PEDRO PAULO MENEZES SCARIOT

DETERMINAÇÃO DA ATIVIDADE ESPONTÂNEA DE RATOS POR GRAVIMETRIA E RELAÇÕES COM A CAPACIDADE AERÓBIA E EXPRESSÃO GÊNICA DE MCTs 1 E 4: EFEITOS DA IDADE E DO TREINAMENTO FÍSICO

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Resumo

A locomoção representa um comportamento extremamente importante e primordial para a vida animal. Diante disso, torna-se notório que o restrito confinamento imposto aos animais de laboratório pode causar negativas implicações fisiológicas, uma vez que diversos comportamentos locomotores naturais são suprimidos. Baseado nessa problemática, desejamos verificar se animais alojados em gaiolas convencionais possuem piores indicadores de desempenho ao exercício, quando comparados a animais expostos a condições aumentadas de espaço físico. Além disso, buscamos analisar se a característica do espaço físico das gaiolas representa um fator mais relevante que a própria aplicação do exercício, haja vista que protocolos de treinamento físico em modelos animais não conseguem promover melhoras expressivas da capacidade aeróbia ao longo da idade. Em outra temática, a locomoção também se apresenta intimamente relacionada a aspectos genéticos, uma vez que marcantes diferenças individuais são distinguidas quando rodas de atividades são disponibilizadas para animais de laboratório. Entendendo que o aspecto genético é de suma importância nesse contexto, nós supomos que animais mais ativos na gaiola, exibiriam maiores vantagens metabólicas e genéticas para a prática de exercício quando comparados a animais mais inativos. Diante disso, buscamos explorar se as atividades espontâneas e voluntárias dos animais já refletem propensões para melhores desempenhos físicos. Embora pareçam similares, tais atividades são consideradas distintas pela literatura, e carecem de serem relacionadas com a capacidade aeróbia, e com respostas moleculares envolvidas com a performance. Tendo em vista todos os temas abordados, o objetivo geral do presente projeto baseia-se em verificar a influência do espaço físico da gaiola, bem como sua interação com as duas principais intervenções experimentais científicas (treinamento físico ou livre acesso à roda de atividade) sobre respostas fisiológicas e moleculares relacionadas com o metabolismo aeróbio e anaeróbio, composição corporal e estresse em ratos ao longo da idade (60, 90 e 150 dias). Além disso, verificar as relações entre a atividade espontânea e voluntária com parâmetros fisiológicos envolvidos com a performance no exercício.

Palavras chaves: *Ratos, Fisiologia, Locomoção, Exercício*

Abstract

Locomotion is extremely important and essential for animal life behavior. Thus, it becomes clear that the restricted confinement and therefore the suppression of many locomotors behaviors imposed to laboratory animals can cause negative physiological implications. Based on these problems, we verified if animals housed in conventional cages have worse exercise performance indicators when compared to animals exposed in increased housing space. Furthermore, we analyzed if the physical space of the cages is a more important factor than implementation of the exercise, given that physical training protocols in animal models fail to promote improvements in aerobic capacity over the age. On another topic, locomotion is closely related to genetic factors. We assumed that animals more active in the cage, exhibit higher metabolic and genetic advantages for the practice of exercise when compared to inactive animals. Therefore, we investigated whether the spontaneous and voluntary activities of animals already reflect propensities for better physical performance. Although they look similar, these activities are considered distinct in the literature. There is scarce information about the relationship of such with aerobic capacity and molecular responses involved with the performance. The overall goal of this project is based on checking the influence of the housing space of the cage as well as its interaction with the two main scientific experimental interventions (physical training or free access to activity wheel) on physiological and molecular responses related aerobic and anaerobic metabolism, body composition and stress in rats along age. In addition, to check the relationship between spontaneous and voluntary activity with physiological parameters involved with the exercise performance.

Key Words: *Rats, Physiology, Locomotion, Exercise*

Sumário

Vencer não é nada, se não se teve muito esforço; fracassar não é nada se foi realizado o melhor possível

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1. INTRODUÇÃO GERAL

Apesar da similaridade de algumas características fisiológicas com os seres humanos, os roedores, assim como a maioria dos animais, possuem atributos diferenciados em relação à locomoção. Isto revela ser relevante, uma vez que negativas implicações fisiológicas podem ser ocasionadas pelos hábitos sedentários induzidos pela constante exposição a restritos confinamentos. Tal afirmação torna-se clara ao observar que, protocolos de treinamento físico aplicados em roedores não conseguem promover melhoras "expressivas" da capacidade aeróbia (Chimin et al., 2009; De Araujo et al., 2012a; Pica e Brooks, 1982). Diante dessa problemática, De Araujo et al. (2012a) especulam que a redução do desempenho aeróbio e anaeróbio é ocasionada pelo longo período que os animais são mantidos em pequenas gaiolas, as quais constantemente induzem uma redução da atividade espontânea dos animais. Além disso, tais autores sugerem que para minimizar os efeitos do sedentarismo durante o período experimental, as gaiolas devem permitir espaço suficiente para a atividade habitual do animal, de maneira próxima como se comportam na natureza.

De fato, tal especulação apresentada acima é altamente pertinente dada a grande importância evolutiva da locomoção animal. Baseado nos estudos de Garland et al. (2011), é evidente que a locomoção representa uma característica primordial da vida diária dos animais (busca de alimento, abrigo, interação com os concorrentes e escape de predadores, migração). Ainda, Garland et al. (2011), enfatizam que a atividade espontânea possui grande contribuição sobre o gasto energético para roedores, sendo que tal atividade assemelha-se nesse quesito, ao exercício físico. Contudo, apesar do conhecimento da essencial importância da locomoção na vida animal, tais comportamentos naturais não são permitidos aos roedores de laboratório, pois os mesmos são persistentemente confinados em pequenas gaiolas. Diante do exposto, parece óbvio o motivo da falta de sucesso em encontrar modificações positivas da capacidade

aeróbia e anaeróbia com a intervenção do exercício físico crônico. Supostamente parece que animais submetidos a treinamento físico se tornam simplesmente menos sedentários, e não de fato treinados. Assim, a abordagem do espaço físico da gaiola representa um interessante meio de exploração científica. Isso é pertinente, uma vez que ambientes contendo maior espaço físico conseguem promover interessantes respostas fisiológicas em animais de laboratório, tais como, menor ganho de peso e maiores níveis de atividade locomotora, atividade enzimática muscular e conteúdo de glicogênio (Spangenberg et al., 2005).

Na literatura podemos definir a atividade voluntária como uma atividade locomotora que não é diretamente necessária para a sobrevivência ou homeostase e não diretamente motivada por qualquer fator externo (Garland et al., 2011). Com base neste conceito, a motivação específica para a prática de determinadas modalidades esportivas podem facilmente denotar a atividade voluntária em humanos, sendo que tal motivação é intimamente ligada a traços de personalidade (Rhodes e Smith, 2006). O uso da roda de atividade (running wheel) é comumente adotado em roedores de laboratório com o intuito de quantificar diferenças existentes na motivação intrínseca para a realização de exercício, bem como mensurar a atividade voluntária do animal (Sherwin, 1998). Ainda no que tange a atividade voluntária, evidências relatam que a corrida em rodas de atividade pode ser viciante em roedores (Werme et al., 2000), bem como pode promover alterações no comportamento (Malisch et al., 2009) e na atividade neuronal de regiões específicas (Rhodes et al., 2003). Apesar da elevada relevância da atividade voluntária, vale destacar que os animais podem se engajar em outras formas de locomoção, dentre as quais se destaca a atividade espontânea (Ravussin et al., 1986). Para alguns autores, a atividade espontânea, também compreendida como non-exercise activity thermogenesis ou NEAT, é considerada uma importante forma de gasto energético que

inclui atividades de vida diária, inquietação muscular, contração espontânea, manutenção de postura, as quais por sua vez não se classificam como exercício voluntário (Levine et al., 1999).

Não distante, a locomoção também se apresenta como um comportamento intimamente relacionado a aspectos genéticos. Evidências apontam marcantes diferenças individuais no comportamento locomotor entre animais de laboratório, haja vista que essas manifestações individuais são notavelmente distinguidas quando rodas de atividades são disponibilizadas para tais. A literatura aponta que tais fenômenos são atribuídos a aspectos genéticos, os quais por sua vez podem estar conectados com a capacidade motivacional ou propensão fisiológica para a realização da atividade física (Friedman et al., 1992). Dessa forma, entendendo que o comportamento motivacional e a capacidade fisiológica para o exercício são derivados de aspectos genéticos, nós hipotetizamos que tais diferenças já sejam refletidas na atividade espontânea e voluntária do animal, mesmo sem o efeito de alguma intervenção experimental, supondo que os animais mais ativos na gaiola, apresentariam maiores vantagens metabólicas e genéticas para a prática de exercício quando comparados a animais mais inativos. Mediante isso, acreditamos que as análises de expressão gênica de proteínas podem ser altamente sensíveis para detectar mínimas diferenças interindividuais. Dentre a infinidade de mecanismos moleculares existentes no organismo, os transportadores monocarboxílicos (MCTs) se destacam na atuação do transporte do lactato a nível celular e que por sua vez apresentam íntima relação com a capacidade aeróbia (Dubouchaud et al., 2000). Apesar de mecanismos que envolvem a atuação do exercício já serem bem explorados, respostas da expressão gênica dos MCTs frente a tópicos relacionados com a locomoção de roedores de laboratório ainda necessitam ser compreendidos. Embora as atividades espontânea e voluntária sejam

amplamente estudadas em áreas comportamentais, tais variáveis são pouco relacionadas com a expressão de MCT 1 e 4 em roedores.

Tendo em vista sua importância, o presente estudo se justifica primordialmente pela necessidade de conhecer a influência do espaço físico da gaiola sobre variáveis fisiológicas envolvidas com a capacidade aeróbia e composição corporal, estas que por sua vez estão intimamente envolvidas com aspectos relacionados ao desempenho no exercício bem como também a eventos patológicos, respectivamente. Nesse sentido, com a realização do presente estudo será possível responder questões pertinentes para pesquisadores que utilizam o exercício como uma intervenção experimental não medicamentosa, possibilitando assim compreender se as respostas fisiológicas oriundas do exercício físico são inibidas devido à forma de confinamento imposto. Assim, o presente projeto pretenderá responder as seguintes questões: I) Animais submetidos a confinamentos em gaiolas convencionais possuem menores capacidades aeróbias quando comparados a animais expostos a um amplo espaço físico com maiores possibilidades de locomoção? II) As características do espaço físico das gaiolas são fatores mais relevantes que a própria aplicação de exercício em modelos animais? III) A capacidade aeróbia se altera ao longo da idade e tem relação com o espaço físico?

2. OBJETIVOS

2.1. Objetivo geral

Analisar a influência do espaço físico da gaiola, bem como sua interação com treinamento físico ou livre acesso à roda de atividade sobre respostas fisiológicas e moleculares relacionadas com o metabolismo aeróbio e anaeróbio, composição corporal e estresse em ratos ao longo da idade (60, 90 e 150 dias de idade). Adicionalmente, verificar as relações existentes entre a atividade espontânea e voluntária com parâmetros indicadores de performance no exercício.

2.2. Objetivos específicos

- *Investigar se a oferta de maior espaço físico da gaiola pode atenuar o declínio da capacidade aeróbia e anaeróbia ocasionado pelo aumento da idade em roedores;*
- *Averiguar os efeitos crônicos de dois tipos de confinamento (espaço convencional vs espaço amplo) sobre parâmetros aeróbios e anaeróbios determinados pelo teste de lactato mínimo, expressão gênica de transportadores monocarboxílicos (MCTs 1 e 4), e conteúdo do tecido adiposo branco e marrom;*
- *Verificar se a inserção do treinamento físico ou roda de atividade podem modular os efeitos do confinamento;*
- *Mensurar as concentrações circulantes de corticosterona como marcador de estresse nos diferentes tipos de confinamento e intervenções estudadas.*
- *Determinar a atividade espontânea dos animais por meio do sistema gravimétrico e quantificar a as rotações da roda de atividade como marcador de atividade voluntária;*

- *Correlacionar os níveis de atividade espontânea e voluntária com indicadores aeróbios e anaeróbios determinados pelo teste de lactato mínimo e níveis de expressão gênica de MCTs 1 e 4;*

3. REVISÃO DE LITERATURA

3.1. Locomoção

Mamíferos gastam energia por meio de diversas maneiras, incluindo em processos de atividade celular, digestão, termoregulação, crescimento e reprodução, sendo que estes processos podem variar substancialmente entre espécies e indivíduos (Garland et al., 2011). Além desses processos supracitados, o gasto energético advindo da locomoção constitui uma parcela altamente significativa (Rezende et al., 2009). A locomoção representa um componente primordial, e que na maioria das espécies de mamíferos, estabelece um elemento importante da vida, tais como busca de alimento, abrigo, interação e escape de predadores e migração (Garland et al., 2011; Rezende et al., 2009).

Tais questões referentes a locomoção valem para os seres humanos, os quais como mamiferos possuem tais atributos indispensáveis e extremamente importantes. Isso torna-se notório uma vez que de forma curiosa, o crescente aumento da inatividade física foi concomitante com o surgimento de várias doenças endócrino-metabólicas na sociedade contemporânea de maneira sem precedentes (Booth e Laye, 2010; Katzmarzyk e Janssen, 2004). A relação direta existente entre inatividade física e doenças crônicas revela ser tão intrigante que alguns pesquisadores têm dedicado atenção em postular algumas hipóteses que explicam mecanismos e adaptações com o intuito de compreender tal temática (Booth et al., 2000; Booth e Laye, 2010). Tendo em vista que doenças endócrino-metabólicas não existiam

nos primórdios, é coerente pensar que inatividade física observada nos indivíduos contemporâneos é fisiologicamente anormal. De fato, a elevada demanda de atividade física prevaleceu ao longo da maioria da história humana. Nossos antepassados evoluíram em um contexto muito mais fisicamente exigente, onde era requerido ao ser humano um estilo de vida nômade e preparado para caça ou fuga. Vale destacar que a atividade física para esses indivíduos era normal e obrigatória para sobrevivência a fim de reunirem seus próprios recursos para a obtenção de alimento, abrigo e defesa (Booth e Laye, 2010). Além disso, é valido destacar que o sucesso na sobrevivência dependia ou estava conectado a melhores capacidades físicas, as quais eram extremamente relevantes naquele contexto. Contudo uma constante disponibilidade de alimentos associada com baixa atividade física observada em indivíduos contemporâneos foi um fator desencadeador para a ocorrência de um desajuste metabólico e o consequente aparecimento de doenças metabólicas, tais como a obesidade.

De maneira bastante similar, tal assunto se repete em animais de laboratório, os quais são armazenados e privados à restritos espaços em gaiolas e de certa forma confinados em um "ambiente obesogênico" o qual é caracterizado pela fácil alimentação disponível (ad libitum) e pela escassa ou quase nenhuma movimentação pela busca de alimento ou outros recursos (Garland et al., 2011). Dessa forma, alguns autores sugerem que os humanos modernos das sociedades industrializadas bem como os animais de laboratório vivem em ambientes "não naturais", uma vez que a locomoção diária tornou-se relativamente baixa em relação aos seus respectivos antepassados (Booth et al., 2002a; Booth et al., 2002b).

3.2. Animais em pesquisas científicas

A busca do conhecimento aprofundado sobre comportamentos fisiológicos e patológicos envolvendo humanos foram ao longo da história muitas vezes limitados devido a dificuldades

referentes às análises invasivas e controle de condições experimentais. Por conta disso, estudos envolvendo o uso de animais, ganharam elevado destaque a fim de produzir conhecimento científico, elucidando fenômenos fisiológicos e patológicos aos seres humanos. De fato, investigações envolvendo o uso de animais são realizadas provavelmente há mais de dois mil anos, com os estudos de Aristóteles, Galeno, e mais recentemente Claude Bernard (1813-1878) na área da fisiologia os quais, realizavam vivissecções em animais afim de melhor compreender estruturas e o funcionamento de sistemas fisiológicos relacionados aos órgãos humanos.

Alguns animais são mais usados devido à elevada similaridade de respostas orgânicas complexas sistemas fisiológicos de humanos. Nesse sentido, os macacos se destacam em estudos com cérebro (Arnsten e Goldman-Rakic, 1998; Roberts et al., 1994), porcos são adotados em estudos relacionados à memória e tarefas cognitivas (Gieling et al., 2011). Entretanto, quando a fim de obter informações sobre os efeitos agudos ou crônicos do exercício físico, diversos estudos utilizam os roedores, sobretudo ratos e camundongos, como objeto de estudo (Applegate et al., 1982; Billat et al., 2005; Cartee e Farrar, 1987; Dawson e Horvath, 1970; Mcardle e Montoye, 1966). O uso desses roedores de laboratório tornou-se largamente difundido em pesquisas no meio científico devido a vários motivos, dentre eles, a possibilidade de intervenções e análises invasivas, fácil manipulação, boa resposta ao exercício, monitoramento constante de variáveis comportamentais (mensuração de ingestão alimentar e atividade espontânea) e possibilidade de controle interno (espécies, linhagem) e externo (biotério, temperatura, manipulação, dieta e sono) (De Araujo et al., 2012a; Gobatto et al., 2001).

3.3. Exercício físico em animais

A aplicação de exercício físico agudo ou crônico têm se mostrado uma importante ferramenta de intervenção não medicamentosa em experimentação animal, e vêm sendo aplicado visando entender os vários e complexos mecanismos fisiológicos em diferentes níveis (De Araujo et al., 2012a; Gobatto et al., 2001). A literatura aponta diferentes maneiras que os animais de laboratório podem ser submetidos ao exercício físico, este que pode ser aplicado forçadamente (imposto pelo pesquisador) ou realizado voluntariamente (motivado pelo próprio animal) (Arida et al., 2004b; Ke et al., 2011; Leasure e Jones, 2008). Considerando uma extensa variedade de fatores intervenientes na escolha do melhor tipo de exercício para esses animais, vale destacar a elevada sensibilidade desses roedores à ativação do sistema nervoso simpático e do eixo hipotálamo-pituitária-adrenal. Mediante as exposições agudas do exercício físico, tal ativação mostra-se como uma resposta de estresse normal. Contudo, quando essa ativação torna-se crônica ou prolongada, adversas e negativas implicações podem afetar alguns aspectos fisiológicos do animal, levando por sua vez a interpretações equivocadas, uma vez que os efeitos do exercício podem ter sido advindos do estresse e não do exercício aplicado (Kregel et al., 2006). Embora sejam muitas vezes negligenciadas, as características relacionadas ao tipo de exercício, adaptação, duração, frequência, intensidade entre outros devem ser detalhadamente escolhidos a fim de obter adaptações fisiológicas sem os efeitos negativos advindos de respostas do estresse crônico. Tais aspectos tornam-se ainda mais evidentes quando animais com patologias são estudados, uma vez que diversos fatores podem limitar o desempenho ou exacerbar a condição fragilizada desses animais (Kregel et al., 2006).

Nesse contexto, a natação (Dawson e Horvath, 1970; De Araujo et al., 2012a; Gobatto et al., 2001; Mcardle e Montoye, 1966) e a corrida (Applegate et al., 1982; Laursen et al., 2007) destacam-se como os principais meios de intervenção. Dentre as vantagens e desvantagens

observadas entre os diferentes tipos de exercício, a natação tem sido amplamente escolhida devido ao baixo custo e garantia de alto desempenho desses animais, uma vez que os ratos são nadadores inatos, sendo, portanto desnecessária a seleção de animais. Além disso, de acordo com a American Physiological Society, em comparação com corrida, a natação é considerada menos fisicamente traumática para os animais, uma vez que não há lesões nos pés ou choques elétricos (Kregel et al., 2006). Além disso, durante o exercício de natação, os animais nadam atados a sobrecargas de chumbo em propulsão vertical, o que permite quantificar e prescrever diferentes intensidades de esforço de acordo com sobrecargas relativizadas ao peso corporal (Gobatto et al., 2001).

No que tange o exercício voluntário, uma elevada quantidade de estudos tem proposto a roda de atividade (running wheel) como um dos principais meios de intervenção, uma vez que permite aos animais escolherem quando e qual intensidade desejam realizar, podendo assim reduzir os efeitos do estresse crônico e depressão nos animais de laboratório (Sexton, 1995; Sherwin, 1998). Além disso, estudos apontam que tal forma de exercício voluntário é capaz até de promover uma variedade de adaptações fisiológicas, por exemplo, o aumento do consumo máximo de oxigênio, economia de corrida (Lambert e Noakes, 1990), aumento da atividade enzimática muscular (Rodnick et al., 1989) adaptações cardiovasculares (Sexton, 1995) e longevidade (Holloszy, 1993).

Dentre as diferentes formas de aplicação de treinamento ou exercício crônico em animais, o exercício de característica aeróbia tem gerado grande interesse por vários pesquisadores. Nesse sentido, alguns estudos mostram diversos adaptações em marcadores fisiológicos do metabolismo aeróbio frente a intervenção do exercício físico aeróbio (Holloszy,

1973; Holloszy, 1975; Holloszy e Booth, 1976; Holloszy e Coyle, 1984; Holloszy et al., 1970; Holloszy et al., 1977).

No entanto, curiosamente, apesar de interessantes modificações mostradas acima, alguns estudos relatam reduções ou estagnações da capacidade aeróbia mesmo em animais ao longo da idade submetidos a programas de treinamento físico (De Araujo et al., 2012a; Pica e Brooks, 1982). Da mesma forma, menores valores de indicadores de capacidade aeróbia também são observados em ratos idosos quando comparados a ratos jovens, mesmo com a intervenção crônica do exercício físico (Cartee e Farrar, 1987; Hepple et al., 2003).

Entendendo que respostas fisiológicas oriundas do envelhecimento são inevitavelmente esperadas, torna-se coerente que o confinamento imposto em animais de laboratório pode acelerar tais processos fisiológicos levando a resultados não expressivos sobre as modificações positivas das capacidades aeróbia e anaeróbia com a intervenção do exercício físico. Parece que a aplicação de exercício físico em animais de laboratório compete com uma simultânea indução ao sedentarismo, tornando os animais submetidos a treinamento físico simplesmente menos sedentários, e não de fato "treinados". Em uma hipótese muito relevante, De Araujo et al. (2013b) especulam que possivelmente, a redução do desempenho aeróbio e anaeróbio de ratos ao longo da idade ocorre devido a forma de manutenção de alojamento, que poderia induzir o sedentarismo e redução da atividade física espontânea. Além disso, os autores sugerem que para minimizar os efeitos do sedentarismo durante o período experimental, as gaiolas devem permitir espaço suficiente para a atividade habitual do animal, de maneira mais próxima como se comportam na natureza.

3.4. Atividade espontânea e voluntária: Definição e mensuração

Na literatura podemos definir a atividade voluntária como uma atividade locomotora que não é diretamente necessária para a sobrevivência ou homeostase e não diretamente motivada por qualquer fator externo (Garland et al., 2011). Com base neste conceito, a motivação específica para a prática de determinadas modalidades esportivas podem facilmente denotar a atividade voluntária em humanos, sendo que tal motivação para o exercício voluntário é intimamente ligada a traços de personalidade (Rhodes e Smith, 2006) bem como pode modular substancialmente o gasto energético (Garland et al., 2011). A roda de atividade (running wheel) é comumente adotada em roedores de laboratório, sendo amplamente utilizada com o intuito de quantificar diferenças existentes na motivação intrísica para a realização de exercício, bem como mensurar a atividade voluntária do animal (Sherwin, 1998). Ainda no que tange o exercício voluntário, evidências relatam que a corrida em rodas de atividade pode ser viciante em roedores (Werme et al., 2000), bem como pode promover alterações no comportamento (Malisch et al., 2009) e na atividade neuronal de regiões específicas de roedores de laboratório (Rhodes e Garland, 2003).

Apesar da elevada relevância da atividade voluntária, vale destacar que os seres vivos podem se engajar em outras formas de locomoção, a qual se destacaa atividade espontânea (Ravussin et al., 1986). Para alguns autores, tal atividade pode ser compreendida como nonexercise activity thermogenesis ou NEAT, sendo esta uma importante forma de gasto energético que inclui atividades de vida diária, inquietação muscular, contração espontânea, manutenção de postura, as quais por sua vez não se classificam como exercicio voluntário (Levine et al., 1999).

Devido à grande importância de parâmetros referentes à atividade do animal, os diferentes métodos existentes para mensuração da atividade espontânea são extremamentes

relevantes em estudos de fisiologia (Garland et al., 2011; Thorburn e Proietto, 2000), farmacologia (Campbell e Mc, 1948) e comportamento animal (Marczinski et al., 1998; Poon et al., 1997; Rezende et al., 2005; Rezende et al., 2006) uma vez que podem fornecer importantes parâmetros e indicadores de atividade e padrões de comportamento específicos (circulação, exploração, etc). Dentre os principais métodos, destacam-se o monitoramento por sistema de vídeos (Poon et al., 1997), fotossensores (Badiani et al., 1995; Minematsu et al., 1995), detectores infravermelhos (Ticher et al., 1994) e plataformas de força (Malisch et al., 2009).

Como princípio de funcionamento, o sistema de monitoramento de vídeo transmite a imagem de um animal a uma câmera de vídeo contraste sensível que registra as coordenadas do animal (figura 1), sendo possível posteriormente obter a distância percorrida e tempo de movimentação. Apesar da elevada sensibilidade de tal método, as câmaras de vídeo requerem um nível de luz, a qual por sua vez pode influenciar substancialmente a ritmo circadiano e consequentemente a atividade dos animais durante as medições no período escuro (Biesiadecki et al., 1999; Vorhees et al., 1992).

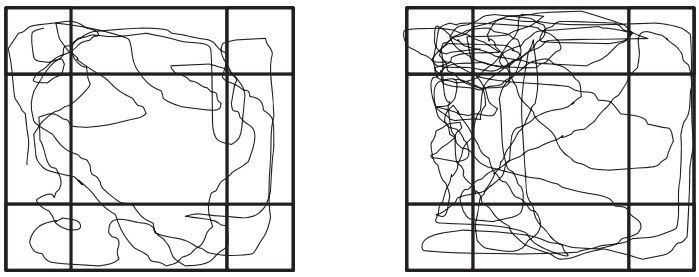

Figura 1: *Determinação da atividade espontânea por monitoramento de vídeo*

A observação direta inclui qualquer método onde a medição dos dados é classificada por um observador humano. Apesar da elevada simplicidade do método, uma vez que não requer nenhum equipamento, a observação direta possui algumas limitações, sendo, portanto menos precisa do que outros métodos devido à variabilidade entre os avaliadores. Além disso, de certa forma a realização de tal método torna-se inviável por longos períodos de tempo bem como em ambientes noturnos.

Além desses métodos citados, grandes sofisticações foram encorajadas nesse âmbito exploração. Os detectores fotoelétricos, como por exemplo, operam como princípio de funcionamento por meio de interrupções dos contínuos raios infravermelhos. No entanto, apesar de interessantes métodos serem disponíveis para a quantificação da atividade animal, tais requerem aparatos caros e exigem ambientes controlados a fim de estabelecer condições ideiais para o funcionamento de equipamentos, sendo, portanto inviável a mensuração em procedimentos experimentais. Mediante a esses intervenientes, métodos alternativos que conseguem obter confiáveis medidas por meio aparatos simples e de baixo custo, têm sido almejados pelos pesquisadores inseridos nesse campo de pesquisa. Nesse sentido, a gravimetria, a qual baseia no princípio de pesagem tem se apresentado como uma interessante alternativa para a mensuração de parâmetros referentes a atividade espontânea, uma vez que tal método pode ser desenvolvido apenas por meio de uma balança (Biesiadecki et al., 1999), a qual pode fornecer ótimas condições de mensuração da atividade espontânea.

3.5. Genética e a relação com a locomoção

Dentre os diversos estudos na área de comportamento animal, autores relatam a existência de significativas diferenças individuais no comportamento locomotor (Friedman et al., 1992) e atribuem essas distinções à aspectos genéticos. Entretanto, outras evidências também

apontam influências genéticas sobre o comportamento motivacional este que por sua vez está intimamente envolvido com a capacidade ou propenção fisiológica dos animais praticarem exercício (Waters et al., 2008).

Com base nesses apontamentos, alguns animais possuem capacidades diferenciadas em relação aos outros, sendo que essas manifestações individuais podem ser usualmente evidenciadas no exercício voluntário. Nesse sentido, Eikelboom (1998), sugere a existência de semelhanças entre o exercício voluntário realizado por humanos e o exercício executado na roda de atividade por animais, uma vez que a voluntariedade na realização de exercícios é determinada por uma motivação intrínsica (Sherwin, 1998). Tal fato pode ser observado por diferentes estudos, ao relatarem uma substancial variação individual na realização de atividade voluntária, dado que alguns animais correm quilômetros por dia, enquanto outros quase não se envolvem em nenhuma atividade na roda (Friedman et al., 1992; Swallow et al., 1998b).

Além das diferenças interindividuais, significativas diferenças em aspectos fisiológicos pode ser encontradas em animais selecionados ao longo de várias gerações. Nesse contexto, usando a roda de atividade como um critério de seleção (Waters et al., 2008), observa-se que animais selecionados "high capacity runners" se envolvem com mais frequência com atividades na roda de atividade quando comparados aos animais "low capacity runners" (Swallow et al., 1998a; Swallow et al., 1998b). Em concordância, evidências apontam que os níveis de expressão de determinados genes definem características fenotípicas, bem como a capacidade do desempenho fisiológico frente ao exercício. Nesse sentido,Tsao et al. (2001) observou que ratos geneticamente modificados para super-expressão de transportadores de glicose - GLUT-4 (Glucose transporter type) no músculo, apresentaram um aumento significativo na atividade voluntária na roda de atividade.

3.6. Transportadores monocarboxílicos (MCTs)

Devido ao aumento das contrações musculares durante o exercício e ao maior requerimento energético especialmente em atividade de curta duração e alta intensidade, elevadas concentrações de íons H^+ e lactato são encontradas no meio intracelular do tecido muscular esquelético (Pilegaard et al., 1999b; Stringer et al., 1992). Mediante ao aumento das concentrações desses produtos e da consequente diminuição do pH, a célula dispõe de mecanismos de defesa, sendo que, tais substâncias são transportadas para o meio extracelular, de forma a evitar o acúmulo de íons H^+ e manter o equilíbrio acido-básico, especialmente em situações extremas (Juel e Halestrap, 1999).

Por muitos anos, acreditava-se que uma vez produzido, o lactato se movia livremente de músculo para o sangue, ou seja esse produto atravessava livremente a membrana celular para o meio extracelular por meio de mecanismo simples de difusão (Hill et al., 1924). No entanto, nos últimos anos foi observado a existência de transportadores específicos, os quais são conhecidos como transportadores monocarboxílicos (MCTs). Dentre as 14 isoformas de MCTs conhecidas (Halestrap, 2012; Juel e Halestrap, 1999), apenas as isoformas MCT1 e 4 são de fato mais conhecidas e que, desempenham funções relevantes para o transporte de lactato (Bonen, 2001; Bonen et al., 1998). O MCT1 está presente principalmente nas fibras musculares oxidativas e no coração, sendo associado à entrada do lactato para o meio intracelular (Pilegaard et al., 1999a; Thomas et al., 2005). Além disso, vale destacar que a MCT1 no músculo é altamente correlacionada com a capacidade oxidativa e enzimas mitocondriais no músculo esquelético (Dubouchaud et al., 2000). Por outro lado, o MCT4 é encontrado principalmente em fibras brancas de contração rápida ou tecidos glicolíticos que promovem grande produção de lactato da célula (Bonen, 2001). Isto demonstra ser um mecanismo altamente precioso uma vez que a rápida eliminação do lactato para o meio extracelular

possibilita condições ideais para a manutenção do fornecimento de energia pela glicólise anaeróbia durante eventos fisiológicos onde existe uma alta demanda energética, tais como em exercício de alta intensidade (Bonen, 2001).

Digno de ser abordado, algumas investigações relatam a importância dos valores de Km (Taxa de Michaelis) dos MCTs 1 e 4. Especificamente em relação ao lactato, foi observado que o MCT1 possui alta afinidade (Km, 5 mmol/L) e que contraditoriamente o MCT4 é um transportador de baixa afinidade (Km, 22 mmol/L) (Coles et al., 2004). Estas características de MCT1 e MCT4 parecem indicar que há uma alta capacidade de transportar lactato para o meio intracelular em situações de repouso ou durante um exercício de intensidade leve ou moderada, onde existe baixas concentrações de lactato. Por outro lado, existe uma alta capacidade de eliminação de lactato para o meio extracelular em situações onde ocorre um aumento exacerbado da produção de lactato, especialmente em situações extremas, como por exemplo durante um esforço intenso ou exaustivo.

Além de suas características e funções fisiológicas supracitadas, os transportadores de lactato também são facilmente modulados sobretudo pela contração muscular. Evidências tem relatado que o exercício de resistência aeróbia influencia facilmente a expressão e o conteúdo de transportadores MCT1 (Bonen et al., 1998). Interessantemente, aumentos na expressão de MCT 1 foram observados em animais que praticaram a roda de atividade (Yoshida et al., 2004). Por outro lado, estudos apontam que o exercício de alta intensidade pode afetar ambos os transportadores MCT 1 e 4 em humanos (Pilegaard et al., 1999a).

4. ARTIGOS

Devido ao enorme conjunto de dados coletados, percebemos que o fracionamento de artigos seria uma eficiente estratégia a ser executada. Assim, alguns resultados obtidos já estão apresentados em 3 artigos, os quais buscam responder alguns dos objetivos do presente trabalho.

*O artigo I intitulado como "**Wide housing space, in conjunction with training exercise, enhances physical fitness in aging rats and benefits aerobic and anaerobic status and adipose tissue**" apresentado no item 4.1 foi confeccionado para tal atender os objetivos primordiais do vigente trabalho. Projetamos e confeccionamos uma gaiola de tamanho não convencional e buscamos averiguar se o aumento do espaço físico da gaiola associado com a aplicação do exercício e inserção da roda de atividade poderiam modular parâmetros aeróbios e anaeróbios e composição corporal de forma a atenuar os efeitos do envelhecimento em ratos de laboratório.*

*O artigo II intitulado como "**Chronic exercise can attenuate the decline of spontaneous physical activity in rats along the age**" apresentado no item 4.2, demonstrou em linhas gerais a descrição e caracterização de um aparato construído para mensurar a atividade espontânea de roedores de laboratório. Vale destacar que a atividade espontânea é uma variável comportamental essencial para o controle de diversos parâmetros fisiológicos, uma vez que esta participa na regulação do balanço energético bem como está intimamente relacionada com ritmos circadianos. Neste manuscrito foi investigado se os efeitos do espaço físico da gaiola associado com a aplicação do exercício e inserção da roda de atividade poderiam modular aspectos relacionados com o comportamento da atividade espontânea.*

*O artigo III intitulado como "**Wide housing space modulate the gene expression of monocarboxylate transporter 1 and 4 and enhances liver glycogen status in rats**"*

apresentado no item 4.3 foi confeccionado com o intuito de apresentar alguns resultados que demonstram o impacto das condições de confinamento de ratos de laboratório sobre modulações da expressão gênica de MCTs, as quais são altamente conectadas ao transporte de lactato a nível celular. Neste manuscrito, pudemos observar que o aumento do espaço físico da gaiola foi capaz de promover um aumento significativo do nível de mRNA de MCT4 no músculo sóleo e gastrocnêmio. Além disso, maiores quantidades de conteúdo de glicogênio foram encontradas no fígado e não foi detectada diferença nos níveis de corticosterona em ambos os confinamentos de habitação. Nossos dados confirmam que um maior espaço de habitação para os ratos, o que permite uma ampla gama de locomoção diária e intensos esforços, parece promover modulações no sentido de aumentar a taxa de efluxo e influxo de lactato, principalmente em fibras glicolíticas. Esta constatação sugere que o maior espaço físico como uma ferramenta poderosa no desenvolvimento de níveis mais elevados de aptidão física e desempenho do exercício de ratos.

4.1. ARTIGO I: Wide housing space, in conjunction with training exercise, enhances physical fitness in aging rats and benefits aerobic and anaerobic status and adipose tissue

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Abstract

Limited opportunities for locomotion can promote adverse physiological consequences in rodents confined in restricted cages. The aim of this study was to analyze the effect of housing space and its interactions with training exercise (Tr) and free access to voluntary wheel running (Ru) on the aerobic and anaerobic parameters and body composition of aging rats. In total, 130 male Wistar rats were kept in two types of housing space, standard (SH) and wide (WH). Animals in the SH group were housed in typical cages, whereas rats in the WH group were housed in a cage with three floors (100 cm in length, 100 cm in width and 33.3 cm in height). Physical activity interventions (Tr or Ru) were planned for each housing condition. The Tr groups swam for 40 min/day, 5 days/week at individual aerobic intensity, whereas the Ru groups had free daily access to a running wheel system. The rats were kept under these interventions from 60 to 150 days of age. At each age studied, ten rats from each group were submitted to the lactate minimum test to assess aerobic and anaerobic parameters and were subsequently euthanized. From the significant interactions ($p<0.05$) obtained by analyses of variance (ANOVA), we found that white adipose tissue content (TWAT) increased with age but that this response was attenuated in the Tr groups. The amplified housing space (WH) promoted a significant decrease in fat contents and delayed the decline of anaerobic and aerobic parameters with age. Greater amounts of brown adipose tissue were found in the Tr-WH group than all other groups. We found higher values for aerobic parameters mainly in the trained animals housed in the wide housing space. Our data indicate that a wide housing space can promote significant improvements, especially in body composition. Additionally, the association between wide housing space and training exercise appears to delay the development of obesity, mainly by increasing the animals' levels of physical fitness.

Keywords*: Rats, Training exercise, Wheel running, Housing conditions, Aerobic capacity*

1. Introduction

Laboratory rodents used in research are commonly, if not solely, kept in cages with limited dimensions of approximately 49 cm in length, 34 cm in width and 18 cm in height. As a consequence of the restricted opportunities for locomotion, these animals tend to exhibit sedentary behavior (Spangenberg et al., 2005). Generally, in addition to their low physical activity, these animals are fed ad libitum (i.e., an obesogenic environment), leading to negative implications for their welfare (Garland et al., 2011; Spangenberg et al., 2005), particularly with regard to physical fitness (i.e., aerobic/anaerobic and body composition parameters). These effects, in turn, are reflected in various metabolic diseases (Fitzgerald et al., 1997; Pimentel et al., 2003). In this regard, studies on rodents kept in traditional cages have reported that, in general, with age, certain aerobic parameters are reduced and white adipose tissue is increased in sedentary rats. Even with physical activity interventions (e.g., forced exercise or access to running wheels), these parameters are not "meaningfully" enhanced (De Araujo et al., 2012b; Gattermann et al., 2004; Laursen et al., 2007; Mazzeo e Horvath, 1986; Pica e Brooks, 1982).

The critical question is not whether types of exercise affect these variables but, rather, whether physiological adaptations are inhibited and what factors contribute to these outcomes. To address this question, we observed that previous studies have indicated that the spontaneous physical activity (SPA) has an essential role and a major effect on energetic metabolism in rodents (Garland et al., 2011; Keesey et al., 1990; Tou e Wade, 2002). These studies led us to propose that cage limits promote the suppression of SPA, which can lead to a pronounced reduction in aerobic components and increase in fat contents with age in captive rodents. Our assumption seems valid, as it has been found that physiological variables related to muscle performance capacity and enzyme activities are positively affected by an increase in housing space (Spangenberg et al., 2005).

Our hypothesis was that by increasing housing space, we would provide rats more locomotion possibilities, thereby increasing their SPA. This higher SPA level should be reflected positively in enhanced aerobic components and diminished fat contents. In addition, we hypothesized that positive effects could also be achieved from the association between increased housing space and training exercise or free access to a running wheel, which are two common scientific experimental interventions. Thus, the aims of our study were to examine the effect of housing space, as well as its interactions with training exercise or free access to a running wheel, on the aerobic and anaerobic parameters during exercise and the body composition of rats during the aging process (from 60 to 90 and 150 days old).

2. Materials and Methods

2.1. Animals

In total, 130 male Wistar rats (Rattus norvegicus albinos) were used in this study. The animals were kept in a room with strictly controlled temperature (23 ± 1 ºC), relative humidity (45-55%) and noise (<80 decibels) with a 12 h light/dark photoperiod in which the room was illuminated from 6:00 a.m. to 6:00 p.m. The rats received balanced standard rodent chow (Nuvilab®), and water was available ad libitum. The experiment was performed with the approval (ID protocol no. 2666-1) of the institutional ethics committee in rigorous accordance with the specific Brazilian resolutions on bioethics in experiments with animals.

2.2. Experimental design

The aerobic and anaerobic parameters of 10 sedentary rats (60-day-old) were evaluated by the lactate minimum test, after which they were euthanized for biochemical analyses to

characterize the baseline values for the study. After this procedure, the remaining 120 rats were randomly allocated into two types of housing space: standard housing space (SH) and wide housing space (WH). Within each housing condition, the rats were randomly subdivided into three groups: the control group (Co n=20), in which rats were kept without any experimental intervention; the training exercise group (Tr n=20), in which rats were submitted to aerobic training; and the voluntary wheel running group (Ru n=20), in which rats were maintained with free access to wheel running. The rats were kept under these interventions from 60 to 150 days old. At 90 days, ten (n=10) animals from each group had their aerobic and anaerobic parameters evaluated by the lactate minimum test and were euthanized thereafter. At 150 days, the remaining rats (n=10) were evaluated and euthanized for biochemical analyses. Fifteen days prior to swimming evaluations, all animals were gradually exposed to progressive adaptation. The timeline of evaluations and events performed during the experimental period is presented in figure 1.

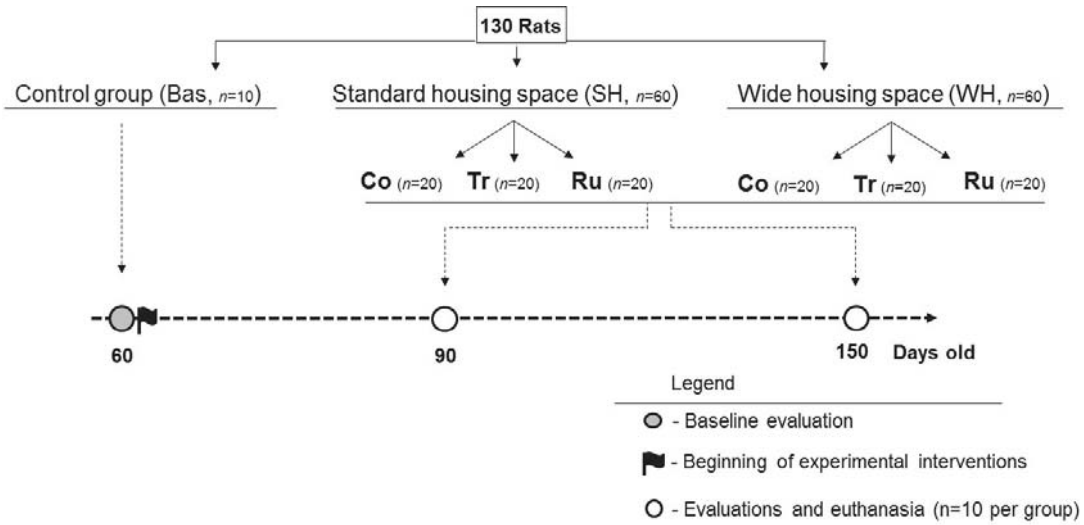

Figure 1. *Timeline of events performed during experiment.*

2.3. Housing conditions

After characterizing the animals' baseline values at 60 days of age, the animals were allocated into the various housing conditions. In the SH group, the rats (5 animals per cage) were maintained in polyethylene cages that were 49 cm in length, 34 cm in width and 18 cm in height. The solo area and total volume in the SH condition were 1,666 cm^2 and 26,656 cm^3, respectively. In the WH group, the rats were allocated into cages with three floors covered with sawdust (100 cm in length, 100 cm in width and 33.3 cm in height). In the WH group, the dimensions of the solo area and total volume were 30.000 cm^2 and 1.000.000 cm^3, respectively, as illustrated in figure 2. The WH group comprised 40 rats (Tr: n=20; Co: n=20) from 60 to 90 days old. Only 20 animals (Tr: n=10; Co: n=10) remained until the age of 150 days. In a separate cage (WH), 20 rats of the Ru group were maintained from 60 to 90 days old, and 10 rats remained until they were 150 days old.

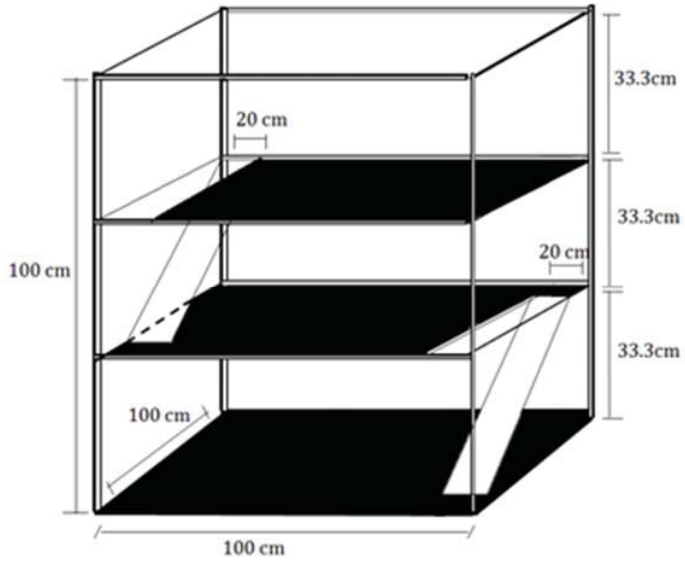

Figure 2. *Design of the wide housing space.*

2.4. Aerobic training and voluntary running wheel interventions

For the trained groups, the animals began the training exercise protocol, which consisted of swimming sessions (40 min/day, 5 days per week), when they were 60 days old. The workload exercise performed by animals was equivalent to 80% of the individual lactate minimum intensity, which was adjusted every two weeks according to the body mass of the animals (%BM).The workloads were imposed upon the animals by attaching small lead weights to their upper back using elastics. The animals were placed individually in cylindrical tanks with a smooth surface and individual compartments (30 cm diameter × 120 cm depth) to prevent the animals from resting on the bottom of the tank or trying to escape by jumping. The exercise sessions were conducted at the same time of day (08:00 a.m.), and the water temperature was kept at 31±1 ºC (Harri e Kuusela, 1986). Concurrently, during the experimental period, all untrained groups were subjected to brief swimming sessions (5 min, two days/week, without workload) to simulate water stress. Voluntary running activity for the Ru groups was carried out in a running wheel of 30 cm diameter and 11 cm width (Gaiolas Bragança®, Bragança Paulista, SP Brazil). In the WH condition, four running wheels were present per cage, whereas in the SH condition, one wheel was present per cage.

2.5. Assessment of aerobic and anaerobic parameters

Prior to beginning the training exercises, all non-trained animals were gradually exposed to an aquatic environment over 15 consecutive days to provide a progressive familiarization to the testing protocol and to reduce stress. The aerobic and anaerobic parameters of all animals were accessed by the lactate minimum test, which has been successfully used in previous studies (De Araujo et al., 2013b; De Araujo et al., 2007). This test consisted of three steps. First,

hyperlactatemia was induced by subjecting the animals to two short bouts of high-intensity exercise at 13%BM, separated by a 30 s passive recovery time. The first bout was 30 s in duration, and the second bout lasted until exhaustion (T_{ANA}), which was taken to be the moment at which the animals could no longer reach the surface for breathing (Mcardle e Montoye, 1966). Subsequently, the animals were submitted to a passive recovery period for 9 min to allow the release of the lactate produced by muscles into the bloodstream. Immediately after the recovery period, the animals were subjected to incremental exercise, in which their workload was gradually increased every 5 min. This incremental phase began with an initial workload of 4%BM, which was increased by 0.5% at each stage, with the exception of the final stage (4, 4.5, 5, 5.5, 6 and 7%BM). Blood samples (25 µL) were collected at each 5 min interval until the animals reached exhaustion.

The lactate concentrations were plotted over time to determine the time corresponding to the lowest lactate value of the ''U-shaped'' curve (Time L), which was obtained from the zero of the second-order polynomial fit and represented the maximal equilibrium between the removal and production of blood lactate (figure 3). Subsequently, to determine the aerobic capacity (AC) expressed relative to body mass, the Time L was applied in an equation obtained from the linear relationship between workload intensities and the time of the incremental phase.

The AC and its lactatemia equivalent in mmol•L^{-1} values[Lac], the total time of the incremental phase (Total T) and the time corresponding to the lowest lactate value of the ''U-shaped'' curve (Time L) were determined and considered as aerobic parameters. The exhaustion time (T_{ANA}) at 13%BM, the blood lactate expressed in mmol•L^{-1} after the hyperlactatemia phase at 9 min [Lac_{9min}] and the time after lactate inflection (Time R) were considered as anaerobic parameters.

The criteria for the success of test were the presence of the fit in the form of a "U" and the coefficient of determination (R^2) of the polynomial fit greater than 0.75. The evaluations that did not meet these criteria were excluded from the analyses of Time L, AC, [Lac] and Time R. However, in this situation, Total T, T_{ANA} and [Lac_{9min}] were considered for analyses. The numbers of rats (n) that completed a successful test are provided in tables 3 and 4.

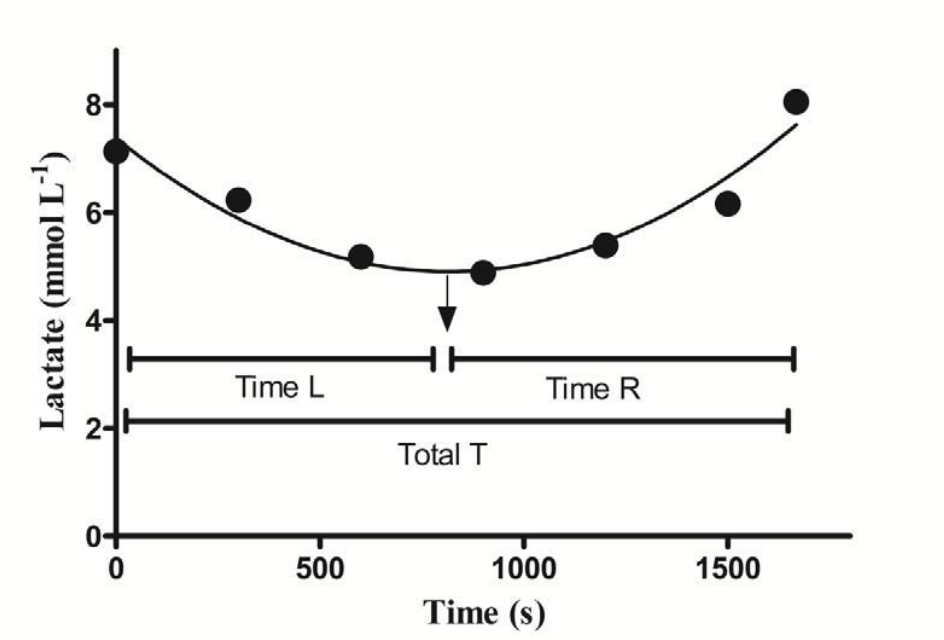

Figure 3: *Example of the lactate minimum test, where x (time) and y (lactate concentration) are plotted in a polynomial fit designed for the determination of the maximal equilibrium between the release and consumption of blood lactate. This figure also exemplifies the total time of the incremental phase (Total T), time referent to lowest lactate value of the "U-shaped" curve (Time L) and time after lactate inflection (Time R).*

2.6. Blood lactate analyses

Blood samples (25 µL) were collected from the rats' distal tails with a capillary tube that had been previously calibrated. The blood was transferred to plastic tubes (1.5 mL) containing 400 µL of trichloroacetic acid ($C_2HCl_3O_2$) at [4%]. The samples were immediately stored at a temperature between 2 and 8 ºC. Blood lactate concentration analyses were carried out on a microplate reader (ASYS Expert Plus UV, Biochrom) by enzymatic methods and were normalized against a calibration curve as previously described (Engel e Jones, 1978).

2.7. Collection of biological material

Twenty-four hours after the lactate minimum test, the animals were anesthetized with 50 mg/kg sodium thiopental. Food was withdrawn 12 h before these procedures. After the loss of cornea and foot reflexes, the animals were weighed (BM) and euthanized by decapitation. Visceral fat deposits, including epididymal (EAT) and retroperitoneal (RAT) fat deposits, were removed. Additionally, the brown adipose tissue (BAT) of the interscapular region was rapidly dissected, cleaned of tissue debris and weighed. The total white adipose tissue (TWAT) corresponded to the sum of the epididymal and retroperitoneal contents and was expressed relative to the animal's body mass.

2.8. Statistical procedures

Statistical analyses were carried out using a software package (Statistic 7.0). The means (M) and standard deviations (SD) were determined for all parameters. The normality of the data was checked using the Shapiro-Wilk test. ANOVA (age x housing space x intervention) was employed to identify the main effects and the interaction among them for all variables studied.

The age effect comprised two groups (90 and 150 days old), the housing space effect comprised two groups (standard and wide housing space), and the intervention effect comprised three groups (control, training exercise and voluntary wheel running). Tukey's HSD post hoc test was used to identify differences among the groups. The data from the 60-days-old group are reported for reference but were not included in the analyses of variance. The significance level was set at $p<0.05$ in all cases.

3. RESULTS

3.1. Body composition

The animals' fat contents and body mass levels are shown in table 1, and the ANOVA p-values are presented in table 2. According to the ANOVA analysis, EAT, RAT and TWAT increased with age (150>90 days old). However, this age-dependent effect was attenuated in the Tr groups, in agreement with a significant age x intervention interaction. The amplified housing space (WH condition) promoted a significant decrease in these variables. Despite lower fat contents (RAT, EAT and TWAT) in animals exposed to the WH condition than in those in the SH condition, the age x housing space interaction did not reach statistical significance (table 2). The percent variation (%) of TWAT for all groups in relation to baseline (60 days old) is shown in figure 4.

BAT increased with age (150>90 days old), but in accordance with a significant housing space x intervention interaction, this variable was higher in trained animals housed in a wide housing space (Tr-WH group). The WH condition promoted a significant decrease in BM. A main effect of intervention was close to statistical significance ($p=0.056$), indicating a tendency for

lower BM values in Tr compared to Co and Ru groups. A significant interaction between housing space and intervention was found (p=0.03), as shown by the opposing BM values of the Co groups under different housing conditions (WH x SH).

Table 2. *The ANOVA p-values of the main effects of age, housing space and intervention and the interaction of these variables with body mass (BM) as well as epididymal (EAT), retroperitoneal (RAT), brown (BAT) and total white adipose deposits (TWAT).*

	Main effects			Interactions			
	Age $F_{(1, 105)}$	**Housing Space** $F_{(1, 105)}$	**Intervention** $F_{(2, 105)}$	**Age x Housing Space** $F_{(1, 105)}$	**Age x Intervention** $F_{(2, 105)}$	**Housing Space x Intervention** $F_{(2, 105)}$	**Age x Housing Space x Intervention** $F_{(2, 105)}$
BM	$F=252.3$ $p=0.00$ (150>90d)	$F=8.49$ $p=0.00$ (SH>WH)	$F=2.95$ $p=0.056$	$F=0.01$ $p=0.90$	$F=1.60$ $p=0.20$	$F=3.37$ $p=0.03$ (Co<Tr, Ru- WH)	$F=1.89$ $p=0.15$
EAT	$F=31.9$ $p=0.00$ (150>90d)	$F=43.1$ $p=0.00$ (SH>WH)	$F=2.38$ $p=0.09$	$F=0.60$ $p=0.44$	$F=5.52$ $p=0.00$ (Tr<Co, Ru- 150d)	$F=2.43$ $p=0.09$	$F=0.03$ $p=0.96$
RAT	$F=21.6$ $p=0.00$ (150>90d)	$F=31.0$ $p=0.00$ (SH>WH)	$F=2.79$ $p=0.06$	$F=0.10$ $p=0.74$	$F=4.40$ $p=0.01$ (Tr<Co, Ru- 150d)	$F=0.58$ $p=0.55$	$F=2.30$ $p=0.10$
BAT	$F=11.0$ $p=0.00$ (150>90d)	$F=14.4$ $p=0.00$ (WH>SH)	$F=77.8$ $p=0.00$ (Tr>Co, Ru)	$F=2.10$ $p=0.14$	$F=27.6$ $p=0.00$ (Tr>Co, Ru- 150d)	$F=22.0$ $p=0.00$ (Tr>Co, Ru- WH)	$F=21.3$ $p=0.00$
TWAT	$F=22.9$ $p=0.00$ (150>90d)	$F=33.4$ $p=0.00$ (SH>WH)	$F=1.48$ $p=0.23$	$F=1.03$ $p=0.31$	$F=7.80$ $p=0.00$ (Tr<Co, Ru- 150d)	$F=1.32$ $p=0.26$	$F=1.45$ $p=0.23$

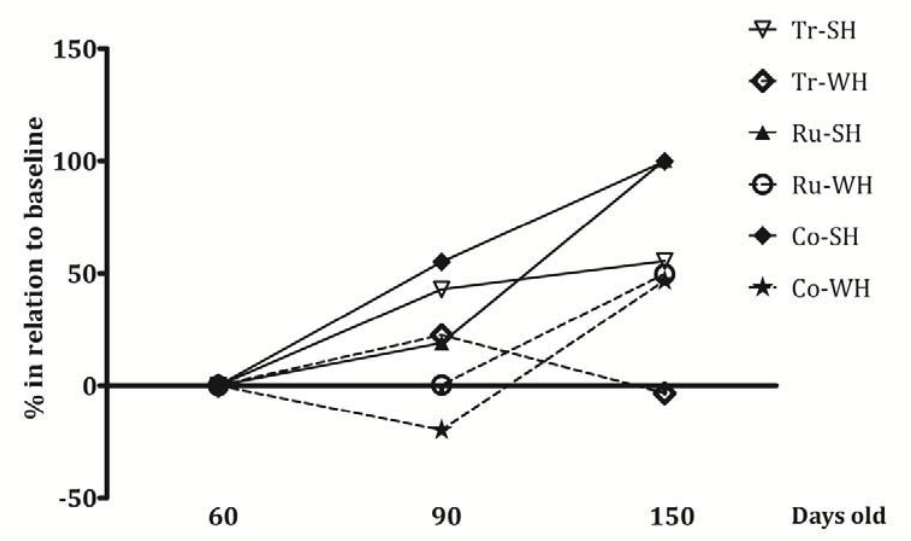

Figure 4. *The percent of variation (%) of total white adipose tissue (TWAT) for the control (Co),voluntary wheel running (Ru) and training (Tr) groups confined in a wide housing space (WH) and standard housing space (SH) at 90 and 150 days old. The data are compared to the baseline readings obtained at 60 days old.*

3.2. Aerobic and anaerobic parameters

The animals' aerobic and anaerobic parameters are presented in tables 3 and 4, respectively. The ANOVA p-values of the main effects and interactions are presented in table 5. The R^2 obtained from the lactate minimum test ranged from 88.0 to 99.0, and no significant differences were found among the groups. The aerobic (Total T, Time L and AC) and anaerobic (T_{ANA} and Time R) parameters decreased with age, revealing a significant main effect for age (90>150 days old). In contrast, [Lac] and [Lac_{9min}] increased with age (150>90 days old). A significant main effect for intervention, as well as age x intervention interaction, was found for the aerobic parameters (Total T, Time L and AC), and both were pronounced by the training exercise intervention. No significant main effect for intervention was found for anaerobic

parameters (T_{ANA} and Time R), except for [Lac_{9min}] values, which were higher in animals exposed to voluntary wheel running. The amount of housing space did not affect aerobic and anaerobic parameters, except for [Lac_{9min}], which was higher in animals housed in a wide housing space. The age x housing space interaction reached statistical significance when Total T was used as an aerobic marker. The decrease in anaerobic parameters (T_{ANA} and Time R) with age was attenuated in animals housed in a wide housing space, in agreement with a significant age x housing space interaction. Finally, significantly higher values for aerobic parameters (Total T, Time L, AC) were found in trained animals housed in a wide housing space, in agreement with the significant housing space x intervention interaction (table 5).

Table 3. *The mean ± SD of the total time of the incremental phase (Total T), time referent to the lowest lactate value of the "U-shaped" curve (Time L), aerobic capacity (AC) expressed as percentage of body mass (%BM) and lactatemia equivalent [Lac] expressed in mmol•L^{-1} for the control (Co), voluntary wheel running (Ru) and training (Tr) groups confined in a wide housing space (WH) and standard housing space (SH) at 60 (Bas), 90 and 150 days old. Percent of variation (**Δ**) in relation to Bas (60 days old). The number of rats (n) that completed a successful lactate minimum test.*

		90 days old						150 days old					
		Standard housing space			Wide housing Space			Standard housing space			Wide housing space		
Aerobic parameters	**Bas** *(n=10)*	**Co** *(n=7)*	**Ru** *(n=8)*	**Tr** *(n=9)*	**Co** *(n=5)*	**Ru** *(n=9)*	**Tr** *(n=9)*	**Co** *(n=3)*	**Ru** *(n=10)*	**Tr** *(n=6)*	**Co** *(n=6)*	**Ru** *(n=4)*	**Tr** *(n=8)*
Total T *(s)*	*1629.8 ±150.1*	*1410.0 ±241.0*	*1380.2 ±259.5*	*1638.7 ±203.8*	*1047.1 ±128.2* e, g	*1182.7 ±301.6* b, e	*1698.1 ±168.5* d	*691.3 ±235.7* b, c, d, e, f, g	*1374.2 ±204.0* m, i	*986.6 ±180.8* b, e, f, g	*1017.0 ±200.2* b, e, f, g, n	*926.6 ±288.7* b, e, f, g, h, n	*1411.8 ±262.1* d, i, m, p
Δ *(%)*		*-13.5*	*-15.3*	*4.2*	*-35.8*	*-27.4*	*0.5*	*-57.6*	*-15.7*	*-39.5*	*-37.6*	*-43.1*	*-13.4*
Time L *(s)*	*953.5 ±178.1*	*744.6 ±208.0*	*523.9 ±170.5*	*819.0 ±201.4*	*467.5 ±257.3*	*585.1 ±190.4* b	*1015.8 ±315.6* d, f	*363.9 ±196.5* b	*819.8 ±172.0*	*531.5 ±56.3* b	*516.7 ±258.3* b	*494.3 ±205.0* b	*780.2 ±167.5*
Δ *(%)*		*-21.9*	*-45.1*	*-14.1*	*-51.0*	*-38.6*	*6.5*	*-61.8*	*-14.0*	*-44.3*	*-45.8*	*-48.2*	*-18.2*
AC *(%BM)*	*5.16 ±0.4*	*4.77 ±0.4*	*4.38 ±0.3*	*4.92 ±0.4*	*4.29 ±0.5*	*4.49 ±0.3* b	*5.28 ±0.6* d, f	*4.07 ±0.4* b	*4.89 ±0.3*	*4.40 ±0.1* b	*4.36 ±0.6* b	*4.33 ±0.4* b	*4.84 ±0.3*
Δ *(%)*		*-7.5*	*-15.0*	*-4.5*	*-16.8*	*-12.9*	*2.3*	*-21.0*	*-5.1*	*-14.7*	*-15.3*	*-16.0*	*-6.2*
[Lac] *(mmol• L^{-1})*	*6.86 ±1.2*	*4.71 ±0.6*	*4.04 ±1.1*	*3.11 ±1.0*	*5.11 ±1.7*	*5.04 ±1.1*	*3.02 ±1.3*	*7.62 ±1.1* b, e, f	*6.38 ±1.5* b, e, f	*6.07 ±2.3* b, e	*7.21 ±0.7* b, e, f	*7.61 ±0.9* b, e, f, g, h	*4.73 ±1.7* p
Δ *(%)*		*-31.3*	*-41.1*	*-54.7*	*-25.5*	*-26.6*	*-56.0*	*11.0*	*-7.0*	*-31.0*	*5.1*	*10.9*	*-11.5*

Sig. Difference *(p<0.05) in relation to* **b***) Tr-WH,* **c***) Ru-WH,* **d***)Co-WH,* **e***)Tr-SH,* **f***)Ru-SH,* **g***)Co-SH for 90 days old and* **h***)Tr-WH,* **i***) Co-SH,* **k***) Ru-WH,* **m***) Tr-SH,* **n***) Ru-SH,* **p***) Co-WH for 150 days old.*

Table 4. *The mean ± SD of exhaustion time (T_{ANA}) at 13%BM, blood lactate expressed at 9 min after the hyperlactatemia phase [Lac_{9min}] and time after lactate inflection (Time R) for the control (Co), voluntary wheel running (Ru) and training (Tr) groups confined in a wide housing space (WH) and standard housing space (SH) at 60 (Bas), 90 and 150 days old. The percent of variation (**Δ**) in relation to Bas (60 days old). The number of rats (n) that reached a successful lactate minimum test.*

		90 days old						150 days old					
		Standard housing space			Wide housing Space			Standard housing space			Wide housing space		
Anaerobic parameters	**Bas** *(n=10)*	**Co** *(n=7)*	**Ru** *(n=8)*	**Tr** *(n=9)*	**Co** *(n=5)*	**Ru** *(n=9)*	**Tr** *(n=9)*	**Co** *(n=3)*	**Ru** *(n=10)*	**Tr** *(n=6)*	**Co** *(n=6)*	**Ru** *(n=4)*	**Tr** *(n=8)*
T_{ANA} *(s)*	*91.8 ±21.0*	*74.4 ±23.1*	*83.4 ±26.4*	*77.2 ±26.1*	*71.0 ±18.2*	*80.8 ±19.5*	*72.1 ±13.4*	*57.3 ±12.2*	*64.7 ±10.0*	*59.6 ±10.9*	*81.2 ±17.5*	*74.2 ±13.6*	*65.4 ±15.7*
Δ *(%)*		*-19.0*	*-9.2*	*-15.9*	*-22.7*	*-12.0*	*-21.5*	*-37.5*	*-29.5*	*-35.0*	*-11.5*	*-19.2*	*-28.7*
[Lac_{9min}] *(mmol• L^{-1})*	*6.89 ±0.6*	*5.54 ±1.3*	*5.31 ±1.0*	*4.16 ±1.2*	*5.71 ±1.3*	*5.92 ±0.9*	*6.48 ±1.2* **e**	*6.89 ±1.9* **e**	*7.82 ±1.0* **c, d, e, f, g**	*6.25 ±1.4* **e**	*6.67 ±1.1* **e**	*7.50 ±1.2* **e, f, g**	*6.52 ±1.0* **e**
Δ *(%)*		*-19.5*	*-22.9*	*-39.6*	*-17.0*	*-14.1*	*-5.9*	*0.1*	*13.6*	*-9.3*	*-3.2*	*8.9*	*-5.3*
Time R *(s)*	*676.3 ±150.7*	*761.8 ±208.1*	*888.4 ±279.3*	*799.5 ±116.6*	*628.5 ±332.3*	*635.7 ±175.5*	*671.0 ±203.2*	*458.8 ±146.2*	*554.4 ±219.1* **f**	*538.5 ±21.4*	*576.3 ±293.9*	*577.0 ±172.0*	*669.9 ±121.1*
Δ *(%)*		*12.6*	*31.4*	*18.2*	*-7.1*	*-6.0*	*-0.8*	*-32.2*	*-18.0*	*-20.4*	*-14.8*	*-14.7*	*-0.9*

***Sig. Difference** (p<0.05) in relation to **c**) Ru-WH, **d**)Co-WH, **e**)Tr-SH, **f**)Ru-SH, **g**)Co-SH for 90 days old.*

Table 5. *The ANOVA p-values of the main effects of age, housing space, intervention and the interaction of these variables for the total time of the incremental phase (Total T), time referent to the lowest lactate value of the "U-shaped" curve (Time L), aerobic capacity (AC) expressed as percentage of body mass and the lactatemia equivalent [Lac], exhaustion time (T_{ANA}) at 13%BM, blood lactate after hyperlactatemia phase [Lac_{9min}] and time after lactate inflection (Time R).*

	Main effects			Interactions			
	Age	**Housing Space**	**Intervention**	**Age x Housing Space**	**Age x Intervention**	**Housing Space x Intervention**	**Age x Housing Space x Intervention**
Aerobic parameters							
Total T	$F_{(1, 101)}=56.6$ $p=0.00$ (90>150d)	$F_{(1, 101)}=0.58$ $p=0.44$	$F_{(2, 101)}=27.1$ $p=0.00$ (Tr>Co, Ru)	$F_{(1, 101)}=9.64$ $p=0.00$ (WH>SH - 150d)	$F_{(2, 101)}=5.48$ $p=0.00$ (Tr>Co, Ru- 150d)	$F_{(2, 101)}=14.2$ $p=0.00$ (Tr>Co, Ru- WH)	$F_{(2, 101)}=10.43$ $p=0.00$
Time L	$F_{(1, 71)}=4.91$ $p=0.02$ (90>150d)	$F_{(1, 71)}=0.03$ $p=0.84$	$F_{(2, 71)}=10.4$ $p=0.00$ (Tr>Co, Ru)	$F_{(1, 71)}=0.10$ $p=0.74$	$F_{(2, 71)}=5.66$ $p=0.00$ (Tr>Co, Ru- 150d)	$F_{(2, 71)}=5.71$ $p=0.00$ (Tr>Co, Ru- WH)	$F_{(2, 71)}=5.46$ $p=0.00$
AC	$F_{(1, 71)}=5.07$ $p=0.02$ (90>150d)	$F_{(1, 71)}=0.07$ $p=0.78$	$F_{(2, 71)}=10.2$ $p=0.00$ (Tr>Co, Ru)	$F_{(1, 71)}=0.11$ $p=0.73$	$F_{(2, 71)}=5.30$ $p=0.00$ (Tr>Co, Ru- 150d)	$F_{(2, 71)}=4.94$ $p=0.00$ (Tr>Co, Ru- WH)	$F_{(2, 71)}=4.80$ $p=0.01$
[Lac]	$F_{(1, 71)}=60.4$ $p=0.00$ (90>150d)	$F_{(1, 71)}=0.17$ $p=0.67$	$F_{(2, 71)}=15.1$ $p=0.00$ (Tr>Co, Ru)	$F_{(1, 71)}=0.94$ $p=0.33$	$F_{(2, 71)}=0.02$ $p=0.97$	$F_{(2, 71)}=3.35$ $p=0.04$ (Co, Ru>Tr - WH)	$F_{(2, 71)}=0.56$ $p=0.57$
Anaerobic parameters							
T_{ANA}	$F_{(1, 103)}=7.68$ $p=0.00$ (90>150d)	$F_{(1, 103)}=1.90$ $p=0.17$	$F_{(2, 103)}=1.55$ $p=0.21$	$F_{(1, 103)}=6.11$ $p=0.01$ (WH>SH - 150d)	$F_{(2, 103)}=0.78$ $p=0.45$	$F_{(2, 103)}=0.73$ $p=0.48$	$F_{(2, 103)}=0.60$ $p=0.54$
[Lac_{9min}]	$F_{(1, 98)}=36.5$ $p=0.00$ (150>90d)	$F_{(1, 98)}=4.03$ $p=0.04$ (WH>SH)	$F_{(2, 98)}=3.66$ $p=0.02$ (Ru>Co, Tr)	$F_{(1, 98)}=5.70$ $p=0.01$ (WH>SH - 90d)	$F_{(2, 98)}=1.81$ $p=0.16$	$F_{(2, 98)}=3.02$ $p=0.053$	$F_{(2, 98)}=1.02$ $p=0.36$
Time R	$F_{(1, 71)}=12.2$ $p=0.00$ (90>150d)	$F_{(1, 71)}=0.70$ $p=0.40$	$F_{(2, 71)}=0.62$ $p=0.53$	$F_{(1, 71)}=7.42$ $p=0.00$ (WH>SH - 150d)	$F_{(2, 71)}=0.19$ $p=0.82$	$F_{(2, 71)}=0.66$ $p=0.51$	$F_{(2, 71)}=0.00$ $p=0.99$

4. DISCUSSION

4.1. Body composition

There is little information in the literature regarding the effects of housing conditions and their interactions with physical interventions (e.g., training exercise and running wheel) in rats. Our study addresses this topic from a unique perspective. Housing space may affect the muscle metabolism of rats, as was previously suggested by Spangenberg et al. (2005). These authors found that muscle performance capacity and enzyme activities were positively influenced by an increase in housing space, but adipose tissue content was not investigated. The major finding of our data was that increased housing space (WH condition) was able to promote a significant decrease in RAT, EAT and TWAT. Such observations are in agreement with a significant main effect for housing space. In light of these results, it seems reasonable to consider that by increasing the opportunities for locomotion and, consequently, energy expenditure among captive rats, the wide housing space promotes the increased mobilization and utilization of fat as an energy substrate (Thompson et al., 2012). This process is an important adaptive response given that a decrease in the accumulation of TWAT has been linked to preventive effects against cardiovascular diseases (Fahlman et al., 2002), insulin resistance (Ross et al., 2004), metabolic syndrome (Stewart et al., 2005) and inflammation markers (Berg e Scherer, 2005). Although the age x housing space interaction did not reach statistical significance, fat content was considerably reduced in the WH animals compared to those housed in SH conditions, as shown in figure 4.

Although we did not find a significant main effect for intervention on TWAT, there was an interaction between age x intervention, suggesting that the increase in TWAT with age is dependent on the intervention to which the rats were subjected. This outcome was improved by training exercise, which had a greater impact on attenuating the increase in adipose content

compared to free access to a running wheel. Classical studies have carried out meticulous analyses of the body composition of animals exposed to training exercise (Garthwaite et al., 1986; Hoffman-Goetz e Macdonald, 1983; Oscai et al., 1973). However, despite providing information on this topic, these studies were conducted using animals housed in standard cages. To the best of our knowledge, our work is the first to investigate the effects of training exercise in conjunction with housing space adjustments. Regarding the effects of wheel running on body composition, our results are somewhat divergent from those reported by Chang et al. (1995), who showed decreased levels of body fat in S5B rats with access to a running wheel in relation to the respective control group. Our data are more similar to those of a study by Gattermann et al. (2004), which found no differences in the relative fat content of golden hamsters with access to running wheels over a period of 52 weeks.

By analyzing the data graphically as percentage values (figure 4), it is possible verify that at 90 days old, exposure to wide housing space alone was effective in reducing TWAT levels. However, at 150 days old, better physiological adaptations occurred in trained animals housed in a wide housing space. From the significant interactions obtained by analyses of variance, our results suggest that housing space is more important than physical interventions for reducing white adipose content. This result was shown statistically by the lack of significance for the housing space x intervention interaction, which suggests that TWAT is lower in animals housed in a wide housing space, independently of the intervention to which the rats are exposed (Tr, Ru and Co).

Regarding BM, a significant main effect was found for housing space. Our results showing that rats in the WH group weighed less than those in the SH group are in agreement with the study by Spangenberg et al. (2005). Moreover, in our study, a main effect of intervention tended

toward statistical significance, indicating a tendency for lower BM values in the Tr group compared to the Co and Ru groups. It has been previously shown that training exercise may reduce (De Araujo et al., 2013a) or have no effect (De Araujo et al., 2012b; De Araujo et al., 2013b) on body weight gain in rats. Thus, based on our results, it seems realistic to consider that training exercise is more efficient than running wheels for decreasing BM values. These results are in contrast to those from Narath et al. (2001), in which the body mass of the running wheel group was significantly lower than that of the trained group. In that study, the rats were exercised by treadmill running, making it difficult to draw direct comparisons with our results due to methodological differences regarding training characteristics; previous studies have reported differences in responses to swimming and treadmill running exercise (Baptista et al., 2008; Contarteze et al., 2008; Snyder et al., 1992). Finally, we found a significant interaction between housing space and intervention as for the Co group, which showed opposing BM values under different housing conditions (WH vs. SH). This finding suggests that body mass is markedly lower in the Co group housed in a wide housing space, indicating that WH may increase locomotion possibilities, counteracting the deleterious effects of a low level of daily activity in sedentary rats.

In agreement with a significant housing space x intervention interaction, higher BAT relative mass was found in trained animals housed in a wide housing space (Tr-WH group), suggesting interesting possible interpretations. Previous studies indicated that UCP-1 is found in BAT, where it regulates energy balance and generates heat by uncoupling oxidative phosphorylation (Cortright et al., 1999) in response to environmental temperature (cold exposure) or diet (regulating energy balance) (Lowell e Spiegelman, 2000). Although we did not evaluate the molecular pathways to guarantee accurate interpretations, an increase in the

amount of BAT gave us morphological indications of thermogenic adaptations in the Tr-WH group. In support of this suggestion, reports have indicated that increased BAT mass (i.e., hypertrophic tissue) can be accompanied by higher UCP protein content (Lowell e Spiegelman, 2000; Oh-Ishi et al., 1996). Additionally, it has been suggested that the circulating levels of irisin, which has powerful effects on the browning of certain white adipose tissues, are increased in response to chronic exercise (Boström et al., 2012).

It was previously reported that swimming can influence BAT activity (Harri e Valtola, 1975). This outcome is most likely induced to compensate for the repeated heat loss caused by the water contact, as these BAT activity changes are not found in treadmill running exercise (Yamashita et al., 1993). Given this finding, it is likely that the type of physical exercise (e.g., swimming vs. running) can have different influences on BAT. However, the majority of studies have used aerobic exercise to study BAT activity, and there is little information on the effects of anaerobic exercise. In contrast to aerobic training, exposure to a running wheel is comparable to interval training due to the intermittent bouts of activity at higher velocities, which can result in improvements in anaerobic metabolism (i.e., muscle hypertrophy) (Jeneson et al., 2007). In line with this pattern, we found thermogenic adaptations in animals submitted to aerobic training exercise intervention, which has a superior impact for increasing BAT contents compared to free access to a running wheel.

4.2. Aerobic and anaerobic parameters

Numerous reports have indicated that aging is associated with declines in aerobic and anaerobic markers. These markers is not enhanced with training exercise intervention, but their decline can be attenuated (De Araujo et al., 2012a; Laursen et al., 2007; Mazzeo e Horvath, 1986; Pica e Brooks, 1982). It is notable that a study by De Araujo et al. (2013b) found no

differences in aerobic capacity between trained and sedentary age-matched animals. Comparisons between our results and the data from De Araujo et al. (2013b) are possible due to the methodological similarities pertaining to the monotonous training proposed in their study. However, all of the studies cited above were conducted using typical cages, and to the best of our knowledge, the current study is the first to investigate the effects of training exercise in combination with wide housing space. Corroborating the literature, the aerobic parameters (Total T, Time L and AC) used in the present study decreased with age, but this response was counteracted by a training exercise intervention, which had a superior impact compared to free access to a running wheel.

Our major finding was that markedly higher values for aerobic parameters (Total T, Time L and AC) were found in trained animals housed in a wide housing space, compatible with a significant housing space x intervention interaction. The mechanisms underlying this result remain to be determined. We speculate that the positive adaptations toward aerobic metabolism of the Tr-WH group can be at least partially attributed to the higher BAT amounts and, therefore, higher UCP-1 protein levels. This condition may have resulted in increased mitochondrial biogenesis (Lowell e Spiegelman, 2000), which has been shown to be related to improved exercise capacity (Ringholm et al., 2013). Spangenberg et al. (2005) found higher enzyme activities (citrate synthase and hexokinase) in rats housed in large cages, but the authors did not provide information on direct aerobic/anaerobic markers in rats exercised at different ages. In accordance with a significant age x housing space interaction, we found that the decline of Total T (aerobic performance marker) was attenuated in animals housed in wide housing conditions, a finding that is unique to our study. Therefore, in light of these results, it seems

reasonable to hypothesize that the reduced opportunities for locomotion in typical restricted cages can significantly inhibit the benefits of training exercise, as previously reported.

The decrease in anaerobic status (T_{ANA} and Time R) with age was attenuated in animals housed in a wide housing space, in agreement with a significant age x housing space interaction. Moreover, the blood lactate at 9 min after the hyperlactatemia phase [Lac_{9min}] was higher in the WH group, suggesting that an improvement in anaerobic metabolism occurred in animals housed in the wide housing space. In general, we expected all these results concerning anaerobic status due to the greater habitual locomotion linked with intense activities (i.e., short and intense bouts of activity) performed by animals housed in a WH condition. In contrast, anaerobic parameters (T_{ANA} and Time R) were not influenced by intervention (Tr and Rr). This result was expected for the Tr groups because evidence has shown reduced or absent anaerobic adaptations in response to aerobic training exercise (De Araujo et al., 2013b; Taylor e Bachman, 1999). However, anaerobic improvements were expected for the Ru groups, as studies have shown muscle hypertrophy in animals exposed to a running wheel, which is comparable to interval training due to the intermittent bouts of activity at higher velocities (Jeneson et al., 2007).

Interestingly, trained animals (WH condition) presented aerobic capacity with overload at ~5.0% of body mass and at blood lactate levels [Lac] of ~4 mmol L^{-1}. This finding was in contrast with the control groups of both housing conditions, which showed aerobic capacity with overload at approximately 4.3% of body mass but at higher blood lactate levels of ~6 mmol L^{-1}. Thus, the AC at a higher relative workload occurring at lower blood lactate concentrations in trained rats housed in a wide housing space, led us to suggest interesting aerobic adaptations toward increased blood lactate removal (clearance). Gobatto et al. (2001) showed that the maximal aerobic capacity (%BM) of trained animals kept in typical cages was improved, but at this

intensity, the blood lactate concentration remained unchanged. However, these authors did not report the blood lactate concentrations at low intensities before and after training. Moreover, it has been suggested that in humans, reductions in blood lactate concentrations occur at a given submaximal oxygen uptake after participation in training programs (Saltin et al., 1969). The literature suggests that these outcomes may be attributed to biochemical alterations related to lower lactate production because of a shift from carbohydrate to lipid oxidation (Mole et al., 1971), increased muscle oxidative capacity resulting from the increased activity of oxidative enzymes (Andersen e Henriksson, 1977) or a higher usage of muscle type I fiber (Ivy et al., 1980). Others studies have suggested that endurance training increases the metabolic clearance rate of lactate (Donovan e Brooks, 1983) in the liver, heart and muscle, which possess biochemical pathways specialized for using lactate as an energy source (Brooks, 2000).

4.3. Training and testing exercise characteristics

It should be emphasized that our training exercise protocol was performed with precise control. Following the principles of the American Physiological Society (Kregel et al., 2006), in the present study, the animals were able to swim continuously, with rare exceptions, in all training sections. Except in cases of climbing, diving and escape swimming behaviors, the continuous swimming behavior is highly recommended. The high incidence of continuous swimming behavior observed in present study may have occurred as consequence of a progressive and gradual familiarization "swimming" exercise. In addition, having the rats swim individually in tanks of deep water may have also promoted a higher incidence of continuous swimming behavior.

Furthermore, we adopted the lactate minimum test, which was developed to evaluate the aerobic capacity of swimming rats by De Araujo et al. (2007). Since then, this test has been

applied for monitoring training in rodents (De Araujo et al., 2013a; De Araujo et al., 2012a). Similarly to De Araujo et al. (2013a), we showed that the lactate minimum test can be effectively employed for monitoring changes in physiological adaptations in response to training. In the current study, the R^2 obtained from the lactate minimum test resulted in values ranging from 88.0 to 99.0, and no significant differences were found among the groups. However, the success of the lactate minimum test was greater in trained animals, showing that training conditions were superior in providing useful test results. Unlike previous studies involving the lactate minimum test (De Araujo et al., 2012a; De Araujo et al., 2007), in our study, the lactate concentrations were plotted over time. We performed this procedure to obtain more information about aerobic performance and not only demonstrate the aerobic capacity expressed in values relative to body mass (%BM). For instance, the total time of the incremental phase (Total T) was used as an aerobic resistance index. Moreover, we calculated the time corresponding to the lowest lactate value of the ‘‘U-shaped’’ curve (Time L), assuming that the lactate minimum represents the maximal exercise intensity at which the capacity of lactate removal is equal to its release. Therefore, Time L should be shifted to the right in response to aerobic adaptations from training exercise. In contrast, the time after lactate inflection (Time R) was calculated to evaluate the exercise tolerance capacity at "supra-threshold" loads (i.e., anaerobic intensities).

4.4. Perspectives and contributions

Many ongoing studies have focused on preventing lifestyle-related diseases or delaying aging-related deleterious effects. In this context, it can be argued that several pathologies are intimately influenced by aerobic capacity and body composition, which have been demonstrated in clinical studies to be of essential importance (Fitzgerald et al., 1997; Pimentel et al., 2003). Considering these aspects, the main contributions of the present study include the

demonstration that successful physiological adaptations in aerobic capacity and body composition resulted from maintaining the study animals in a wide housing space. In terms of the relevant applications of this study, wide housing associated with training exercise represents an unquestionably constructive strategy for researchers attempting to enhance the welfare of study animals. Additionally, our findings suggest an attractive model that can be used to prevent the development of metabolic diseases.

5. CONCLUSION

In summary, wide housing space, by means of increasing locomotion opportunities, affected the physiological responses of the study animals, reducing their TWAT and BM and increasing their BAT levels, and delayed the decline of anaerobic and aerobic parameters with age. We conclude that with regard to BAT and aerobic parameters, the best physiological adaptations occurred in trained animals maintained in a wide housing space. This finding may be useful in developing strategies to prevent diseases related to obesity.

Conflicts of interest

The authors have no conflicts of interest.

Acknowledgments

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4.2. ARTIGO II: Chronic exercise can attenuate the decline of spontaneous physical activity in rats along the age

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Abstract

Spontaneous physical activity (SPA) is key regulator of energy expenditure. Remaining to be addressed, we analyzed the effect of housing space and its interactions with chronic exercise (Tr) and free access to voluntary wheel running (Ru) on the SPA in aging rats by means of an apparatus of gravimetric monitoring. Additionally, we investigate if our experimental interventions could modulate SPA behavior regarding dark and light periods. From 60 days to 150 days old, 130 male Wistar rats were kept into two types of housing space: standard (SH) or wide (WH). Physical activities interventions (Tr or Ru) for each housing condition were planned. Tr groups swam for 40min/day, 5 days/week at aerobic intensity, which was individually prescribed whereas the rats of Ru groups had free daily access to a running wheel system. The cage with five rats was placed on the platform to record SPA of rats at 60, 90, and 150 days-old by the acquisition data device at a frequency of 30 Hz for 20hours (10/10, light/dark period). Rats were removed from WH condition during SPA evaluations, which were executed in identical cage for all groups. From percentage comparisons in relation to rats with 60 days old, we found that SPA levels were decreasing along the age in control and Ru groups of both housing conditions, but this response was attenuated principally in the Tr-SH group at 150 days old. From of interactions ($p<0.05$) obtained by analyzes of variance (ANOVA), diminished SPA levels were found in all animals of the WH group, proposing that the restriction of environmental space during evaluations somehow interfere SPA levels for the WH group. The SPA levels at dark period for all groups were always higher (2.2 to 6.9 fold) than light period, indicating a preservation of this pattern irrespective of experimental intervention. The main finding of our study was that traditional decrease aged-induced of SPA in sedentary rats can be attenuated in rats submitted to chronic physical exercise housed in typical cages, but new interpretations can be found when evaluations of SPA were performed in the wide housing space. Our finding may be useful in developing strategies to prevent diseases related to energy expenditure, such as obesity.

Keywords: *Rodents, Spontaneous physical activity, Gravimetric method, Chronic exercise*

INTRODUCTION

Laboratory rodents can engage in physical activities, as example forced physical exercise (Contarteze et al., 2008; Dawson e Horvath, 1970; De Araujo et al., 2013b) or self-motivated activities (i.e. access to running wheels) (Sherwin, 1998; Yoshida et al., 2004), where each one could promote specific physiological responses (Arida et al., 2004a; Leasure e Jones, 2008). Furthermore, rodents also perform unconscious physical activities such as muscle tone, grooming, rearing, fidgeting and ambulatory locomotion behavior, that has been categorized as spontaneous physical activity (SPA) (Garland et al., 2011; Kotz et al., 2008), which oscillates in line with the circadian rhythm, showing high activity during the night and relatively quiet at the day for nocturnal rodents (Verwey et al., 2013). The SPA in rodents can be evaluated through of a myriad of methods. However, according to Garland et al. (2011), it is necessary to be very careful when reading the literature to note what a particular study essentially means by 'locomotor activity', as it is often used to define either activity in home cages after a period of habituation or in acute tests in novel arenas (e.g. open-field tests). For instance, the locomotion measured in open-field test most refers to ambulation (i.e. exploratory activity) (Skalicky et al., 1996), but SPA refers to all activities, including non-ambulatory events, such as grooming and rearing behavior (Garland et al., 2011). Given this context, we chose to evaluate the SPA through a gravimetric apparatus (Biesiadecki et al., 1999), believing that an ideal system would record all motion changes exerted by animals during long periods, preferentially by means of inexpensive and easy procedures.

Age-dependent decrease in the locomotor activity have been reported in many different methods in a varied range of animal's species (Cass et al., 2005; Goodrick, 1966; Goodrick, 1967; Hagen et al., 1998; Ingram, 2000; Nemoz-Bertholet e Aujard, 2003; Skalicky et al.,

1996; Teske et al., 2012), and this response is associated to reduced quality of life and longevity (Ingram, 2000; Nemoz-Bertholet e Aujard, 2003). In addition, the SPA has an essential role and a major effect on daily energy expenditure in rodents, where reduced SPA levels are related to the development of metabolic diseases, such as obesity (Levine, 2004; Levine e Kotz, 2005; Teske et al., 2012). In view of these considerations, it is noteworthy that non-pharmacological therapies represent an interesting way for attenuate these problems in laboratory rodents. We address this issue in the present study because there is evidences that the age-dependent decrease of exploratory activity measured by the open field test can be delayed when animals are exposed to chronic exercise (Ingram, 2000; Skalicky et al., 1996). Moreover, it has been found that several physiological variables are positively affected by increasing housing space (Spangenberg et al., 2005).

To address these questions, the aims of our study were to examine the effect of housing space, as well as its interactions with chronic exercise or free access to a running wheel, on the SPA assessed through a gravimetric apparatus of rats during the aging process (from 60 to 90 and 150 days old). Secondly, we investigate if our experimental interventions could modulate SPA behavior at dark and light periods. Our hypotheses were that chronic exercise or free access to a running wheel could attenuate the age-dependent decline of SPA in rats, and that such attenuation could be potentiated from the wide housing space availability. Our investigation seems essential in order to expose to researchers about the strong influence of housing conditions and physical activities as non-pharmacological therapies for enhance SPA levels in aging rats.

Materials and Methods

Animals

One hundred and thirty (n= 130) male Wistar rats (Rattus norvegicus albinos) were employed in this study, which was previously approved by the institutional ethics committee (CEUA-UNICAMP, ID protocol: 2666-1). The rats received balanced standard chow (Nuvilab®) and water ad libitum during the experimental period and were kept at room with controlled temperature (23 ± 1ºC), relative humidity (45-55%), noise (<80 decibels) and a photoperiod of 12:00h light/dark cycle (lights switched on at 06:00h). All experiments were conducted in agreement with the guidelines of the European Convention for the Protection of Vertebrate Animals for research involving animals.

Study design

At 60 days old, 10 control rats had their SPA measured for characterize the baseline (Bas) values. The remaining 120 rats were allocated randomly into two types of housing space: Standard Housing Space (SH) and Wide Housing Space (WH). Afterward, for each housing condition, rats were kept from 60 to 150 days old under three interventions: control (Co, n=20), which rats were kept without any experimental intervention, chronic exercise (Tr, n=20) and voluntary wheel running (Ru, n=20). Conformed illustrated in figure 1, at 90 days, ten (n=10) animals from each group had their SPA evaluated, and at 150 days, other rats (n=10) from each group had their SPA evaluated. The rats excluded after at 90 days old were used in another set of experiments.

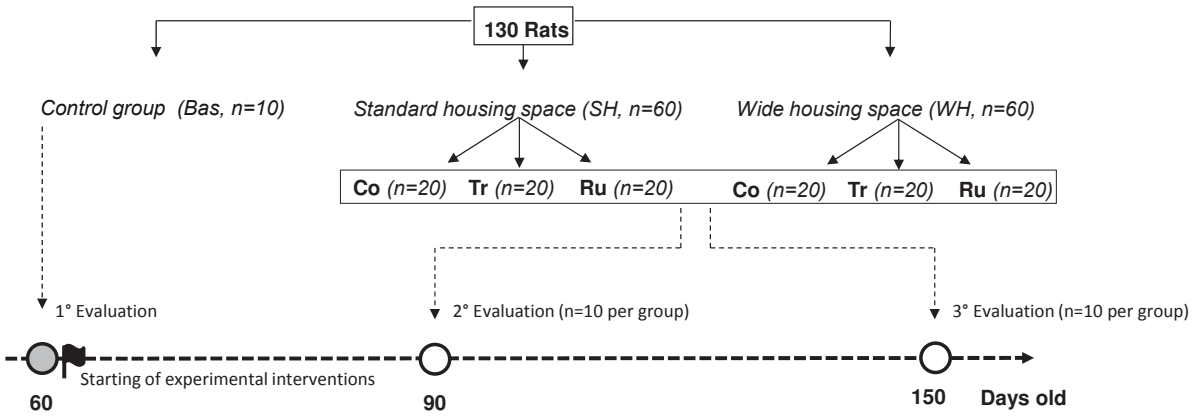

Figure 1. *Timeline of evaluations at different ages (60, 90 and 150 days old) for the control (Co), chronic exercise (Tr) and voluntary wheel running (Ru) groups confined into standard (SH) and wide housing space (WH).*

Housing space conditions

In wide housing space (WH), rats were allocated into cage with three floors covered with sawdust at sizes of 100cm of length, 100 cm of width and 33.3cm of height). The dimensions of solo area and total volume were 30.000 cm^2 and 1.000.000cm^3, respectively. In the standard housing space (SH), rats were maintained in polyethylene cages (5 animals per cage) at sizes of 49cm of length, 34cm of width and 18cm of height. For SH condition, the solo area and total volume were 1.666 cm^2 and 26.656cm^3, respectively. In the WH, 40 rats (Tr: n=20; Co: n=20) were allocated from 60 to 90 days old. After 90 days old, only 20 animals (Tr: n=10; Co: n=10) remained until the age of 150 days. In other cage (WH), 20 rats of Ru group were maintained from 60 to 90 days old while at 90 days old 10 rats remained until 150 days old.

Chronic exercise and voluntary running wheel interventions

During experimental period, Tr groups swam for 40min/day, 5 days/week at aerobic exercise intensity. Regarding this, for the Tr groups, animals with 60 and 90 days old were submitted to the lactate minimum test (De Araujo et al., 2007) to individually estimate the maximal aerobic capacity, and thus to adjust the workload exercise, which was equivalent to 80% of the individual lactate minimum intensity. Exercise intensity was imposed upon the animals using elastics by attaching small lead weights (relative to animal`s body mass (%BM)) to their upper back.

All swimming exercise bouts were conducted in cylindrical tanks (30 cm diameter×120 cm depth) with a smooth surface in order to avoid resting at the tank bottom or jumping for escape tentative, beyond stimulates continuous swimming behavior. Exercise sessions were always conducted at the same time of the day (08:00h) for all animals in water temperature kept at 31±1 °C (Harri e Kuusela, 1986). In addition, before the lactate minimum test, all rats, with exception of Tr group, were submitted to adaptation period (15 days) in order to provide a gradual familiarization to the lactate minimum test reducing the water-induced stress without physical training features.

Voluntary running activity for the Ru groups was carried out in a running wheel of 30 cm diameter and 11 cm width (Gaiolas Bragança ®, Bragança Paulista, SP, Brazil). In the WH condition, four running wheels were present per cage, whereas in the SH condition, one wheel was present per cage. Simultaneously, for the duration of the experimental period, Co and Ru groups were subjected to short-term swimming sessions (5 min, two days/week, without workload) to simulate water stress of the Tr group.

Apparatus of spontaneous physical activity detection

The SPA was measured gravimetrically by a signal acquisition system. In our system (Figure 2), a load cell (PLA30Kg, Lider Balanças ®) as primary sensor element was coupled between two iron platforms (47X40 centimeters). The signals were amplified (MKTC5-10®, MK control and instrumentation™), processed in a USB-6008® signal-conditioning module and transmitted to digital acquisition software (LabView Signal Express® 2009, National Instruments™). The animal's cage was placed on the platform to record movements at a frequency of 30 Hz.

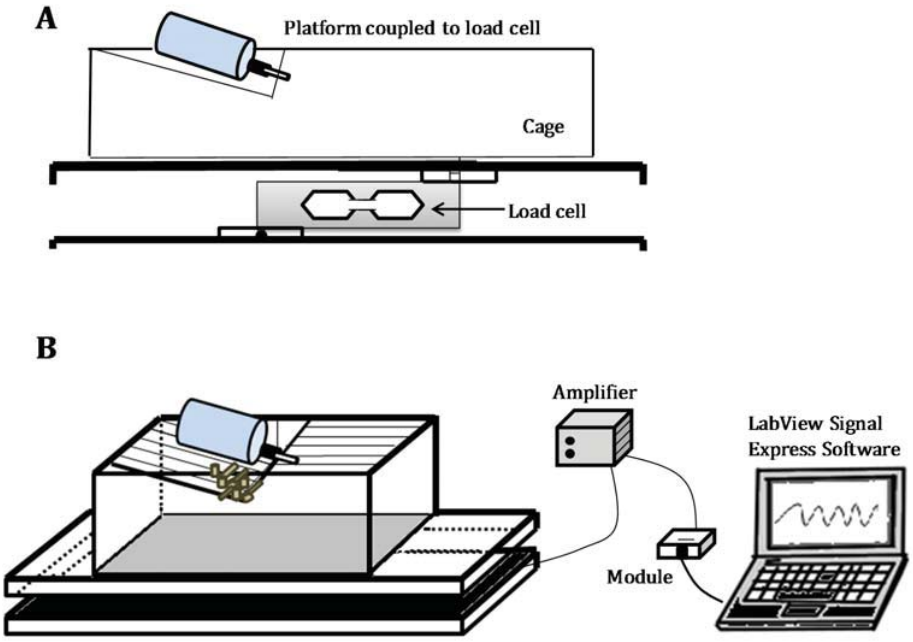

Figure 2. *Side (A) and frontal view (B) of gravimetric apparatus.*

All recordings were carried out during continuous twenty-hours (10/10h, light/dark), considering 20:00h to 16:00h as total period, being divided into dark period (20:00h to 05:59h)

and light period (06:00h to 16:00h). The 4h period was eliminated from analysis to avoid disturbances related to human access and to allow for rat habitualization to the environment. In order to simulate real sociability conditions, all recordings were executed with five rats per cage. It has long been observed that social isolation is deleterious for rats, and that alters physiological and behavioral characteristics (Wiberg e Grice, 1963). All experimental interventions were interrupted during the period of evaluation of SPA. Rats of WH condition were removed from wide housing (5 per group) during SPA evaluations, which were executed always in identical cage (49cm of length, 26cm of width and 18cm of height). The signal acquisition system was calibrated by applying known mass. Regression equations (R^2= 0.99) were then computed enabling conversions of mv signals to kilograms (Kg) units.

Spontaneous physical activity treatment

After data acquisition, digital signal were treated using MatLab® 7.0 (MathWorks™) software. Spontaneous physical activity (SPA) was calculated following Biesiadecki et al. (Biesiadecki et al., 1999), where the difference values among each consecutive samples (Cs) were squared, taken the square root and summed to each hour. After, data were adjusted by dividing the SPA values by the body mass (grams) of five rats of each cage (Kg • g^{-1}). Moreover, SPA as percentage values to each hour were summed over light and dark period considering total 20-h period as 100% (Figure 6). The SPA equation is defined as:

$$SPA = \sum_{i=1}^{n} \sqrt{(Cs_{i+1} - Cs_i)^2}$$

The raw data was processed by a Butterworth digital low-pass filter, of 4th order and 5 Hz corner frequency to remove the non-systematic high frequency noise above 5 Hz. Moreover, the signal of our data acquisition system had a systematic low frequency noise introduced. To

correct the error generated by the low frequency noise, we determined the lower value of SPA during 1 minute within the 20 hours and subtracted from all samples of 1 minute from the signal. We believe that the lower value of SPA represents the moment when all animals were statics by sleeping and the SPA at this moment was generated by low frequency noise.

Statistical Procedures

Statistical analyses were carried out using a software package (Statistic 7.0). Data regarding SPA were reported as the mean (M) and standard deviation (SD) for total (SPA-TOTAL), dark (SPA-DARK) and light (SPA-LIGHT) period. Were checked data normality using Shapiro-Wilk. The age effect has 2 levels (90 and 150 days old), intervention effect compound of 3 levels (control, chronic exercise and voluntary wheel running), housing space effect was constituted of 2 levels (standard and wide housing space) and day-period effect was constituted of 2 levels (dark and light period). Thus, ANOVA (age, intervention, housing space and day-period) was employed to verify the main effects and interactions. Tukey HSD post hoc test was used to locate group's difference. Data from the 60 days old group are reported for reference, but were not included in the analyses of variance. The significance level was set at $p < 0.05$ in all cases.

RESULTS

Spontaneous physical activity (SPA) values are exhibited in the table 1. Regarding SPA-TOTAL, ANOVA revealed a significant main effect for age ($F_{1, 456}$= 12.3, p<0.01, 90>150days old), day-period ($F_{1, 456}$= 685.0, p<0.01, dark>light) and housing space ($F_{1, 456}$= 16.9, p<0.01, SH>WH). No statistical significance was found for main effect of intervention ($F_{2, 456}$= 0.19,

p=0.82), and for interaction between intervention and housing space ($F_{2, 456}$= 0.41, p=0.66), showing always lower SPA-TOTAL values in animals housed in wide housing space, irrespective of interventions.

The age x intervention interaction was near to a statistical significance ($F_{2, 456}$= 2.44, p=0.08), indicating that the decay of SPA-TOTAL along the age was attenuated in Tr group dissimilarly than Co and Ru groups. A significant age x housing space interaction was found ($F_{1, 456}$= 10.8, p<0.01), indicating that SPA-TOTAL levels were diminished along the age in animals housed in the wide housing space. The age x housing space x intervention interaction ($F_{2, 456}$= 1.38, p=0.25) not reached statistical significance.

SPA behavior throughout 20 hours is presented in figure 3. The SPA as percentage values at light period were ranging from 12.8 to 30.9% whereas at dark period ranging from 69.1 to 87.2% (figure 4). In accordance with this discourse, there was no significant interactions among day-period effect with age ($F_{1, 456}$= 3.62, p=0.05) and intervention ($F_{2, 456}$= 0.15, p=0.85). A significant day-period x housing space interaction ($F_{1, 456}$= 22.5, p<0.01, SH>WH at dark period) was found.

Post hoc indicated SPA-LIGHT to be significantly lower compared to SPA-DARK in all groups studied. This result attests that irrespective of experimental intervention, SPA levels were always higher and lower at dark and light periods, respectively.

Table 1. *Mean ± SD of spontaneous physical activity (SPA) for entire period (TOTAL- 20:00h to 16:00h), dark period (DARK - 20:00h to 05:59h) and light (LIGHT - 06:00h to 16:00h) for the control (Co), voluntary wheel running (Ru) and chronic swimming exercise (Tr) groups confined into standard (SH) and wide housing space (WH) at 60, 90 and 150 days old. Data obtained in Kg were normalized by the mass (g) of five rats of cage (Kg•g^{-1}). The percent of variation (Δ) in relation to Bas (60 days old).*

		90 days old						150 days old					
		Standard housing space			Wide housing space			Standard housing space			Wide housing space		
	Bas	Co	Ru	Tr	Co	Ru	Tr	Co	Ru	Tr	Co	Ru	Tr
SPA-TOTAL (Kg•g^{-1})	*0.185 ±0.15*	*0.138 ±0.11*	*0.140 ±0.11*	*0.128 ±0.08*	*0.137 ±0.09*	*0.130 ±0.10*	*0.127 ±0.11*	*0.130 ±0.10* **g**	*0.122 ±0.09*	*0.150 ±0.12* *g,* **s**	*0.083 ±0.05* **m**	*0.102 ±0.07* *h*	*0.099 ±0.07*
SPA-TOTAL (Δ)		*-25*	*-25*	*-31*	*-26*	*-30*	*-32*	*-30*	*-34*	*-19*	*-55*	*-45*	*-47*
SPA-DARK (Kg•g^{-1})	*0.298 ±0.13*	*0.220 ±0.09*	*0.224 ±0.09*	*0.186 ±0.08*	*0.203 ±0.07*	*0.207± 0.07*	*0.205± 0.10*	*0.219 ±0.06* **g,** **s**	*0.189 ±0.07* **g, h**	*0.262 ±0.06* **g, r,** **s**	*0.120 ±0.04* **m, p**	*0.147 ±0.05* *i, h*	*0.137 ±0.07* **s**
SPA-LIGHT (Kg•g^{-1})	*0.072 ±0.07*	*0.056 ±0.03**	*0.055 ±0.03**	*0.070 ±0.05**	*0.070 ±0.05**	*0.052 ±0.02**	*0.048 ±0.03**	*0.040 ±0.02**	*0.056 ±0.03**	*0.038 ±0.02**	*0.047 ±0.03**	*0.057 ±0.06**	*0.061 ±0.05**

Comparisons *(P < 0.05)* ***among groups at similar age*** **(150 days old): g)** *Co-WH,* **h)** *Tr-SH,* **i)** *Co-SH and* **s)** *Tr-WH;*

Comparisons *(P < 0.05)* ***between different ages (90 x 150 days old)****:* **m)** *Co-WH,* **p)** *Co-SH,* **r)** *Tr-SH and* **S)** *Tr-WH;*

Comparisons *(P < 0.05)* ***between periods (Dark x Light):*** * *within of respective group and age.*

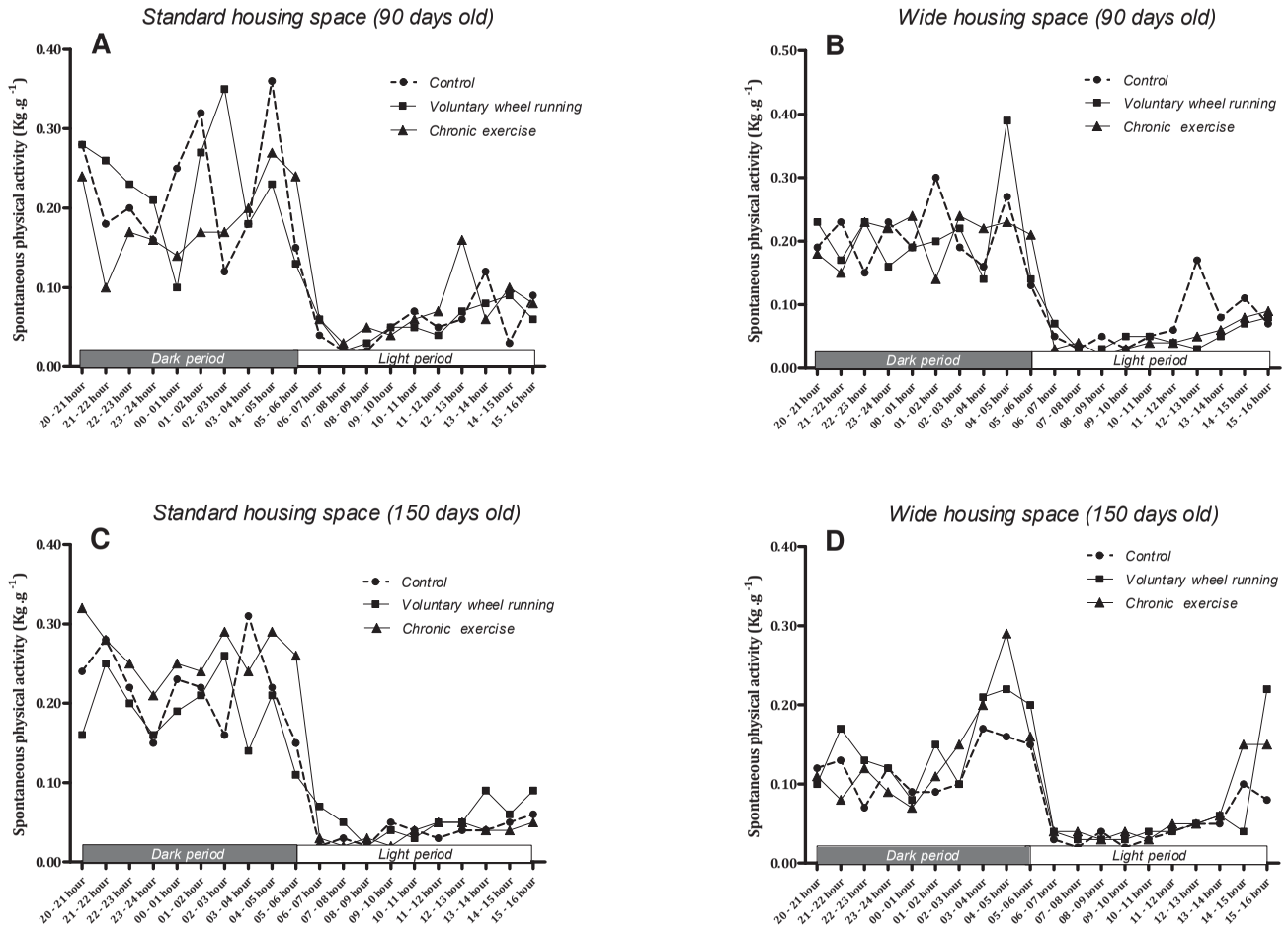

Figure 3. *Spontaneous physical activity behavior during 20 hours for the control, voluntary wheel running and chronic exercise groups confined in standard housing space (A and C) and wide housing space (B and D) at 90 and 150 days old. Data are means of two cages per group.*

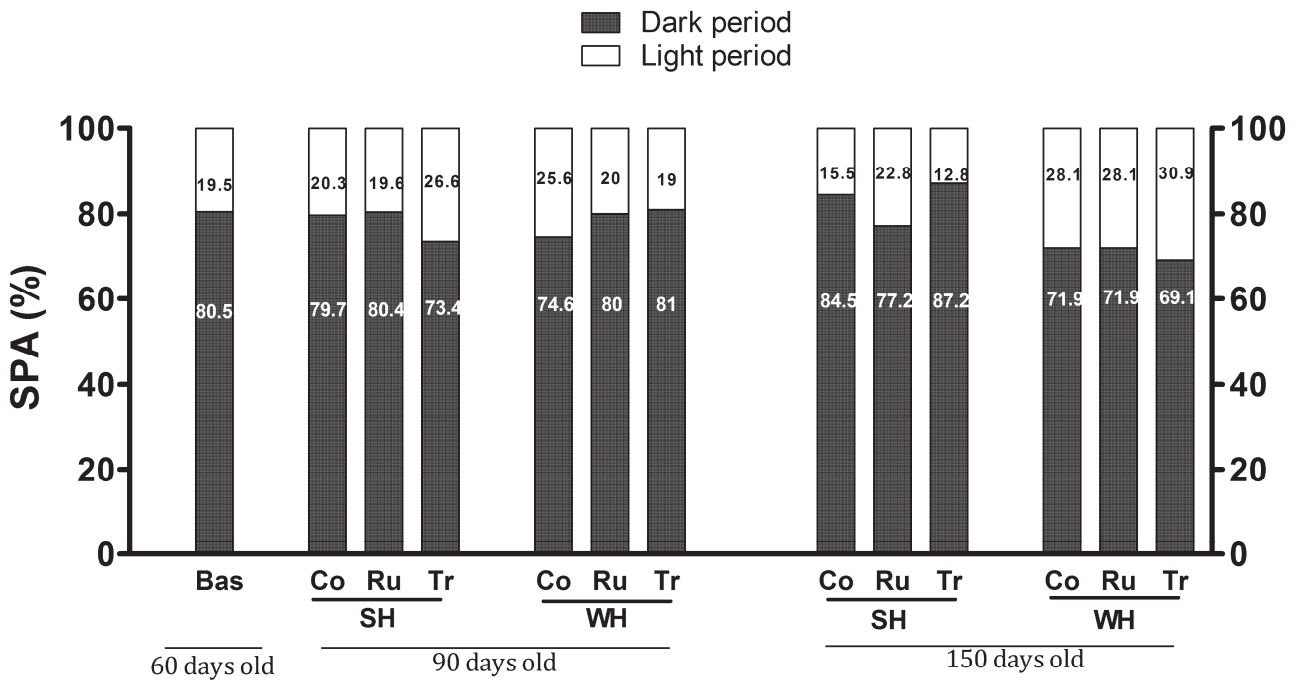

Figure 4. *Spontaneous physical activity (SPA) as percentage values summed over dark period (20:00h to 05:59h) and light period (06:00h to 16:00h) for the control (Co), voluntary wheel running (Ru) and chronic exercise (Tr) groups confined into standard (SH) and wide housing space (WH) at 60 (Bas), 90 and 150 days old.*

DISCUSSION

In the present study we found an age-dependent decay of SPA levels, however, this effect seems be dependent on the intervention to which the rats were subjected. Such observations are in agreement with the percentage values (table 1). It is possible verify that at 150 days old, exposure to chronic exercise alone was effective in attenuating the age-dependent decrease of SPA levels. In light of our results, it seems rational to affirm that better physiological adaptations occurred in trained animals. Our result is consistent with the literature, which previously showed that "exploratory activity" is better preserved in trained animals (Ingram, 2000; Skalicky et al., 1996). These studies has suggested that exercise can promote a slowing of sensorimotor impairment with age, and that possibly improve the maintenance of dopaminergic and cholinergic systems. Nevertheless, the advanced neurobiological mechanisms remain to be further determined by the scientific literature. Indeed, some evidences exposed that the dopamine function, which appears to be the major neurotransmitter involved in locomotor activity (Ingram, 2000), is enhanced by the chronic exercise intervention (Macrae et al., 1987).

There is little information regarding the effects of housing conditions and their interactions with physical interventions (e.g., chronic exercise and running wheel) in rats. Our study addresses this topic from a unique perspective. Housing space may affect the metabolism of rats, as was previously suggested by Spangenberg et al. (2005). They found increased activity levels in rats housed in large floor pen than their control rats kept in typical cages. Contradicting these authors, we found lower SPA-TOTAL values in animals confined in the wide housing (WH) than compared to standard housing (SH), and this occurred irrespective of interventions. Such observations are in agreement with a lack of interaction between intervention and housing space. Regarding this unexpected result, is valid be discussed that we did not evaluate the SPA of rats

in the wide housing condition, as opposed to Spangenberg et al. (2005), which quantified the physical activity levels of rats in their own home. This methodological difference may explain the ensuing in our study. In light of the literature, it seems reasonable to speculate that when rats of the WH group were allocated in typical cages during gravimetric test, they appeared less interest to locomotion and so, SPA levels were suppressed probably by the neurobiological modulations in hippocampus. The consequences of the restriction of environmental space were related by Mitsushima et al. (1998), which found decreased hippocampal acetylcholine release, and locomotor activity in rats after they were allocate in small cages after a period of habituation in large cages. It has been argued that an acute restriction of environmental space can attenuate the response of septo-hippocampal cholinergic neurons to release acetylcholine, which in turn can explain our results. It is established that the acetylcholine is one of the most important neurotransmitters that regulate conscious awareness (Perry et al., 1999) and it has been argued that the hippocampus shows functions in spatial learning and spatial memory performance (Fadda et al., 2000; Stancampiano et al., 1999). Despite of our result, others set of data of our experiment such as lower fat mass give us further indications that the WH condition really stimulated higher SPA levels in our rats (data unpublished). Of note, future studies are needed to evaluate the SPA with a greater specificity and to examine in detail the physiological consequences when rats living in the wide housing space.

Regarding circadian rhythm for rats, it is established that SPA shows high activity during the night in relation to day period (Verwey et al., 2013), but this normal pattern can be influenced by some experimental interventions such as pathological situations (Stephan e Zucker, 1972). Modulations in the normal pattern of SPA can in turn could lead to negatives implications for animal welfare (i.e. modulations in hormonal and sleep patterns). Given this, it is necessary

check the influence of physical activities interventions or housing conditions on SPA behavior/patterns at dark and light periods. We performed this analyze, assuming that the SPA behavior pattern should not be affected. Accepting our assumption, we found higher SPA values were frequently observed at dark and lower at light period, suggesting a similar SPA pattern irrespective of experimental interventions. This result can be visualized in accordance by the post hoc analysis and by the figure 3, which indicated lower SPA values at light contrasting with at dark period. Our findings are analogous from existing literature, which related that ~74 % of total locomotion activity occur at dark period (Ikeda et al., 2000) in animals submitted to systematic light/dark cycles.

A number of studies have previously addressed the evaluation of SPA in rodents from the myriad of methods, which typically relies on system of infrared light matrix (Young et al., 1993), video tracking (Sams-Dodd, 1995; Vorhees et al., 1992), dielectric constant sensor (Kikuchi et al., 2013) or detectors of position change (Masuo et al., 1997). In addition, in the most of studies the locomotion activity was assessed employing the open field test, which is characterized by acute tests in a circular or square arena during 10 min of recordings (Skalicky et al., 1996). Although important, it presents limitations: short periods of evaluation, which in turn might not express the normal rats' behavior and treatment of outcomes from video analyses (i.e. two-dimensional computer techniques), which cannot quantify all sensitive ambulatory movements (i.e. grooming, rearing, fidgeting). In our viewpoint without assuring the adequacy of SPA evaluation itself, any attempt to collect physiological data from advanced analyzes is frail and subjected to criticism. Thus for this reason, our choose to evaluate SPA thought gravimetric apparatus because of the robustness and sensibility, being incontestably accurate and ideal to record all motion changes exerted by animals during long periods. Such method opens an

interesting way of scientific exploration to be applied in several animal models experiments. Originally, Biesiadecki et al. (1999) confirmed the efficacy of gravimetric test as well as this method has confirmed that could be suitable in different applications (Moes e Holden, 2014). In the present study, the SPA was successfully measured by the mathematical approach previously described (Biesiadecki et al., 1999) but, comparison between our results and those from this study are not straightforward because of methodological differences. Of note, we believe that it is necessary to adjust the SPA by the body mass when using animals submitted different experimental interventions (i.e. aging and chronic exercise), which can modulate dramatically the body composition. Our assumption is valid since that conflicting interpretations were found when SPA values were not adjusted by the body mass of rats.

Recently, Teske et al. (2012) related high SPA levels in the obesity resistant rats, and it was presumed that enhanced SPA contributes to the lean phenotype. Indeed, it is know that obesity is generally related to reduced non-exercise activity thermogenesis (i.e. SPA) (Levine, 2004; Levine e Kotz, 2005), and that levels of SPA is involved in mediating energy expenditure, and consequently to the development of metabolic diseases (i.e. obesity). Apart from this, age-dependent decrease in the locomotor activity have been reported in a varied range of non-human species (Cass et al., 2005; Goodrick, 1966; Goodrick, 1967; Hagen et al., 1998; Ingram, 2000; Nemoz-Bertholet e Aujard, 2003; Skalicky et al., 1996; Teske et al., 2012) and this response could be implicated in the decrease in quality of life and longevity (Ingram, 2000; Nemoz-Bertholet e Aujard, 2003). Therefore, the possibility of counteracting the lowering of SPA levels by means of non-pharmacological therapies represent an interesting way and may be useful in developing strategies to prevent diseases in animal's models. In light of our results, the chronic exercise have substantial implications in this context. Based on others set of data of our

experiment such as lower fat mass, it is possible cogitate that the wide housing space really can be employed as a non-pharmacological therapy for stimulate higher SPA levels in rats.

CONCLUSION

We conclude that traditional decrease aged-induced of SPA in sedentary rats can be attenuated in rats submitted to chronic exercise housed in typical cages. However, the SPA levels were suppressed when all rats housed in the wide housing were allocated in typical cages during SPA evaluation. Finally, patterns of SPA at dark and light period were preserved irrespective of chronic exercise and type of housing confinement.

Acknowledgments

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4.3. ARTIGO III: Wide housing space modulate the gene expression of monocarboxylate transporter 1 and 4 and enhances liver glycogen status in rats

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Abstract

It is essential to document the positive physiological effects related to housing conditions. Remaining to be addressed, the aim of study was to analyze the effect of housing space on monocarboxylate transporter (MCT)1 and MCT4 expression in the skeletal muscle, serum corticosterone levels and muscle and liver glycogen content of rats along the age. Male Wistar rats were kept in two types of housing space: standard (SH) and wide (WH). Animals in the SH group were housed in typical cages, whereas rats in the WH group were housed in a cage with three floors (100 cm in length, 100 cm in width and 33.3 cm in height). Rats were kept under these interventions from 60 to 150 days old. Ten rats of each group were euthanized for blood sample collection and tissues excision. From of significant interactions ($p<0.05$) obtained by analyzes of variance (ANOVA), we found that the amplified housing space (WH) was able to promote a significant increase of mRNA level of MCT4 in soleus and gastrocnemius skeletal muscle. Similar findings were observed for mRNA MCT1 for gastrocnemius, but not for MCT1 of soleus muscle. Greater amounts of glycogen contents were found in the liver of animals housed in WH. Through evaluation of the measured by enzyme-linked immunosorbent assay, it was not detected difference in the corticosterone levels in the both housing confinements. Our data support that the wide housing space, which enables a wider range of daily locomotion and intense efforts, seems modulate gene expression of proteins involved with both the anaerobic and the aerobic pathways, probably promoting improvements towards the rate of lactate efflux and influx mainly in glycolytic fibers. This finding suggest a powerful and useful tool in developing higher levels of physical fitness and exercise performance of rats.

Keywords: *Rats, Housing conditions, Glycogen; Lactate, MCT1; MCT4; Messenger ribonucleic acid.*

INTRODUCTION

Lactate and H^+ ions produced in glycolysis are transported through tissues by a transport system involving proton-linked monocarboxylate transporters (MCTs) (Juel e Halestrap, 1999). Commonly associated to muscle pH regulation, it has been suggested that MCT4 is related to extrusion of lactate from the glycolytic muscle cell whereas MCT1 is appears to be responsible for the lactate uptake from blood, being predominantly in oxidative muscle (Bonen et al., 2000; Pilegaard et al., 1999b; Wilson et al., 1998). It is well known that chronic electrical stimulation (Bonen et al., 2000; Mccullagh et al., 1997), acute/chronic physical exercise (Baker et al., 1998; Pilegaard et al., 1999a) and voluntary wheel running (Yoshida et al., 2004) are components that activate the lactate/H^+ transport via MCT1 and 4 proteins. Additionally, others evidences has indicated that loss of contractile activity results in a decrement of lactate transport (Mccullagh e Bonen, 1995).

Given this, it is notorious that the synthesis of MCTs is regulated in essence by the muscle contractile activity. So, it is likely that there are muscle stimulations able to induce MCTs modulations when rats are housed with increased locomotion opportunities, and it is important if considered the restrict space in common maintenance of laboratory animals. The current typical cages provide little choice for rats to perform their natural behaviors or to engage in most forms of physical activity, leading to a sedentary life (Garland et al., 2011), which in turn can inhibits several physiological responses. Our work hypothesis was that increasing the housing space, we would give to rats the opportunity to express a wider range of their natural behaviors, increasing daily locomotion (i.e. aerobic) as well as short and intense efforts (i.e. anaerobic), which ought to be reflected in physiological requirements on monocarboxylate transporters 1 and 4.

Our assumption seems consistent, as it has been found that physiological variables related to muscle performance capacity, such as glycogen content and enzymes activities are positively affected by the increasing of housing space (Spangenberg et al., 2005). Based on these observations, it was investigated the effects of housing space on modulations of gene expression of MCT 1 and 4 in the gastrocnemius and soleus muscle in aging rats. Additionally, were determined the amounts of glycogen in muscle and liver as well as blood corticosterone levels as stress marker in two different housing confinements, a wide cage and a typical cage to laboratory maintenance of rats.

Materials and Methods

Animals

Forty (n=40) male Wistar rats (Rattus norvegicus albinos) were used in this investigation. The animals were kept in an environment controlled room, including temperature (23 ± 1 ºC), relative humidity (45-55 %), noise (< 80 decibels) and a photoperiod of 12:00 h light/dark cycle room, which was illuminated from 6.00am to 6.00pm. The rats received balanced standard chow (Nuvilab®) for rodents and water ad libitum. The experiment was performed under approval (UNICAMP – CEUA protocol no. 2666-1) of the institutional ethics committee following rigorously the specific Brazilian resolutions on bioethics in experiments with animals.

Study Design

After completing 60 days old, forty rats (n=40) were allocated randomly into two types of housing space: standard housing (SH, n=20) and wide housing (WH, n=20). After 4 weeks (90 days old) and at the end of the study period (150 days old), ten rats of each group (n=10) had

their physiological parameters evaluated by means of the blood sample collection and tissues excision (liver, soleus and gastrocnemius muscle).

Housing conditions

In the standard housing space (SH), rats were maintained in polyethylene cages (5 animals per cage) of 49cm length, 34cm width and 18cm height. For SH condition, the solo area and total volume were 1666 cm^2 and 26656 cm^3, respectively. The wide housing space (WH) was building with three floors at sizes of 100cm length, 100 cm width and 33.3cm height. The dimensions of solo area and total volume were 30000 cm^2 and 1000000cm^3, respectively. Thus, in area, the WH was 18.0 higher than SH and, in volume, 37.5 higher than SH. All cages were lined with autoclave-sterilized sawdust that was replaced three times a week.

Collection of biological material

After 12h food withdrawn, the animals were anaesthetized by 50 mg/kg sodium thiopental and, after the loss of cornea and foot reflexes, they were euthanized by decapitation method. Blood samples were immediately collected and centrifuged for serum separation. Samples were stored at -80°C. The liver, soleus (red fiber) and gastrocnemius (white fiber) muscles were dissected, immediately frozen in liquid nitrogen and stored at -80°C.

Enzyme-linked immunosorbent assay

Corticosterone levels were analyzed by ELISA corticosterone kit (Enzo® Life Sciences, Catalog No. ADI-900-097) using a microplate reader (ASYS Expert Plus UV, Biochrom) and measured at 450nm based on the manufacturer's instructions. The data are perceptually

compared to the control readings (i.e. animals confined into standard housing) obtained at same age.

Muscle glycogen content

The liver tissue (500 mg), soleus and the gastrocnemius (200–250 mg) were digested in potassium hydroxide (KOH) [30%]. Sodium sulfate (Na_2SO_4) was added for glycogen precipitation and ethanol [70%] for refinement. The glycogen concentration of tissues samples was analyzed according by colorimetric method using 10 µL of phenol (C_6H_5OH) and 2.0 mL of sulphuric acid (H_2SO_4) with a absorbance was determined at 490 nm following (Dubois et al., 1956).

Quantitative Real Time PCR

Total RNA was extracted from gastrocnemius and soleus muscle using Trizol reagent (Life Technologies Corporation, CA, USA) according to the manufacturer's recommendations. Total RNA was quantified using a Nanodrop ND-2000 (Thermo Electron, WI, USA). Reverse transcription was performed with 3 µg of total RNA using High-Capacity cDNA Reverse Transcription kit (Life Technologies Corporation, California, USA). Relative expression was determined using primers with TaqMan detection system for target genes: MCT1 (Rn00561420_m1), MCT4 (Rn00563409_m1) and GAPDH as endogenous control (Life Technologies Corporation, CA, USA). The analysis of gene expression by Real-time PCR was performed on ABI Prism 7500 Fast platform using 20 ng of cDNA. The data were analyzed using the Sequence Detection System 2.0.5 (Life Technologies Corporation, CA, USA), and expressed

as a relative value using the comparative threshold cycle (Ct) method (2−ΔΔCt), according to the manufacturer's recommendation.

Statistical Procedures

Statistical analyses were carried out using a software package. Mean (M) and standard deviation (SD) were determined for all parameters. Data normality was checked using Shapiro-Wilk. ANOVA (age x housing space) was employed to verify the main effects and interaction among them for all variables studied. The age effect comprised two groups (90 and 150 days old), and housing space effect comprised two groups (standard and wide housing space). Tukey HSD post hoc test was used to locate group's difference. The significance level was set at $p < 0.05$ in all cases.

RESULTS

Analyses of mRNA level of monocarboxylate transporters 1 and 4 in soleus and gastrocnemius skeletal muscle are presented in figure 1. According to significant main effects of ANOVA, the amplified housing space was able to promote a significant increase of MCT4 mRNA of both oxidative (soleus) and glycolytic (white gastrocnemius) muscle. Moreover, MCT4 mRNA increased along age (150>90 days old), and this age-dependent effect was pronounced in the WH condition, as viewed by the significant age x housing interaction. Similar findings were observed for MCT4 mRNA for gastrocnemius, but not for MCT1 mRNA of soleus muscle.

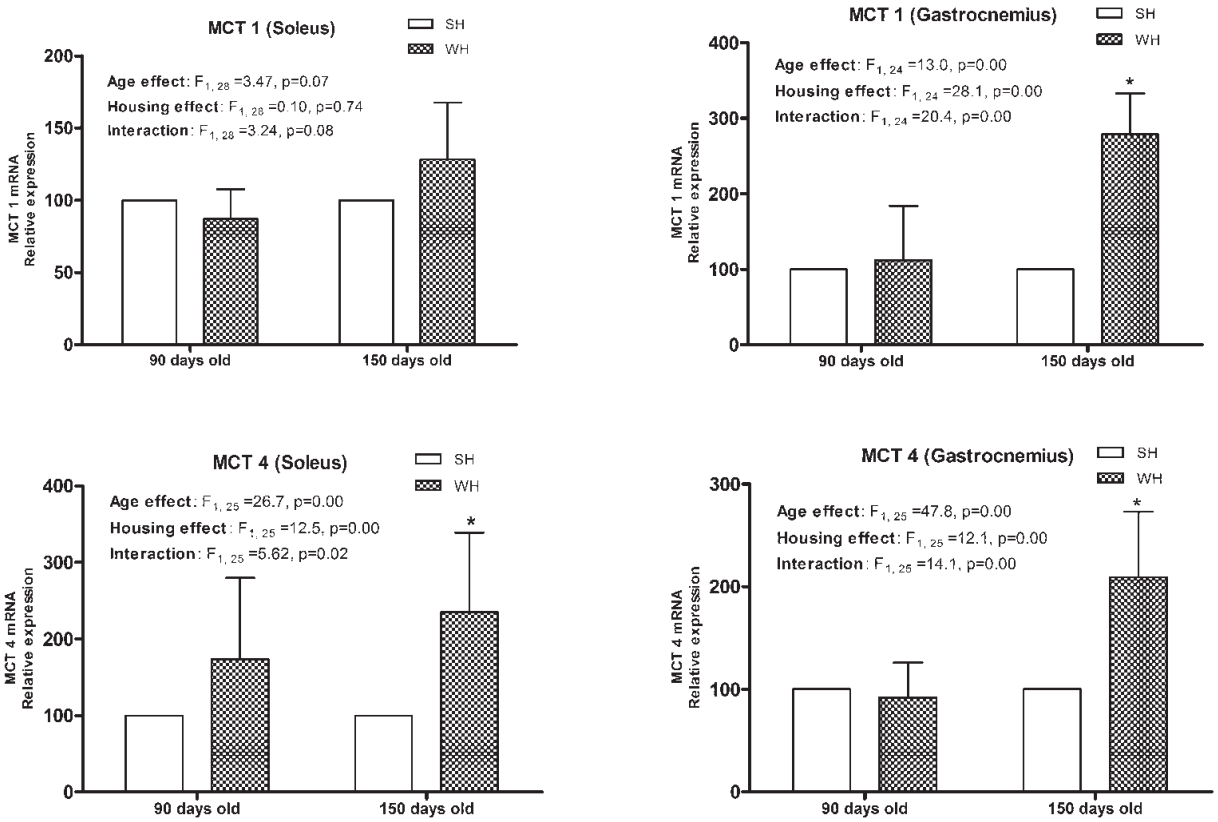

Figure 1. *mRNA level of monocarboxylate transporters (MCT)1 and 4 in soleus and gastrocnemius muscle of rats confined into a wide housing space (WH) and standard housing space (SH) at 90 and 150 days old.* Sig diff p<0.05 from all groups.*

Regarding corticosterone levels, none significant main effect was found for age (p=0.10), housing space (p=0.75) and interaction between these factors (p=1.00) (figure 2). The animals' glycogen content, and the ANOVA p-values are shown in table 1.

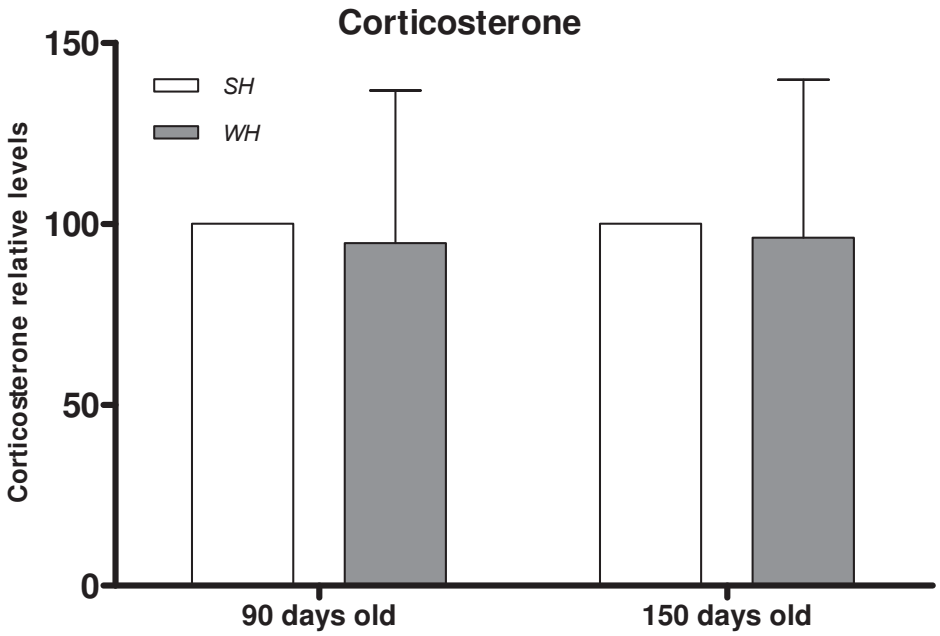

Figure 2. *Corticosterone relative levels measured by enzyme-linked immunosorbent assay for rats confined into a wide housing space (WH) and standard housing space (SH) at 90 and 150 days old. The data are compared (%) to the control readings obtained at same age.*

Table 1. *Mean ± SD values of tissue glycogen contents (mg/100mg of tissue) in animals confined into standard (SH) and wide housing space (WH) at 90 and 150 days old.*

	90 days old		150 days old		ANOVA p-value		
	SW	WH	SW	WH	*Age*	*Housing*	*Age* x *Housing*
Liver *(mg/100mg of tissue)*	1.08± 0.55	0.92± 0.43	1.34± 0.46	2.27± 1.01[a]	p=0.002 (150> 90d)	p=0.044 (WH> SH)	p=0.044 (WH> SH- 150d)
Gastrocnemius *(mg/100mg of tissue)*	0.15± 0.09	0.26± 0.13	0.22± 0.05	0.22± 0.08	p=0.475	p=0.077	p=0.077
Soleus *(mg/100mg of tissue)*	0.27± 0.05	0.18± 0.09	0.33± 0.05*	0.23± 0.04	p=0.056 (150> 90d)	p=0.001 (SH >WH)	p=0.947

*Sig diff p<0.05: *from WH group (90 days old), [a] from all others groups*

DISCUSSION

In general, our study reveal that higher locomotion, by means of increasing the housing space of rats, can be a powerful factor for influence physiological adaptations to the interest parameters. In rodents, the majority studies are conducted, if not solely, using cages with small dimensions. To the best of our knowledge, our work is the first study intended to investigate the effects of housing space on mRNA of MCTs, placing our study in a unique perspective for addressing this topic. Our data presented in this paper showed that only wide housing confinement of rats was able to up-regulate expression of MCT4 in white gastrocnemius and soleus muscles. This reflects that the higher locomotion possibilities to increase the rate of lactate efflux of both oxidative and glycolytic fibers. Moreover, this response was pronounced in the aged (150 days old) when compared with younger rats (90 days old). These results were expected for rats confined in WH condition due to greater habitual locomotion linked with intense

activities. In fact, the availability of wide space led the experimental group to show a wild behaviors of great prominence, which could not be measured, but were easily identified by us. So, predominantly anaerobic, behaviors such as offensive and defensive (i.e. chasing, biting, encounters to fighting, flee, jumps) preferentially utilize glycolysis, and obviously produce lactate. Is valid remember that MCT4 on the cell surface in the skeletal muscle appears to be important for several tissues which are capable of high rates of glycolysis, producing large quantities of lactate that require rapid efflux (Bonen, 2001). Although we did not evaluate the MCTs proteins contents to guarantee accurate interpretations, an increase in the mRNA MCT4 gave us indications that the animals housed in a wide space tended to present a rapidly exit of lactate from the intracellular pool. In support of this interpretation, reports have indicated that the increases in MCT expression can result in increased rates of lactate flux, as this has been observed in previous studies when these proteins have been experimentally up-regulated (Coles et al., 2004).

Pilegaard et al. (1999a) have appointed that the decline in muscle force during development of fatigue in high-intensity exercise is associated with accumulation of lactate and a concomitant lowering of pH in the muscle. This condition established can interfere negatively the excitation-contraction muscle activity (Favero et al., 1997; Fitts, 2010). However, previous studies indicated that high-intense exercise training can increase muscle sarcolemmal lactate/H^+ transport in human skeletal muscle as well as improve the ability of the muscle to release lactate and H^+ during contractions (Pilegaard et al., 1999a). Therefore, in light of these considerations, it seems reasonable to consider that the enhanced opportunities for locomotion among captive rats promotes, if not a "high-intense training effect", almost a reduced sedentary behavior (or obesogenic environment), as had considered for any authors (Lake e Townshend, 2006). So,

we suggest that in WH the removal of lactate and H^+ from skeletal muscle is likely to be importance to the animals maintained in this conditions, and this process is an important adaptive response as strategy to maintain an efficiency in anaerobic efforts during daily habits. MCT1 is perhaps the most remarkable trigger for all aerobic adaptations. The higher activity of this protein is likely to be of importance to promote the lactate uptake, being highly correlated with the oxidative muscle capacity (Bonen et al., 2000; Pilegaard et al., 1999b; Wilson et al., 1998). Increased mRNA MCT1 was observed also in the gastrocnemius skeletal muscle. It seems reasonable to consider that the wide housing space, by means of increased daily locomotion, appears affects both aerobic as anaerobic metabolism. We assume that the increase in MCT 1 and 4 in the gastrocnemius muscle of rats might be responsible for physiological adaptations towards intensification of lactate efflux and influx, simultaneously. Of note, in the present study, our wide housing space not affected the stress of rats, because it has been shown that levels of corticosterone, released by the hyperactivity of the hypothalamo-pituitary-adrenal system, appears associated with affective disorders such as depression, and manifestation of several catabolic consequences (Holsboer e Barden, 1996; Karten et al., 1999).

Glycogen concentrations were different between animals of two housing confinements, likely due to their different levels of locomotion. The wide housing space was able to promote an increase of glycogen stocks in the liver. This physiological adaptation could be responsible to maintain the homeostasis of glycemic levels (i.e. avoiding the hypoglycemia) during daily activities, which in turn are higher in animals housed in the wide housing continent (Baldwin et al., 1975; Galbo et al., 1979). Moreover, we found a statistical tendency ($p=0.07$) for higher glycogen content in the white gastrocnemius muscle of rats housed in the WH condition. This is fascinating because it is know that glycolytic fibers are recruited when the excitatory input into

the motorneurons increases to high levels (i.e. during intense work activities) (Baldwin et al., 1975). Thus, as the rats of WH condition must be ready to transient intense works that requiring high rates of glycolysis, we believe that the main function of glycogen preservation in the skeletal gastrocnemius muscle, is to serve as an energy store in "fight or flight" situations (i.e. offensive and defensive activities). Is valid be argued that, the type I muscle fibers rely more on free fatty acid (FFA) oxidation during aerobic activities, whereas type II fibers are capable of rapid contractions and rely primarily on glycolytic pathways for energy production, which is important for survival during acute emergencies activities reactions (Greenberg et al., 2006; Jensen et al., 2011). For that reason, we speculate that in the soleus muscle, the wide housing space led to a noticeable adaptation towards to oxidize FFA, which probably inhibited the glycogen synthesis (glycogen synthase kinase-3) via protein kinase B (PKB), as previous reported in cells incubated (Schmitz-Peiffer et al., 1999). This may explain the lower storage of glycogen in soleus muscle in rats of WH condition.

Taken all results, it seems reasonable to cogitate that the reduced locomotion possibilities of restricted typical cages really inhibit the gains of physiological adaptations. Nevertheless, we showed in this paper that the wide housing confinement increase the expression of both MCT1 and MCT4, notably in the glycolytic muscle. The modulations in MCTs, could contribute to the reductions in the circulating lactate concentrations, and are important adaptive responses to maintain an efficiency, especially in anaerobic efforts during daily habits. Finally, in our viewpoint, the wide housing represents an unquestionably and attractive model for enhance the physical fitness and exercise performance.

5. CONCLUSION

In summary, the wide housing space affected the physiological responses of the study animals, rising their mRNA of MCT4 and MCT1 in gastrocnemius muscle. We conclude that a wider range of daily locomotion and intense efforts, seems modulate the messenger ribonucleic acid of proteins involved with both the anaerobic and the aerobic pathways, probably promoting improvements towards increasing the rate of lactate efflux and influx mainly in glycolytic fibers. This finding suggest a useful way in developing higher levels of exercise performance of rats.

Conflicts of interest

The authors have no conflicts of interest.

Acknowledgments

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5. DISCUSSÃO E CONCLUSÃO GERAL

Discussão específica e mais aprofundada dos dados obtidos por ocasião dos experimentos encontra-se nos artigos apresentados, no entanto, realizaremos algumas conclusões pertinentes neste momento. De nosso conhecimento, nossas investigações tratam de um achado inédito, sendo o primeiro estudo a analisar a influência do espaço físico da gaiola associado com a intervenção do exercício físico ou roda de atividade sobre diversos parâmetros em ratos. Coletivamente, nossos dados sugerem que uma maior disponibilidade de locomoção oferecida por uma gaiola contendo maior espaço pode trazer importantes reduções no conteúdo de gordura corporal, o que sem dúvida possui um potencial alvo terapêutico. No entanto, é valido ser destacado que a interação entre o treinamento físico e o alojamento em gaiolas com amplo espaço parece ser mais interessante, uma vez que os efeitos do envelhecimento sobre parâmetros aeróbios foram mais atenuados adotando essa condição. Em linhas gerais, é possível observar que realmente o espaço físico tem grande contribuição sobre o metabolismo e gasto energético de roedores de laboratório. Acreditamos que nossos estudos serão impactantes para a comunidade cientifica uma vez que obtivemos um conjunto de dados revelam que o maior espaço físico pode atuar como uma ferramenta poderosa no desenvolvimento de níveis mais elevados de aptidão física de ratos. Futuros estudos que ainda estão em fase de confecção irão fornecer informações preciosas a respeito da influência do espaço físico associados ao exercício físico crônico ou livre acesso a roda de atividade sobre

aspectos genéticos (MCTs), as quais desempenham papel crucial para a regulação do equilíbrio ácido-base e transporte de lactato a nível celular.

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7. ANEXOS

7.1. Parecer de aprovação da Comissão de Ética no Uso de Animais

UNICAMP

CEUA/Unicamp

Comissão de Ética no Uso de Animais
CEUA/Unicamp

C E R T I F I C A D O,

Certificamos que o projeto **"DETERMINAÇÃO DA ATIVIDADE ESPONTÂNEA DE RATOS POR GRAVIMETRIA E RELAÇÕES COM A CAPACIDADE AERÓBIA E EXPRESSÃO GÊNICA DE MCTs 1 E 4: EFEITOS DA IDADE E DO TREINAMENTO FÍSICO"** (protocolo nº **2666-1**), sob a responsabilidade de **Prof. Dr. Claudio Alexandre Gobatto / Pedro Paulo Menezes Scariot**, está de acordo com os **Princípios Éticos na Experimentação Animal** adotados pela **Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL)** e com a legislação vigente, **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais, e o **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**.

O projeto foi aprovado pela Comissão de Ética no Uso de Animais da Universidade Estadual de Campinas - CEUA/UNICAMP - em **26 de março de 2012**.

Campinas, 26 de março de 2012.

Profa. Dra. Ana Maria A. Guaraldo
Presidente

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7.2. Financiamento da pesquisa

DETERMINAÇÃO DA ATIVIDADE ESPONTÂNEA DE RATOS POR GRAVIMETRIA E RELAÇÕES COM A CAPACIDADE AERÓBIA E EXPRESSÃO GÊNICA DE MCTs 1 E 4: EFEITOS DA IDADE E DO TREINAMENTO FÍSICO

Esse trabalho foi financiado pela "FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO"

FAPESP

Processo n° 2011/16222-7