates.

All manuscripts should be submitted in English. American spelling should be used throughout except for direct quotations from primary sources. For example, use "analyze," not "analyse"; "behavior," not "behaviour"; "modeling," not "modelling"; and "fulfill," not "fulfil." Please consult *Merriam-Webster's Collegiate Dictionary, 11th Edition*, for guidance. Proper names containing British spelling should be expressed as given (e.g., "BC Centre for Disease Control"; "Minister of Employment, Workforce, and Labour").

Abbreviations

All abbreviations, including abbreviations for elements (e.g., Fe, Cu) and chemical compounds [e.g., polychlorinated biphenyls (PCBs), carbon dioxide (CO_2)], should be defined in the text on first use with abbreviations used thereafter.

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Sample Alphabetical List

Slotkin TA. 2004a. Cholinergic systems in brain development and disruption by neurotoxicants: nicotine, environmental tobacco smoke, organophosphates. Toxicol Appl Pharmacol 198:132–151.

Slotkin TA. 2004b. Guidelines for developmental neurotoxicity and their impact on organophosphate pesticides: a personal view from an academic perspective. Neurotoxicology 25:631–640.

Slotkin TA. 2005. Developmental neurotoxicity of organophosphates: a case study of chlorpyrifos. In: Toxicity of Organophosphate and Carbamate Pesticides (Gupta RC, ed). San Diego:Elsevier Academic Press, 293–314.

Slotkin TA, MacKillop EA, Ryde IT, Tate CA, Seidler FJ. 2007. Screening for developmental neurotoxicity using PC12 cells: comparisons of organophosphates with a carbamate, an organochlorine and divalent nickel. Environ Health Perspect 115:93–101.

Slotkin TA, Persons D, Slepetis RJ, Taylor D, Bartolome J. 1984. Control of nucleic acid and protein synthesis in developing brain, kidney, and heart of the neonatal rat: effects of a difluoromethylornithine, a specific, irreversible inhibitor of ornithine decarboxylase. Teratology 30:211–224.

Slotkin TA, Seidler FJ. 2007. Comparative developmental neurotoxicity of organophosphates in vivo: transcriptional responses of pathways for brain cell development, cell signaling, cytotoxicity and neurotransmitter systems. Brain Res Bull 72:232–274.

Footnotes

Do not use footnotes. Place all textual information within the manuscript and all references in the proper form both in text and in the reference list.

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Each table must begin on a new page after the References. Tables must be numbered with Arabic numerals, followed by a brief title (not to exceed 25 words). Tables should contain no more than two layers of column headings. A column heading must be provided for each column. Additional column heads should not be placed in the middle of a table. Tables must be created using the Table feature in Microsoft Word. List abbreviations and definitions under each table. Type footnotes directly after the abbreviations, beginning on the next line. General footnotes to tables must be indicated by lowercase superscript letters beginning with “a” for each table. Footnotes indicating statistical significance must be identified in the following order: asterisks (*, **), number signs (#, ##), and daggers (†, ††). The comparison to

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TYPES OF REFERENCES

Journal article—conventional reference:

Waalkes MP, Liu J, Diwan BA. 2007. Transplacental arsenic carcinogenesis in mice. Toxicol Appl Pharmacol 222:271–280.

Journal article—advance publication:

Latendresse JR, Bucci TJ, Olson G, Mellick P, Weiss C, Thorn B, et al. 2009. Genistein and ethinyl estradiol dietary exposures in multigenerational and chronic studies induce similar poliferative lesions in mammary gland of male Sprague-Dawley rats. Reprod Toxicol; doi:10.1016/j.reprotox.2009.04.006 [Online 19 April 2009].

Journal article—published online only:

Glas AM, Floore A, Delahaye LJ, Witteveen AT, Pover RC, Bakx N, et al. 2006. Converting a breast cancer microarray signature into a high-throughput diagnostic test. BMC Genomics 7:278; doi:10.1186/1471-2164-7-278.

Journal article, "in press":

Holmes AK, Maisonet M, Rubin C, Kieszak S, Barr DB, Calafat AM, et al. In press. A pilot study of exposures to endocrine-disrupting compounds in pregnant women and children from the United Kingdom. Int J Child Adolesc Health.

Article in non-English language:

Rateau JG, Broillard M, Morgant G, Aymard P. 1986. Etude experimental chez le lapin de l'effet de la cholestyramine dans le traitement des diarrhees infectieuses d'orgine cholerique [in French]. Actualite Therapeut 22:289–296.

Magazine article:

Grant M. 1997. The cell from hell. People, 19 May:101–103.

Newspaper article:

Clabby C. 2001. Study details how centuries of fishing depleted sea life. News and Observer (Raleigh, NC) 27 July: B1.

Book:

Luna LG. 1968. Manual of Histopathologic Staining Methods of the Armed Forces Institute of Pathology. 3rd ed. New York:McGraw-Hill.

Book, edited:

Gross TL, Ihrke PJ, Walder EJ, eds. 1992. Veterinary Dermatopathology. St. Louis, MO:Mosby Year Book.

Chapter in edited book:

Gurevitch J, Hedges LV. 1993. Meta-analysis: combining the results of independent experiments. In: The Design and Analysis of Ecological Experiments (Scheiner SM, Gurevitch J, eds). New York:Chapman & Hall, 378–398.

Book chapter, "in press":

McCoy KA, Guillette LJ. In press. Endocrine disruptors. In: Amphibian Biology. Vol 8. Conservation and Decline of Amphibians (Heatwole HF, ed). Chipping Norton, New South Wales, Australia:Surrey Beatty & Sons.

Agency monograph:

IARC (International Agency for Research on Cancer). 1993. Cadmium and cadmium compounds. IARC Monogr Eval Carcinog Risk Hum 58:119–237.

Agency as author:

CDC (Centers for Disease Control and Prevention). 2005. Fourth National Report on Human Exposure to Environmental Chemicals. Atlanta, GA:Centers for Disease Control and Prevention. Available:http://www.cdc.gov/exposurereport/ [accessed 14 January 2010].

Proceedings:

Ibrahim K. 1994. The status of marine turtle conservation in Peninsular Malaysia. In: Proceedings of the first ASEAN Symposium Workshop on Marine Turtle Conservation, 6–10 December 1993, Manila, Philippines (Nacu A, Trono R, Palma JA, Torres D, Agas F Jr, eds). Manila, Philippines:ASEAN, 87–103.

Technical paper:

NTP. 2006. Toxicology and Carcinogenesis Studies of Bromodichloromethane (CAS No. 75-27-4) in Male F344/N Rats and Female B6C3F$_1$ Mice (Drinking Water Studies). TR 532. Research Triangle Park, NC:National Toxicology Program.

Dissertation/thesis

Gelobter M. 1993. Race, Class, and Outdoor Air Pollution: The Dynamics of Environmental Discrimination from 1970 to 1990 [PhD Dissertation]. Berkeley, CA:University of California, Berkeley.

Software manual:

SAS Institute Inc. 2001. SAS/STAT Guide for Personal Computers, Version 8. Cary, NC:SAS Institute, Inc.

Website:

CDC (Centers for Disease Control and Prevention). 2003. National Health and Nutrition Examination Survey Homepage. Available: http://www.cdc.gov/nchs/nhanes.htm [accessed 6 August 2008].

Online database:

National Center for Biotechnology Information. 2011. PubMed. Available:http://www.ncbi.nlm.nih.gov/pubmed/ [accessed 14 July 2011].

Abstract:

Barbeito AG, Guelfi N, Varga MR, Pehar M, Beckman J, Barbeito L, et al. 2005. Chronic low-level lead exposure increases survival of G93A SOD-1 transgenic mice [Abstract]. In: Amyotrophic Lateral Sclerosis: Beyond the Motor Neuron. Available:http://iibce.edu.uy/ALSmeeting/abstract.htm [accessed 14 April 2008].

Federal regulation:

U.S. Environmental Protection Agency. 2001. National primary drinking water regulations. Arsenic and clarifications to compliance and new source contaminants monitoring. Final rule. Fed Reg 66:6076–7066.

Executive order; federal regulation:

Clinton WJ. 2000. Executive Order 13148. Greening of the government through leadership in environmental management. Fed Reg 65:24595–24606.

U.S. Government document:

U.S. Environmental Protection Agency. 2004. Air Quality Criteria for Particulate Matter. EPA/600/P-99/002aF. Research Triangle Park, NC:U.S. Environmental Protection Agency.

State document:

State of Maryland. 1998. Water Quality Improvement Act of 1998. Annapolis, MD:General Assembly.

Law:

Food Quality Protection Act of 1996. 1996. Public Law 104-170.

Court case:

Leach *v*. E.I. du Pont de Nemours & Co. 2002. Civil Action No. 01-C-608, 2002 WL 1270121. Circuit Court of Wood County, West Virginia, 10 April 2002.

ABBREVIATIONS

All nonstandard abbreviations [e.g., organochlorine (OC) pesticides, limit of detection (LOD), polymerase chain reaction (PCR)] and abbreviations for elements (e.g., Fe, Cu, Ag) and chemical compounds [e.g., polychlorinated biphenyls (PCBs), carbon dioxide (CO_2)] should be defined in the text on first use and abbreviated thereafter.

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